NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF PUBLIC HEALTH SCIENCE

COURSE CODE: PHS 818

COURSE TITLE: PUBLIC HEALTH MICROBIOLOGY AND ENTOMOLOGY

COURSE GUIDE

PHS 818
PUBLIC HEALTH MICROBIOLOGY AND ENTOMOLOGY
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NATIONAL OPEN UNIVERSITY OF NIGERIA

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Published by

National Open University of Nigeria

Printed 2014

ISBN: 978-058-978-0

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INTRODUCTION

The course, PHS 808 Public Health Microbiology and Entomology is a three-credit unit course. In this course we shall consider the study of Public Health Microbiology and Entomology in terms of the infectious diseases associated with the underlined words. Public Health can be defined as the science and art of promoting and protecting health and well-being, preventing ill-health and prolonging life through the organised efforts of society. While Microbiology is the study of small living things. Generally, this means living things that are too small to see without the use of a microscope. In other words, it is the study of microscopic organisms, those that are unicellular (single cell), multicellular (cell colony), or acellular (lacking cells). All these can be sub divided into specialized areas of study; Such as:

- Microbiology(Bacteriology),
- Parasitology(Malariology)
- Entomology
- Virology,
- Immunology,
- Mycology

Entomology is the study of insects, including their relationships with other animals, their environment, and human beings. Entomological research can also give us broader insights into ecology, evolution, and social behaviour.

This course guide tells you what to expect from reading this course material.

WHAT YOU WILL LEARN IN THIS COURSE

The study of Public Health Microbiology and Entomology explains the integration of Public Health, Bacteriology, virology, Mycology, Parasitology, and Entomology in infectious diseases establishment in human and his environment. We shall discuss the essential scientific features of the various components as listed above, how relevant they are in Public health and in infectious diseases dissemination and prevention.

Microbiology

In this section, we shall look at the structure of typical bacterial, viral, parasitic, mycological cells and the ways in which they liberate energy from complex organic molecules. For example, various aspects of bacterial structure and metabolism are the basis of bacterial identification and taxonomy. Bacteria are constantly accumulating mutational changes as their environment imposes a strong selective pressure on them. Thus, they constantly and rapidly evolve. In addition, they exchange genetic information, usually between members of the same species but occasionally between members of different species. We shall see how this occurs.

Bacteria have parasites, the viruses called bacteriophages which are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery. Eventually, these lyze the infected bacterial cell liberating new infectious phage particles. The interrelationships of bacteria and the phages will be discussed.

Entomology

This deals with the study of insects and associated diseases. The role played by insects as vectors of disease and other critical functions shall be examined under Entomology. Finally, we shall look at general aspects of bacterial, viral, parasitic and mycological diseases and their pathogenesis, and how bacteria, virus, etc., damage the host organism, before examining some specific microbial diseases a variety of human diseases that are caused by them.

COURSE AIM

The aim of this course is to provide a good understanding of Public Health Microbiology and entomology for better management of infectious disease

and the provision of quality human / animal health, and habitable environment.

COURSE OBJECTIVES

After going through this course, you should be able to:

- define and explain the concepts of public health microbiology and entomology
- know the structures of bacteria, viruses, parasites, fungi,
- Know how to identify infectious agent without prior culture
- Understand bacterial, viral, parasitic, fungal, identification in the diagnostic laboratory versus taxonomy.
- Understand taxonomic characterization of bacteria, viruses etc.
- Know simple approaches to rapid diagnosis of these infectious agents
- Describe Nutrition, Growth and Energy Metabolism of microorganisms
- Describe Cell Envelope, spores and Macromolecular Biosynthesis of microrganisms
- Describe structure and synthesis of the cell walls of gram-positive and Gram negative bacteria.
- Describe the activity of Antibiotics – on Cell Envelope.
- Understand the mode of action of beta-lactam antibiotics.
- Know the role antibiotics in protein synthesis, nucleic acid synthesis and metabolism of microrganisms.
- Know the various types of insects and arthropods.
- Describe the various diseases associated with these.
- Know some immunological basis of how the body defends itself against micro-organism attack/invasion and simple laboratory technics involving these strategies.

WORKING THROUGH THIS COURSE

This course has been carefully put together bearing in mind that you might be new to the course. However, efforts have been made to ensure that adequate explanations and illustrations are available to enhance better understanding of

the course. You are therefore advised to spend adequate time to study this course and ensure that you attend tutorial sessions where you can, ask questions and compare your knowledge with that of your course mates.

COURSE MATERIALS

This course comprises of two parts. They are as listed below:

i. A course guide

ii. Study units

STUDY MODULES/ UNITS

These comprise of seven modules broken down into some specified number of units per module. They are as listed below:

MOUDLE TWO

In module one, unit one, section A, we shall look at introduction to general microbiology such as simplified overview of the bacterial cell; in unit two we shall consider sterilization and disinfection being the backbone of successful public health microbiology practice and application: while in unit three we shall briefly look at microscopy; and in unit four of this module we shall be introduced to bacterial nutrition.

In Module two, section B, part two of the introduction to microbiology would be considered. Here, we shall consider bacterial cell envelope, spores, and macromolecular biosynthesis in unit one, in unit two we shall briefly look at the effect of antibiotics on bacterial cell envelope,…while in unit three we shall take an in-depth look at bacteriophage exchange of genetic information, and genetic regulatory mechanisms in bacteria; bacteria pathogenesis; enterobacteriacae; while in unit four we shall discuss chlamydia and rickettsia.

In module three, section C unit one introduces us to entomology.

In module four, section D, we shall consider Malaria parasites as being typical of parasites due to its endemicity, and relevance in the tropics

Module five, section E, takes us to virology; in unit one we shall look at Hepatitis B, in unit two we shall consider Human Immunodeficiency Virus, HIV, while in unit three we shall look at the common cold disease virus and in unit four we shall discuss the dengue fever virus and its ill public health implications; Unit five introduces us to Ebola virus disease, and unit six takes

us to us to Lassa fever virus and its medical and public health importance; finally in this module we shall consider chicken pox virus disease.

In Module six, section F, shall introduce us to immunology and its relevance in public health.

The last but not the least Module, seven, shall introduce us to mycology and its impacts in public health microbiology.

MODULE ONE

Unit 1: THE BACTERIAL CELL

CONTENTS

1.0 INTRODUCTION TO BACTERIOLOGY

Prokaryotes and eukaryotes**:** "True" bacteria (which include all bacteria that infect man) are members of one kingdom (the eubacteria, bacteria).

In addition, a group of organisms often found in extreme environments form a second kingdom (archaebacteria, Archaea). Morphologically, the two kingdoms of organisms appear similar, especially in the absence of a nucleus, and thus are classified together as prokaryotes. (Table 1) However, they have major biochemical differences. Most archaea live in environments such as hot sulphur springs where they experience temperatures as high as 80 ^{0}C and a pH of 2. These are called thermoacidophiles. Others live in methane-containing (methanogens) or high salt (extreme halophiles) environments.

2.0 OBJECTIVES

At the end of this Unit, you will be able to:

- Identify bacteria in the diagnostic laboratory
- Carry out Taxonomy and Taxonomic characterization of bacteria.
- Be predisposed to approaches to rapid diagnosis, Culture and identification of infectious agents.

3.0 MAIN CONTENT

3.1: CHARACTERISTICS OF EUBACTERIA, ARCHAE AND EUKARYOTES

	Eubacteria	**Archaea**	**Eukaryotes**
Nucleus	No	No	Yes: membrane-bound
Nucleosomes/histones	No	**Yes**	**Yes**
Operons/polycistronic mRNAs	Yes	Yes	No
Introns	No	No	Yes
TATA Box binding protein	No	**Yes**	**Yes**
Organelles	No	No	Yes: mitochondria, lysosomes, endoplasmic reticulum etc.
Chromosomes	One Circular	One Circular	More than one
RNA polymerase	One (simple)	**More than one (complex)**	**More than one (complex)**
Protein initiator amino acid	N-formyl methionine	**Methionine**	**Methionine**
Protein synthesis sensitivity to diphtheria toxin	Insensitive	**Sensitive**	**Sensitive**

Peptidoglycan	Yes	**No**	**No**
Protein synthesis	• initiation factors • ribosomal proteins • elongation factors of Archaea are more similar to those of eukaryotes than eubacteria		

Table 1: Similarities between Archaea and Eukaryotes

3.2: Archaea;

Based on DNA sequence similarities, it appears that the archaea and eukaryotes arose from the eubacteria before they diverged from each other (figure 1.1.1a) and in some ways, archaea are biochemically more like eukaryotes than they resemble the eubacteria. For example, the RNA polymerase of archaea is as complex, in terms of number of subunits, as the eukaryote nuclear polymerases and there is considerable amino acid homology with some of the eukaryotic subunits.

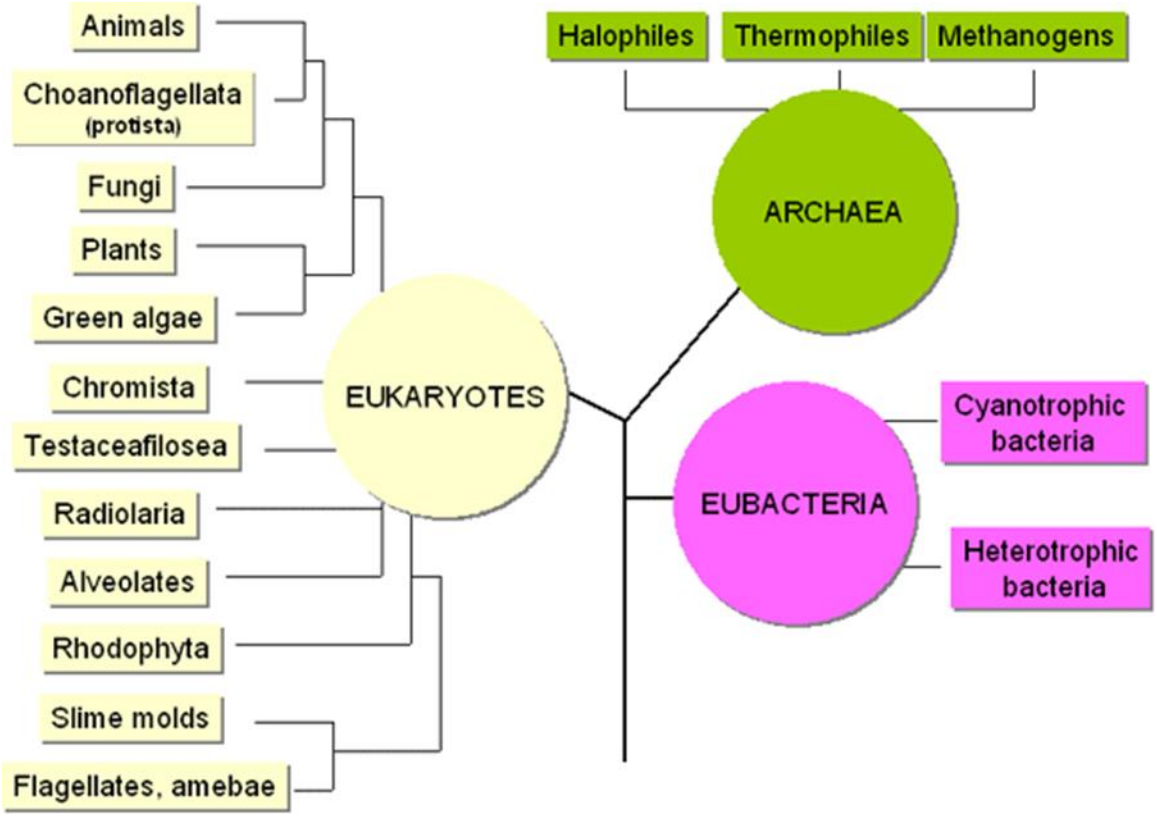


Figure 1.1.1a Adapted from Dr Alvin Fox, Emeritus Professor of Microbiology at the University of South Carolina USA:

Gene promoter structure in archaea is also more similar to that of eukaryotes than eubacteria, although, like the eubacteria, archaea have operons and transcribe these to polycistronic mRNA. Similarity also exists between the protein synthesis factors of archaea and eukaryotes suggesting that the overall protein synthesis mechanisms of eukaryotes and archaea may be similar. The 16S rRNAs of the eubacteria and the archaea are quite distinct in sequence. Eubacteria (with the exception of the genera Mycoplasma and Chlamydia) possess peptidoglycan (synonyms: murein, mucopeptide, cell wall skeleton). Peptidoglycan contains a unique sugar, muramic acid, not found elsewhere in nature. Archaebacteria contain a pseudomurein that is different in structure from eubacterial murein. In view of the increasing number of similarities between the archaea and the eukaryotes, the term archaebacteria is no longer used. All other cellular forms of life (including plants, animals, and fungi) are referred to as eukaryotes. Members of the Archaea are not human pathogens and will not be discussed further. (Alvin Fox, 1996)

3.3 Differences between prokaryotes/eukaryotes

The prokaryotic cell, in contrast to the eukaryotic cell, is not compartmentalized. Nuclear membranes, mitochondria, endoplasmic reticulum, Golgi body, phagosomes and lysosomes are not present (Figures 1.1.1b,1.1.2 and 1.1.3). Prokaryotes generally possess only a single circular chromosome. Since there is no nuclear membrane, the chromosome is bound to a specific site on the cell membrane - the mesosome. Prokaryotic ribosomes are 70S (S stands for Svedberg unit, a measure of size), whereas eukaryotic ribosomes are larger (80S). Prokaryotic ribosomal subunits are 30S and 50S (eukaryotic are larger). The 30S ribosome has 16S RNA, whilst the 50S ribosome contains 23S and 5S RNA. Ribosomal RNA is larger in eukaryotes (e.g. 18S versus 16S rRNA). Bacterial membranes generally do not contain sterols (e.g. cholesterol).

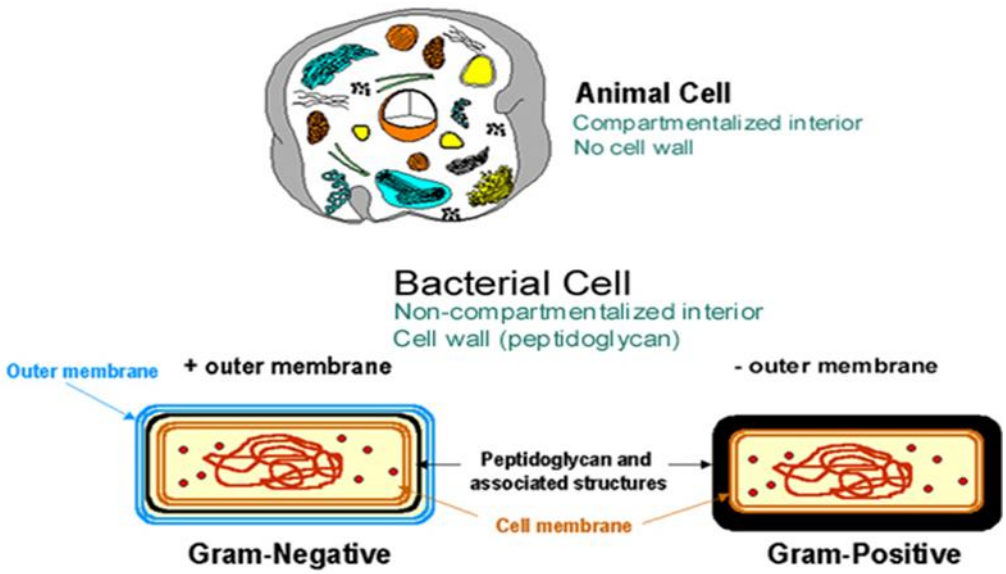


FIGURE 1.1.1B

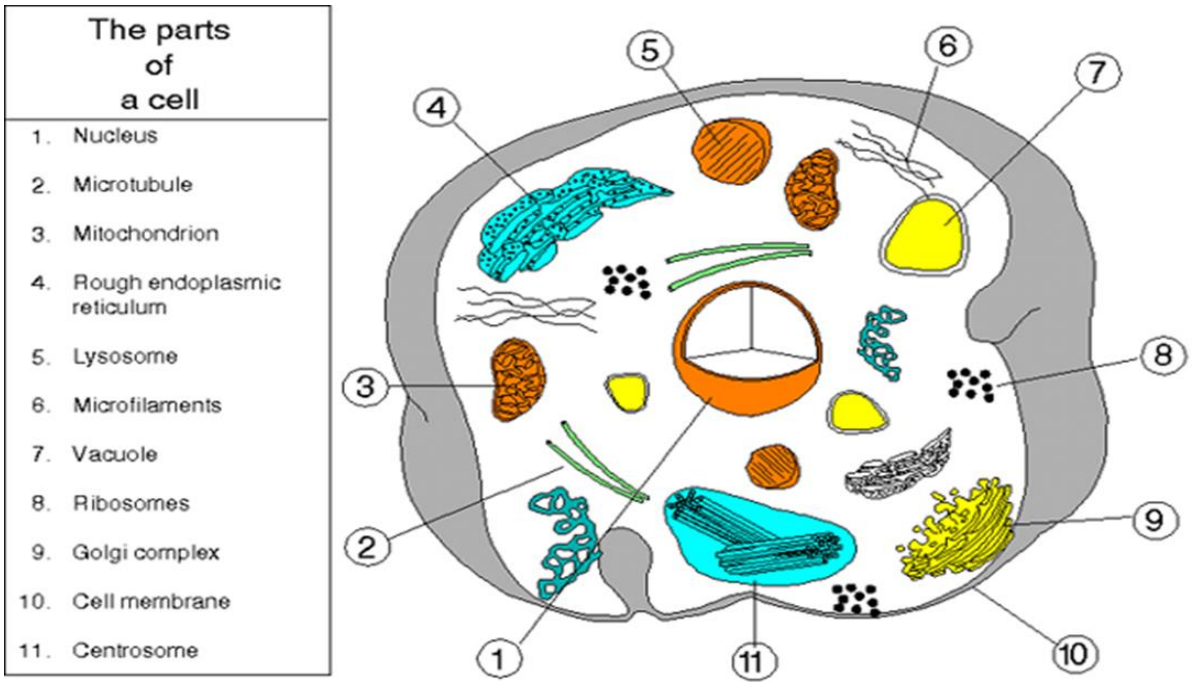


FIGURE 1.1.2

Adapted from Dr Alvin Fox, Emeritus Professor of Microbiology at the University of South Carolina USA

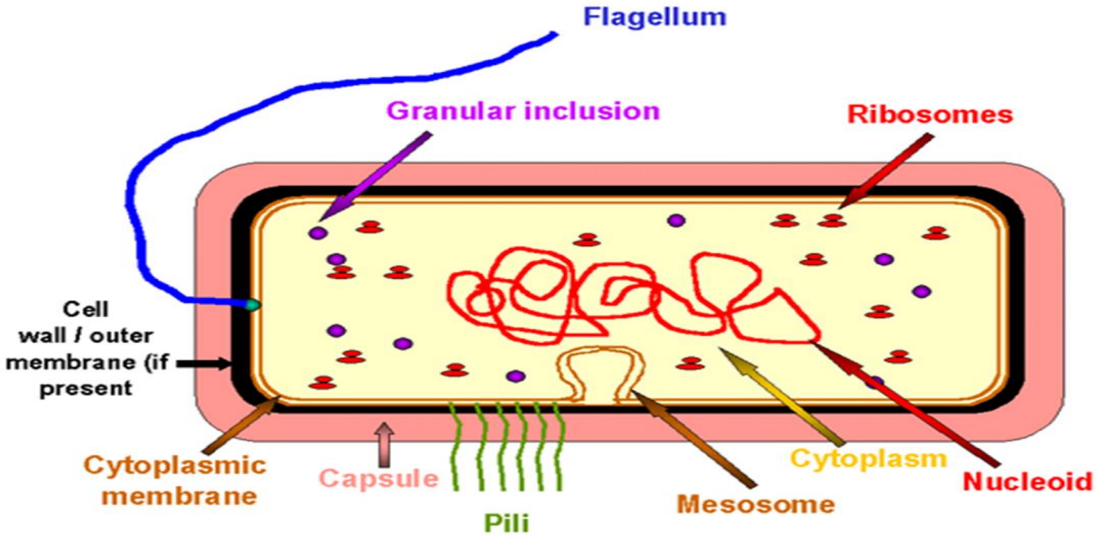


FIGURE 1.1.3

ALL COLOURED DIAGRAMS ADAPTED FROM DR ALVIN FOX, EMERITUS PROFESSOR OF MICROBIOLOGY AT THE UNIVERSITY OF SOUTH CAROLINA USA(FIGURES 1.1.4- 1.1.8)

3.40 Bacterial Structures

Despite their lack of complexity compared to eukaryotes, a number of eubacterial structures may be defined. Not all bacteria possess all of these components.

3.41 Plasmids: These are extra-chromosomal DNA, usually present in multiple copies

that often code for pathogenesis factors and antibiotic resistance factors. Some forms are also involved in bacterial replication.

3.42 The cell envelope: Bacteria can be divided into two groups on the basis of staining with the Gram stain; Gram positive bacteria remain stained by crystal violet on washing, Gram negative organisms do not. All bacteria have a cell membrane where oxidative phosphorylation occurs (since there are no mitochondria). Outside the cell membrane is the cell wall which is rigid and protects the cell from osmotic lysis. In Gram positive bacteria, the cell wall peptidoglycan layer is a much thicker layer than in Gram negative bacteria. Gram negative bacteria have an additional outer membrane. The outer membrane is the major permeability barrier in Gram negative bacteria. The space between the inner and outer membranes is known as the periplasmic space. Gram negative bacteria store degradative enzymes in the periplasmic space. Gram positive bacteria lack a periplasmic space; instead they secrete exoenzymes and perform extracellular digestion. Digestion is needed since large molecules cannot readily pass across the outer membrane (if present) or cell membrane.

3.43: Wall-less forms of Bacteria

When bacteria are treated with:

1) enzymes that are lytic for the cell wall e.g. lysozyme

or

2) antibiotics that interfere with biosynthesis of peptidoglycan, wall-less bacteria are often produced. Usually these treatments generate non-viable organisms. Wall-less bacteria that cannot replicate are referred to as spheroplasts (when an outer membrane is present) or protoplasts (if an outer membrane is not present). Occasionally wall-less bacteria that can replicate are generated by these treatments (L forms).

3.44 Flagella

Some bacterial species are mobile and possess locomotory organelles - flagella (Figure 1.1.4). Those that do are able to perceive taste their environment and respond to specific chemical foodstuffs or toxic materials and move towards or away from them (chemotaxis). Flagella are embedded in the cell membrane, extend through the cell envelope and project as a long strand. Flagella consist of a number of proteins including flagellin. They move the cell by rotating with a propeller like action. Axial filaments in spirochetes have a similar function to flagella. Binding

proteins in the periplasmic space or cell membrane bind food sources (such as sugars and amino acids) causing methylation of other cell membrane proteins which in turn affect the movement of the cell by flagella.

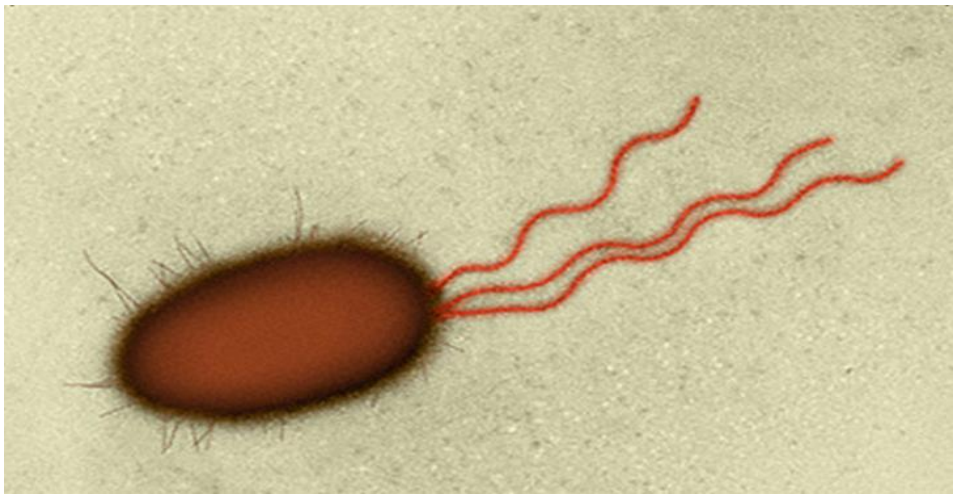


FIGURE 1.1.4

Permeases are proteins that then transport these foodstuffs through the cell membrane. Energy and carbon sources can then be stored when necessary in cytoplasmic "storage granules" which consist of glycogen, polyhydroxybutyrate or polyphosphate

3.45 Pili (synonym: fimbriae)

The types of pili (or whether they are produced at all) varies both among and between species. Pili are hair-like projections of the cell (Figure 1.1.5). Some are involved in sexual conjugation and others allow adhesion to host epithelial surfaces in infection

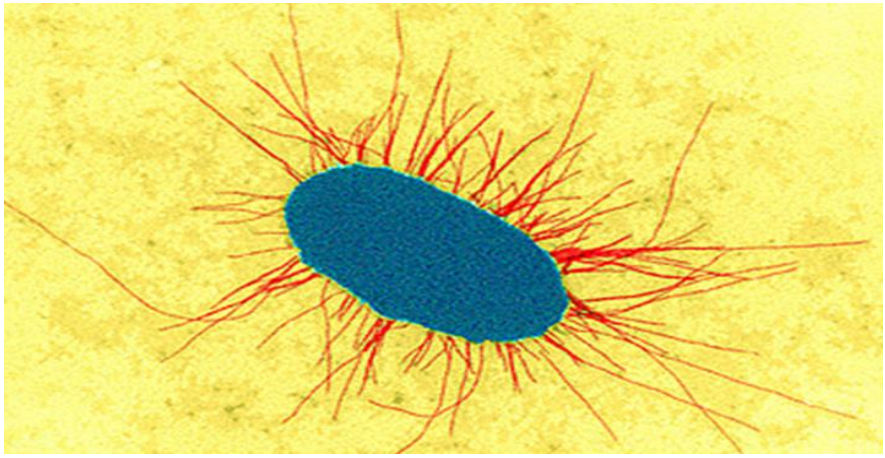


FIGURE 1.1.5

3.46: Capsules and slime layers (Figure 1.1.6)

These are structures surrounding the outside of the cell envelope. When more

defined, they are referred to as a capsule when less defined as a slime layer or glycocalyx. They usually consist of polysaccharide; however, in certain bacilli they are composed of a polypeptide (polyglutamic acid). They are not essential to cell viability and some strains within a species will produce a capsule, whilst others do not. Capsules of pathogenic bacteria inhibit ingestion and killing by phagocytes. This enhances micro-organisms ability to establish infection in their host. Capsules are often lost during *in vitro* culture.

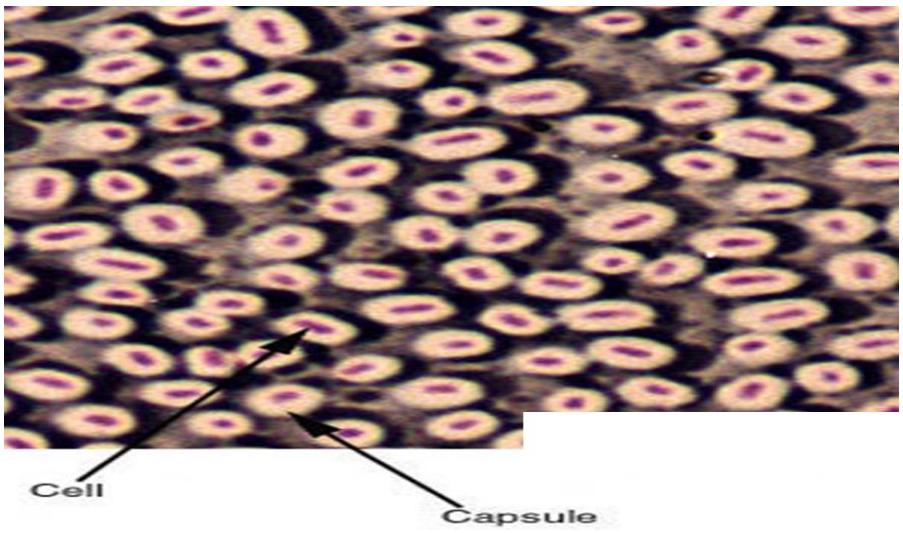


FIGURE 1.1.6

3.47 Endospores (spores)

Endospores are a dormant form of a bacterial cell produced by certain bacteria when starved (figure 1.1.7); the actively growing form of the cell is referred to as vegetative. The spore is resistant to adverse conditions (including high temperatures and organic solvents). The spore cytoplasm is dehydrated and contains calcium dipicolinate (dipicolinic acid – figure 1.1.8) which is involved in the heat resistance of the spore. Spores are commonly found in the genera *Bacillus* and *Clostridium*. These are strictly anaerobic bacteria that thrive in the absence of oxygen.

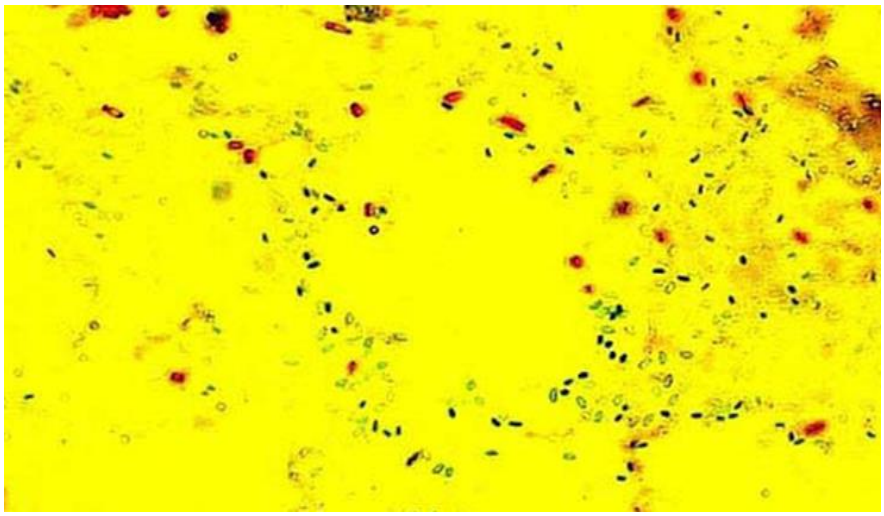


FIGURE 1.1.7A

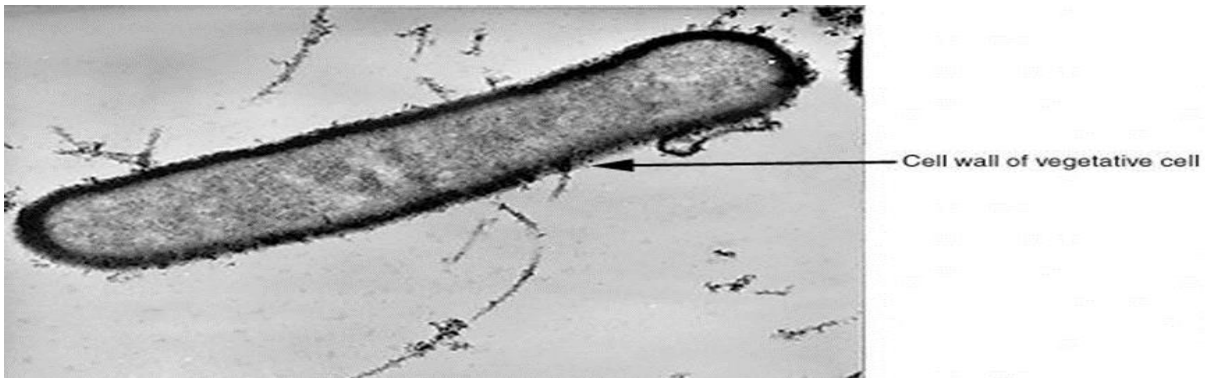


FIGURE 1.1.7B

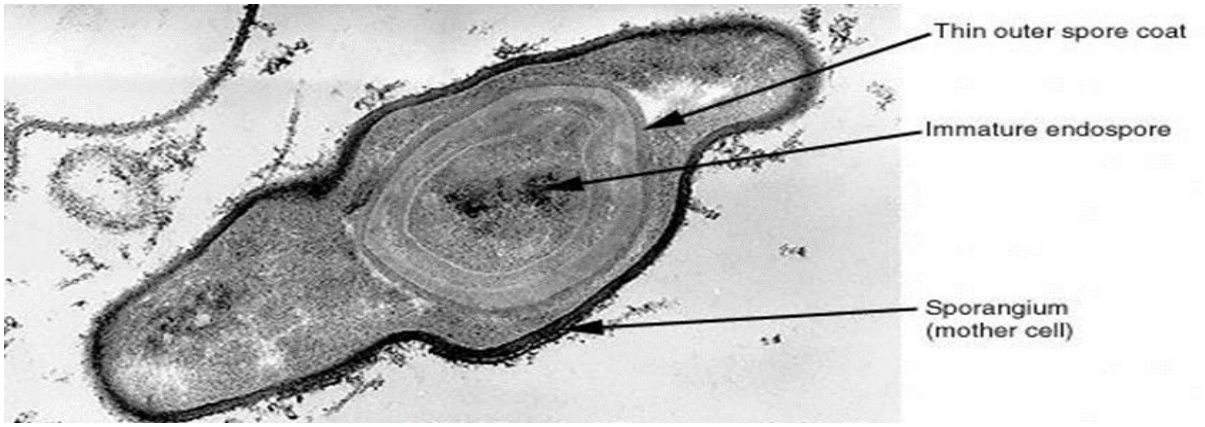


FIGURE 1.1.7C

FIGURE 1.1.7D

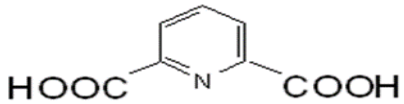


FIGURE 1.1.8

3.5: Bacterial identification in the diagnostic laboratory versus taxonomy

Isolation and identification of bacteria from patients' aids treatment. This is because infectious diseases caused by different bacteria have a variety of clinical courses and consequences. Susceptibility testing of isolates (i.e. establishing the minimal inhibitory concentration or MIC) can help in selection of antibiotics for therapy. Recognizing that certain species (or strains) are being isolated atypically may suggest that a disease outbreak has occurred e.g. from contaminated hospital supplies or poor aseptic technique on the part of hospital personnel.

When patients are suspected of having a bacterial infection, it is usual to isolate visible colonies of the organism in pure culture (on agar plates), and then speciate the organism. The identification is based on taxonomic principles applied to the clinical microbiological situation. In the diagnostic laboratory, many samples must be characterized each day and results obtained as quickly as possible. Tests must be easily learned, low in cost and rapidly performed. These classical methods for speciation of bacteria are based on morphological and metabolic characteristics. The diagnostic tests have been selected on the basis that empirically they provide discriminating information. There are numerous different tests for each of the many target pathogens. Additionally, molecular biology techniques (for characterization of specific genes or gene segments) are now commonplace in the clinical laboratory.

Modern taxonomic approaches often employ technically more complex methodology and are concerned with profiling the structural composition of bacteria. This often involves "molecular biology" or "analytical chemistry" -based approaches. It is now recognized that many of the classical schemes for differentiation of bacteria provide little insight into their genetic relationships and in some instances are scientifically incorrect. New information has resulted in renaming of certain bacterial species and in some instances has required totally reorganizing relationships within and between many bacterial families.

3.51

Taxonomic terms (classification)
Family: a group of related genera.
Genus: a group of related species.
Species: a group of related strains.
Type: sets of strain within a species (e.g. biotypes, serotypes).
Strain: one line or a single isolate of a particular species.
The most commonly used term is the species name (e.g. *Streptococcus pyogenes* - abbreviation *S. pyogenes*). There are always two parts to the species name, one defining the genus in this case "*Streptococcus*" and the other the species (in this case "*pyogenes*"). The genus name is always capitalized but the species name is not. Both species and genus are underlined or in italics.

3.52 Steps in diagnostic isolation and identification of bacteria

Step 1.Body fluids samples (e.g. blood, urine, cerebrospinal fluid) are streaked on culture plates and isolated colonies of bacteria (which are visible to the naked eyes) appear after incubation for one to several days (Figure 1.1.9). Each colony consists of millions of bacterial cells. Observation of these colonies for size, texture, colour, and (if grown on blood agar) haemolysis reactions, is highly important as a first step in bacterial identification. Whether the organism requires oxygen or not (i.e. aerobic, microaerophilic or anaerobic) for growth is another important differentiating characteristic.

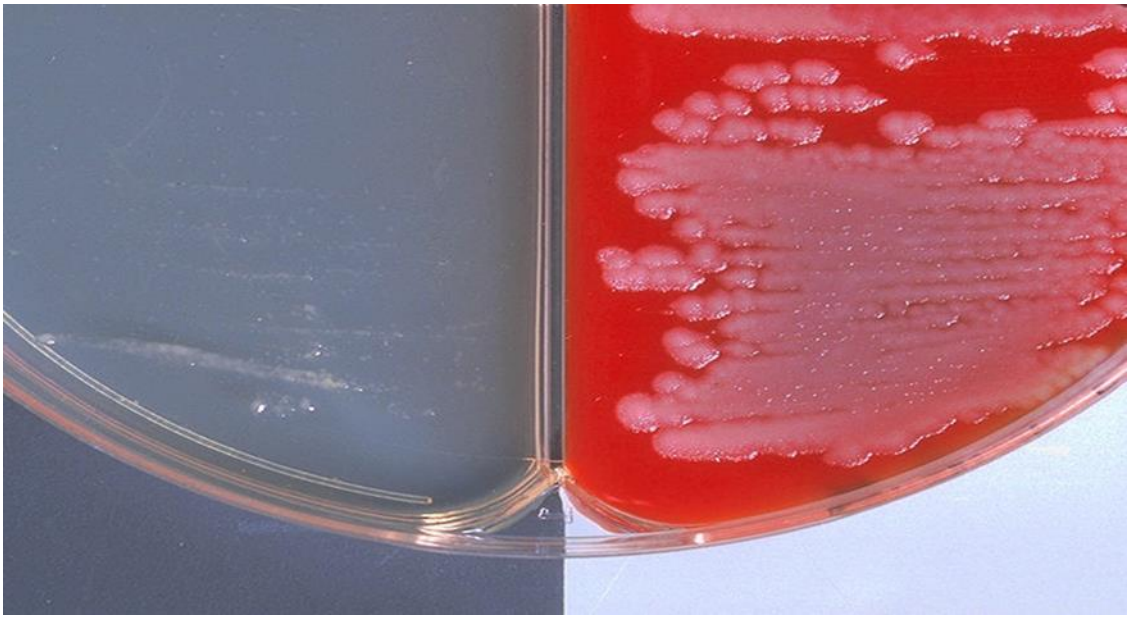


FIGURE 1.1.9

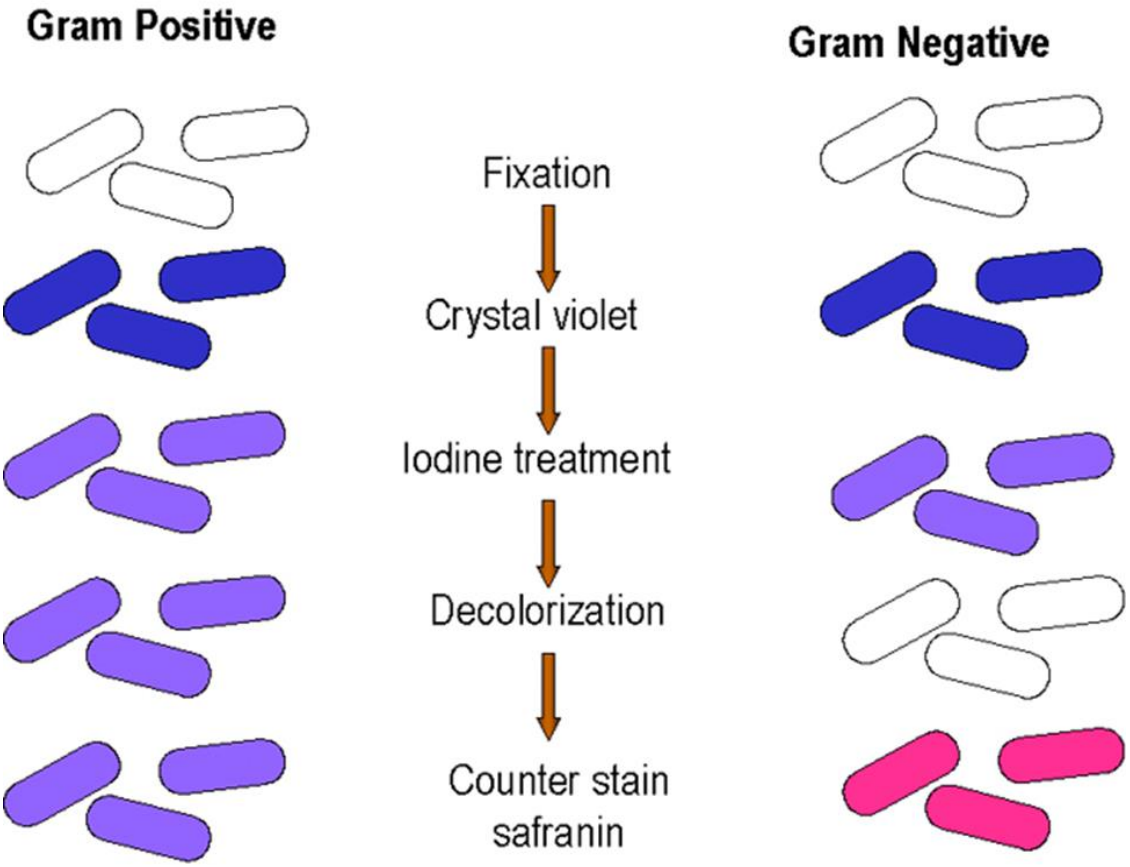


FIGURE 1.1.10

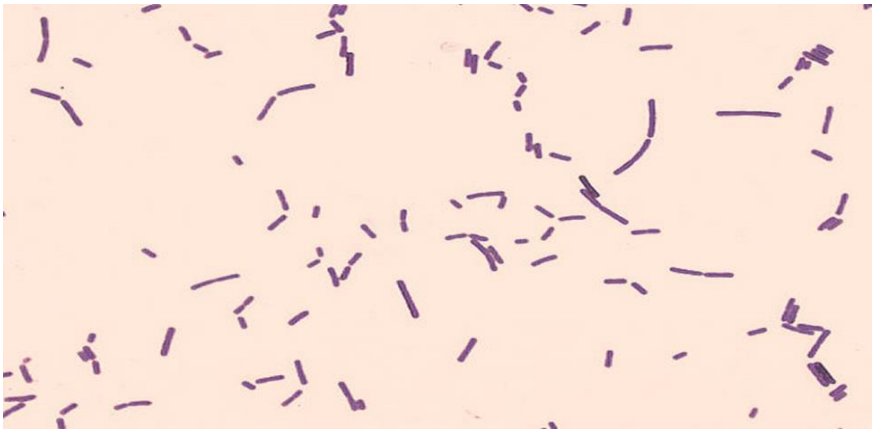


FIGURE 1.1.11A

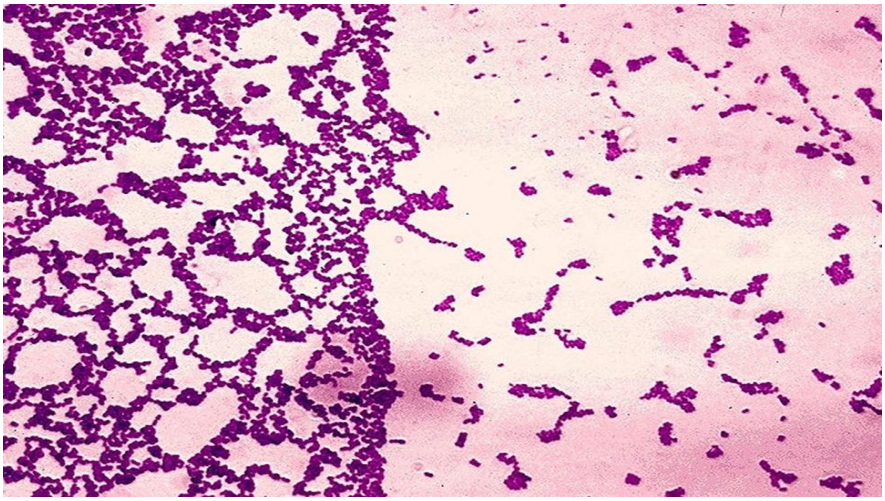


FIGURE 1.1.11B

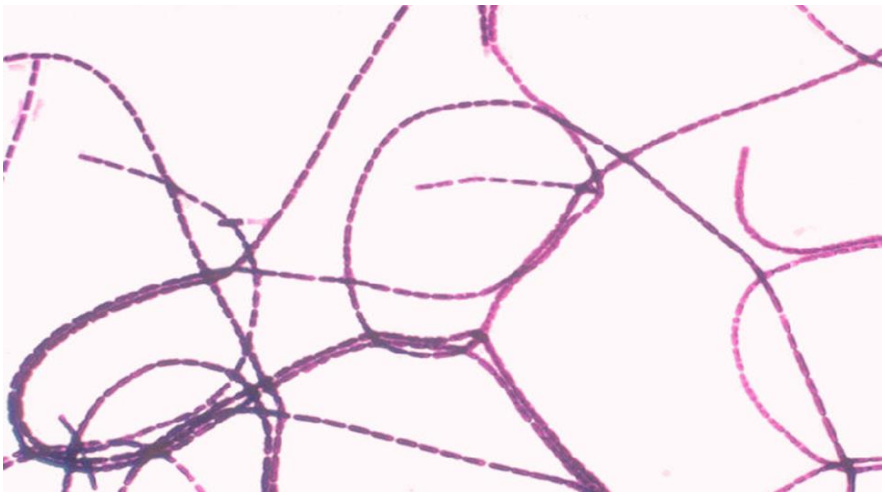


FIGURE 1.1.11C

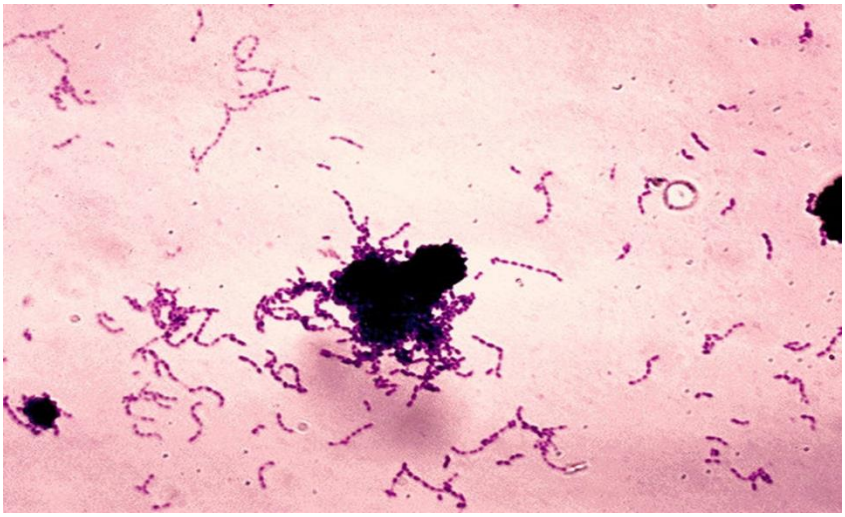
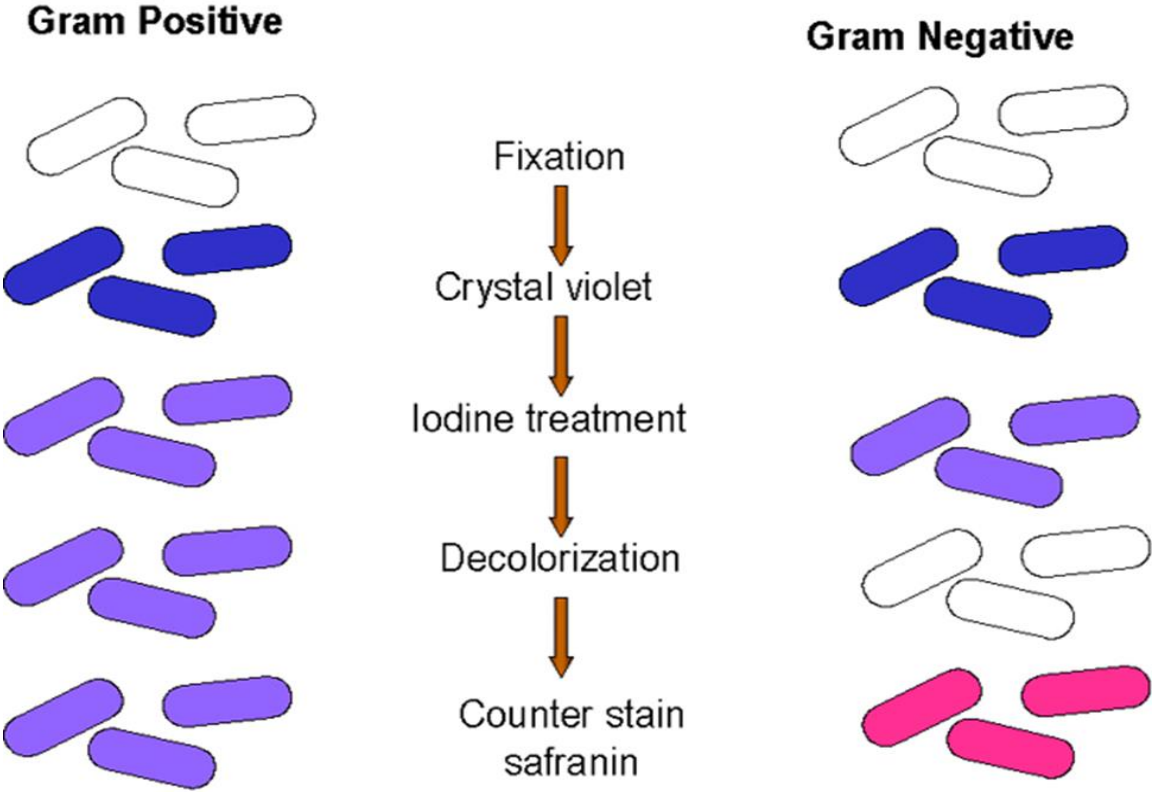


FIGURE 1.1.11D

Step 2. Colonies are Gram stained and individual bacterial cells observed under the microscope.
Step 3. The bacteria are speciated using these isolated colonies. This often requires an additional 24 hours of growth

The Gram Stain
A colony is dried on a slide and treated as follows (Figures 10 and 11):



Step 1. Staining with crystal violet.
Step 2. Fixation with iodine stabilizes crystal violet staining. All bacteria remain purple or blue.

Step 3. Extraction with alcohol or other solvent. Decolorizes some bacteria (Gram negative) and not others (Gram positive).
Step 4. Counterstaining with safranin. Gram positive bacteria are already stained with crystal violet and remain purple. Gram negative bacteria are stained pink.
Under the microscope, the appearance of bacteria is observed. Questions to be asked include:

- Are they Gram positive or negative?
- What is the morphology (rod, coccus, spiral, pleomorphic [variable form] e.t.c.)?
- Do cells occur singly or in chains, pairs e.t.c.?
- How large are the cells?

Beside the Gram stain, there are other less commonly employed stains available (e.g. for spores and capsules).
Another similar colony from the primary isolation plate is then examined for biochemical properties; for example, will the bacteria ferment a sugar such as lactose? In some instances, the bacteria are identified (e.g. by aggregation) with commercially available antibodies recognizing defined surface antigens. Other commercial molecular tests are now widely used.

3.60 Taxonomic characterization of bacteria

There is considerable diversity even within a species. Thus comparisons of species involve comparisons of multiple strains for each species. Comparisons are primarily based on chemical or molecular analysis.

3.61 Chemical analysis

Sophisticated tools are available for studying the structural composition of bacteria (most commonly fatty acid, carbohydrate or ubiquinone profiling). Characterization of secreted metabolic products (e.g. volatile alcohols and short chain fatty acids) is also helpful.

3.62 Molecular analysis

It would be ideal to compare sequences of entire bacterial chromosomal DNA, but this is currently not feasible. Millions of nucleotides have to be sequenced for each strain. In the past several years, sequencing of the entire genomes of one representative (i.e. a strain) of a few bacterial species has been achieved. In each case, this has involved massive amounts of work by large research groups dedicated to the task of sequencing. Alternatively, genomic similarity has been historically assessed by the content of guanine (G) plus cytosine (C), usually expressed as a percentage (% GC). This has been replaced by two alternatives -

hybridization and sequencing (most commonly of the gene coding for 16S rRNA). DNA-DNA homology (or how well two strands of DNA from different bacteria bind [hybridize] together) is employed to compare the genetic relatedness of bacterial strains/species. If the DNA from two bacterial strains display a high degree of homology (i.e. they bind well), the strains are considered to be members of the same species. DNA from different bacterial species (unless closely related) display no homology.
In the last few years, sequencing of 16S ribosomal RNA molecules (16S rRNA) has become the "gold standard" in bacterial taxonomy. The molecule is approximately sixteen hundred nucleotides in length. The sequence of 16S rRNA provides a measure of genomic similarity above the level of the species allowing comparisons of relatedness across the entire bacterial kingdom. Closely related bacterial species often have identical rRNA sequences. The technique thus provides complementary information to DNA-DNA hybridization. Determinations of the sequence of 16S rRNA genes and other genetic regions are used in identification in the clinical microbiology laboratory.

3.63 Approaches to rapid diagnosis without prior culture

Certain human pathogens (including the causative agents of tuberculosis, Lyme disease and syphilis) either cannot be isolated in the laboratory or grow extremely poorly. Successful isolation can be slow and in some instances impossible. Direct detection of bacteria without culture is possible in some cases. A simple approach to rapid diagnosis (as an example of antigen detection) is used in many doctor's offices for the group A streptococcus. The patient's throat is swabbed and streptococcal antigen extracted directly from the swab (without prior bacteriological culture). The bacterial antigen is detected by aggregation (agglutination) of antibody coated latex beads.
Bacterial DNA sequences can be amplified directly from human body fluids (the polymerase chain reaction, PCR). In this fashion large amounts of specific genes or portions of genes can be generated and readily detected. For example, great success has been achieved in rapid diagnosis of tuberculosis.
Finally, direct microscopic observation of certain clinical samples for the presence of bacteria can be helpful (e.g. detection of *M. tuberculosis* in sputum). Serologic identification of an antibody response (in patient's serum) to the infecting agent can only be successful several weeks after an infection has occurred.

4.0 CONCLUSION

In this unit, we have defined and explained the concepts of public health microbiology and entomology. By now you should have known at least the structures of some bacteria relevant in human infectious diseases. In subsequent modules we shall also look at the structures of viruses, parasites, fungi of public health relevance. You have learnt how to culture bacteria on solid agar media. Simple colonial features of common bacteria on various media must be known as earlier described. We have also demonstrated and learnt about how to identify infectious agent without prior culture as this becomes very necessary during periods of urgent diagnosis in health settings-as earlier explained. Equally important is bacterial identification in the diagnostic laboratory versus taxonomy, you must pay rapt attention to the details involved and commit all areas emphasized to memory. Please do this alongside the taxonomic characterization of bacteria that was clearly stated above. Approaches to rapid diagnosis of infectious agents need no further emphasizes at least from the trend of our earlier discussion.

5.0 SUMMARY

In this unit we have learnt that:

- Prokaryotes "True" bacteria (which include all bacteria that infect man) are members of one kingdom (the eubacteria, bacteria).
- A group of organisms often found in extreme environments form a second kingdom (archaebacteria, Archaea) with biochemical features that closely resemble Eukaryotes (organisms with well-defined nuclear and other organelle membranes).
- Morphologically, the two kingdoms of organisms appear similar, especially in the absence of a nucleus, and thus are classified together as prokaryotes.
- Steps in diagnostic isolation and identification of bacteria using Gram staining reaction.
- Bacterial identification and culture in the diagnostic laboratory versus taxonomy.
- Taxonomic characterization of bacteria.
- Approaches to rapid diagnosis without prior culture.

6.0 TUTOR-MARKED ASSIGNMENT

Ques. 1 Clearly differentiate between Prokaryotes and Eukaryotes.

Ques. 2 What are the simple approaches to rapid diagnosis without prior culture?

Ques.3 Describe the Gram staining technique.

Ques.4 In tabular form only show the similarities between Archaea and Eukaryotes.

Ques.5 Write a comprehensive account on the taxonomic classification of the microorganisms we have learnt in this unit.

7 Suggested further reading / references

Atlas R.M: *Principles of Microbiology*, St Louis,1995, Mosby USA

Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2012, Mosby USA.

Murray P.R. *et al*, editor: *Medical Microbiology,* ed.5 St. Louis 2005, Mosby USA.

Schmidt H. Hensel M.: Pathogenicity islands in bacterial pathogenesis. *Clinical Microbiological Review* 17: 14, 2014 USA**.**

Todar's Online textbook of Bacteriology: Kenneth Todar,PhD

University of South Carolina: Online Microbiology Teaching Aids and Resources, 2013.

ALL COLOURED DIAGRAMS Adapted from Dr Alvin Fox, Emeritus Professor of Microbiology at the University of South Carolina USA(figures 1.1.4- 1.1.8)

******Unit 2: STERILIZATION AND DISINFECTION**

CONTENTS

3.24: Mailing biohazardous materials

3.25: Rejection of unacceptable specimens

3.26: Specimen processing

3.27: Extent of identification required

3.28: Communication of laboratory findings

4.0 Conclusion

5.0 Summary

6.0 Tutor-Marked Assignment

7.0 References/Further Reading

1.0: Introduction: Sterilization and Disinfection

Sterilization is a process whereby all forms of microbial life, including bacterial spores, are killed. Sterilization may be accomplished by physical or chemical means. Disinfection is a process whereby pathogenic organisms, but not necessarily all microorganisms or spores, are destroyed. As with sterilization, disinfection may be accomplished by physical or chemical methods.

2.0 : Objective

At the end of this unit you should be able to know the various

- Methods of sterilization
- Methods of disinfection
- Safety Methods
- Methods of engineering controls in the laboratory
- Classification of Biologic Agents Based on Hazard
- Various methods of mailing biohazardous materials
- Rejection of unacceptable specimens
- Various Methods of Specimen processing
- Communication of laboratory findings

3.0 : CONTENTS

3.10 Methods of sterilization

The physical method of sterilization includes the following:

- Incineration
- Moist heat
- Dry heat
- Filtration
- Ionizing (gamma) radiation

Incineration is the most common method of treating infectious waste. Hazardous material is literally burned to ashes at temperatures of 870^{o} to 980^{o}C. Toxic air emmisions and the presence of heavy metals in ash have limited the use of incineration in most large U.S. cities, however.

Moist heat (steam under pressure) is used to sterilize biohazardous trash and heat-stable objects; an autoclave is used for this purpose. An autoclave is essentially a large pressure cooker. Moist heat in the form of saturated steam under 1 atmosphere (15 psi [pounds per square inch]) of pressure causes the irreversible denaturation of enzymes and structural proteins. The most common type of steam sterilizer in the microbiology laboratory is the gravity displacement type shown in figure 1.2.1 Steam enters at the top of the sterilizing chamber and, because steam enters at the top of the sterilizing chamber and, because steam is lighter than air, it displaces the air in the chamber and forces it out the bottom through the drain vent. The two common sterilization temperatures are 121^{o} C (250^{o}F) and 132^{o}C (270^{o}F). Items such as media, liquids, and instruments are usually autoclaved for 15 minutes at 121^{o}C. Infectious medical waste, on the other hand, is often sterilized at 132^{o}C for 30 to 60 minutes to allow penetration of the steam throughout the waste and the displacement of air trapped inside the autoclave bag. Moist heat is the fastest and simplest physical method of sterilization.

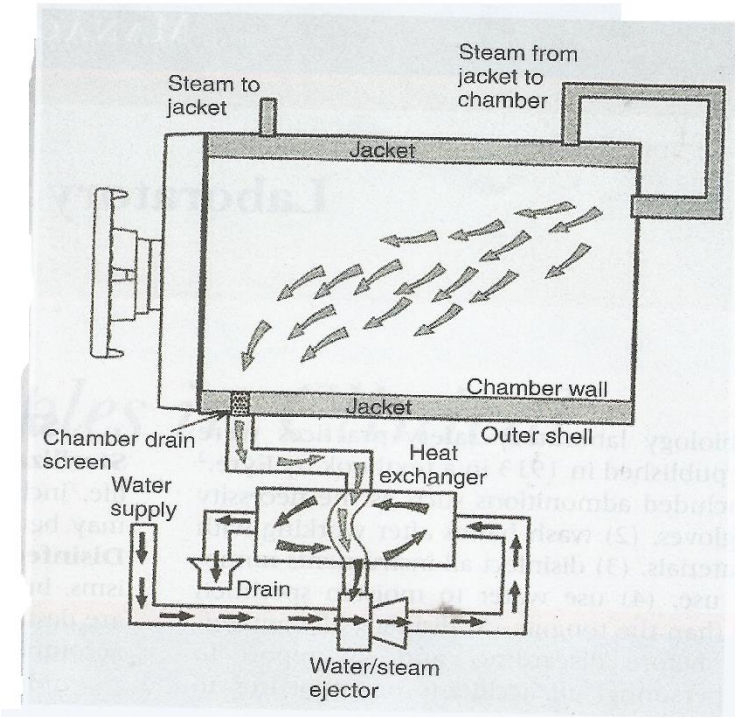


Figure 1.2.1: Gravity Displacement Autoclave

Courtesy AMSCO International Inc. Ohio

Dry heat: This requires longer exposure times (1.5 to 3 hours) and higher temperatures than moist heat (160^{o} to 180^{o}C). Dry-heat ovens are used to sterilize items such as glassware, oil, petrolatum, or powders.

Filtration is method of choice for antibiotic solutions, toxic chemicals, radiosotopes, vaccines, and carbohydrates, which are all heat sensitive. Filtration of liquids is accomplished by pulling the solution through a cellulose acetate or cellulose nitrate membrane with a vacuum. Filtration of air is accomplished using high efficiency particulate air (HEPA) filters designed to remove organisms larger than 0.3.µm from isolation rooms, operating and biological safety cabinets (BSCs). Ionizing radiation used in microwaves and radiograph machines are short wave length and high energy gamma rays. **Ionizing radiation** is used for sterilization disposables such as plastic syringes, catheters, or gloves before use. The most common chemical sterilant is ethylene oxide (Et O), which is used in gaseous form for sterilizing heat sensitive objects. Formaldehyde vapor and vapor-phase hydrogen peroxide (an oxidizing agent) have been used to sterilize HEPA filters in BSCs. Glutaraldehyde, which is sporicidal (kills spores) in 3 to 10 hours, is used for medical equipment such as

bronchoscopes, because it does not corrode lenses, metal, or rubber. Peracetic acid, effective in the presence of organic material, has also been used for the surface sterilization of surgical instruments. The use of gglutaraldehyde or peracetic acid is called cold sterilization.

3.11 : Methods of disinfection

Physical method of disinfection

The three physical method of disinfection are:

- Boiling at 100^{o}C for 15 minutes, kills vegetative bacteria
- Pasteurizing at 63^{o}C for 30 minutes or 72^{o}C for 15 seconds, which kills food pathogens
- Using nonionizing radiation such as ultra violet (UV) light

UV rays are long wavelength and low energy. They do not penetrate well and organisms must have direct surface exposure, such as the working surface of a BSC, for this form of disinfection to work.

3.12: Chemical methods of disinfection

Chemical disinfectants comprise many classes, including the following:

- Alcohols
- Aldehydes
- Halogens
- Heavy metals
- Quaternary ammonium compounds
- Phenolics

When chemicals are used to kill all life they are called chemical sterilants, or biolcides; however, these same chemicals used for shorter periods are disinfectants. Disinfectants used on living tissues (skin) are called antiseptics.

A number of factors influence the activities of disinfectants such as the following:

- Types of organisms present

- Temperature and pH process
- Number of organisms present (microbial load)
- Concentration of disinfectant
- Amount of organics (blood, mucus, pus) present
- Nature of surface to be disinfected (e.g., potential for corrosion: porous vs. non-porous surface)
- Length of contact time
- Type of water available (hard vs. soft)

Resistance to disinfectants varies with the type of microorganism present. Bacterial spores such as Bacillus spp., are the most resistant, followed by mycobacteria (acid-fast bacilli); NON-LIPID virus, for example, poliovirus; fungi; vegetative (nonsporulating) bacteria, for example, herpes simplex virus, which are the most susceptible to the action of disinfectants. The environmental protection agency (EPA) registers chemical disinfectants used in the United States and requires manufactures to specify the activity level of each compound at the working dilution. Therefore, microbiologist who must recommend appropriate disinfectant should check the manufacturers cut sheets (product information) for the classes of microorganisms that will be killed generally, the time necessary for killing micro-organisms increase in direct proportion with the number of organisms (microbial load). This is particularly true of instrument contaminated with organic material such as blood, pus, or mucus. The organic material should be mechanically removed before chemical sterilization to decrease the microbial load. This is analogous to removing dried food from utensils before placing them in a dishwasher and is important for cold sterilization of instruments such as bronchoscopes. The type of water and its concentration in a solution are also important. Hard water may reduce the rate of killing of microorganisms, and surprisingly, 70% ethyl alcohol is more effective as a disinfectant than 95% ethyl alcohol.

Ethyl or isopropyl alcohol is non-sporicidal (does not kill spores) and evaporates quickly. Therefore, either is best used on the skin as an antiseptic or on thermometers and injection vial rubber septa as a disinfectant. Because of their irritating fumes, the aldehydes (formaldehyde and glutaraldehyde) are generally not used as surface disinfectants. The halogens, especially chlorine and iodine, are frequently used as disinfectants. Chlorine is most often used in the form of sodium hypochlorite (NaOCl), the compound known as house bleach. The centers for disease

control and prevention (CDC) recommends that tabletops be cleaned following blood spills with a 1:10 dilution of bleach. Iodine is prepared either as tincture with alcohol or as an iodophor coupled to a neutral polymer, for example, povidone-iodine. Both iodine compounds are widely employed antiseptics. In fact, 70% ethyl alcohol, followed by an iodophor, is the most common compound used for skin disinfection before drawing blood cultures or surgery. Because mercury is toxic to the environment, heavy metals containing mercury are no longer recommended, but an eye drop solution containing 1% silver nitrate is still instilled in the eyes of newborns to prevent infections with *Neisseria gonorrhoea.* Quaternary ammonium compounds are used to disinfect bench-tops or other surfaces in the laboratory. However, organic materials, such as blood, may inactivate heavy metals or quaternary ammonium compounds, thus limiting their utility. Finally, phenolics, such as the common laboratory disinfectant amphyl, are derivatives of carbohlic acid (phenol). The addition of detergent results in a product that cleans and disinfects at the same time, and at concentrations between 2% and 5% these products are widely used for cleaning bench-tops.

The most important point to remember when working with biocides or disinfectants is to prepare a working solution of the compound exactly according to the manufacturer's package insert. Many people think that if the manufacturer says to dilute 1:200, they will be getting a stronger product of they dilute it 1:10. However, the ratio of water to active ingredient may be critical, and if sufficient water is not added, the free chemical for surface disinfection may not be released.

3.13: Chemical Safety

In 1987, the United States Occupational safety and Health Administration (OSHA) published the hazard Communication standard, which provides for certain institutional educational practices to ensure all laboratory personnel have a thorough working knowledge of the hazards of chemicals with which they work. Similar organizations in Nigeria had done likewise of recent, such as NESTRA, NEPAD, and e.t.c. This standard has also been called the "employee right-to-know." It mandates that all hazardous chemicals in the workplace be identified and clearly marked with a National Fire Protection Association (NFPA) label stating the health risks, such as carcinogens (cause of cancer), mutagens (cause of mutation in DNA or RNA), or teratogens (cause of birth defects), and the hazard class for example, corrosive (harmful to mucus membranes, skin, eyes or tissue), poison, flammable, or oxidizing.

Examples of these labels are shown in Figure below

FIGURE 1.2.2

Each laboratory should have a chemical hygiene plan that concludes guidelines on proper labeling of chemical containers, manufacturer's material safety data sheets (MSDSs), and the written chemical safety training and retaining programs. Hazardous chemicals must be inventoried annually. In addition, laboratories are required to maintain a file of every chemical that they use and a corresponding MSDS sheets for every hazardous chemical; some manufacturers also provide letters for nonhazardous chemicals, such as saline, so these can be included with the other MSDSs. The MSDSs include information on the nature of the chemical, the precaution to take if the chemical is spilled, and disposal recommendations. The sections in the typical MSDS include:

- Substance name
- Name, address, and telephone number of manufacturer
- Hazardous ingredients
- Physical and chemical properties
- Fire and explosion data
- Toxicity
- Health effects and first aid
- Stability and reactivity

- Shipping data
- Spill, leak, and disposal procedures
- Personal protective equipment
- Handling and storage

Employees should become familiar with each other MSDS so that they know where to look in the event of an emergency.

Fume hoods (figure 1.2.3) are provided in the laboratory to prevent inhalation of toxic fumes. Fumes hoods protect against chemical odor by exhausting air to the outside but are not HEPA-filtered to trap pathogenic microorganisms.

It is important to remember that a BSC is *not* a fume hood. Work with toxic and noxious chemicals should always be done in a fume hood or when wearing a fume mask, gloves, impervious (impenetrable to moisture) apron, and goggles. Acid and alkaline, flammable, and radioactive spill kits are available to assist in rendering any chemical spill harmless.

3.14: Fire safety

Fire safety is an important component in the laboratory safety program. Each laboratory is required to post fire evacuation plans that are essentially blueprints for finding the nearest exit in case of fire. Fire drills conducted quarterly or annually depending on local laws ensure that all personnels know what to do in case of fire. Exit ways should always remain clear of obstructions, and employees should be trained to use fire extinguishers. The local fire department is often an excellent resource for training in the types and use of fire extinguishers.

Type A fire extinguishers are used for trash, wood and paper; **type B** are used for chemical fires; type C are used for electrical fires. Combination type ABC are found in most laboratories, so personnel need not worry about which extinguisher to reach for in case of a fire. However, **type C** extinguishers, which contain carbon dioxide (CO_2) or another dry chemical to smother fire, are also used because this type of extinguisher will not damage equipment.

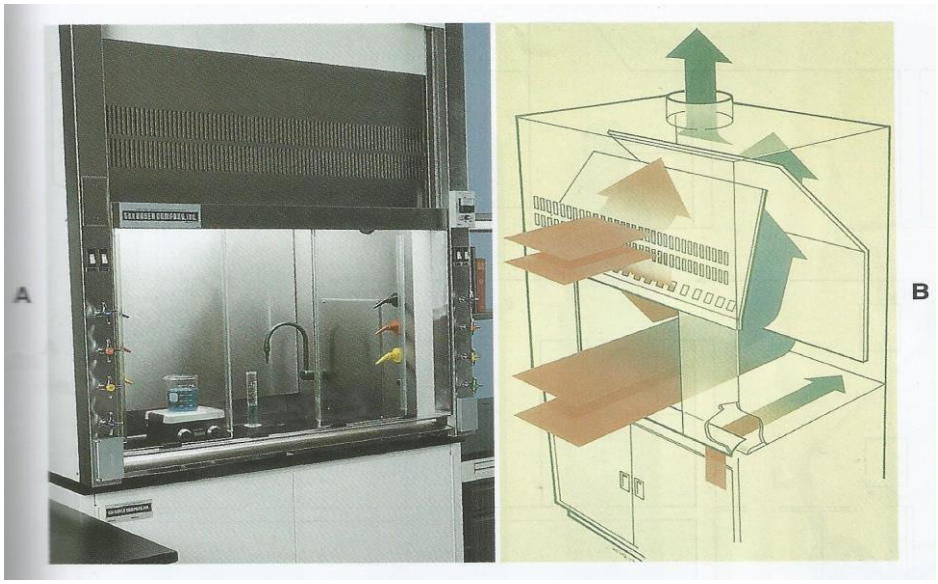


FIGURE 1.2.3 :

COURTESY AMSCO INTERNATIONAL INC. OHIO

The important actions in case of fire and the order in which to perform tasks are remembered as the standard acronym RACE:

1. Rescue any injured individuals.
2. Activate the fire alarm.
3. Contain (smother) the fire, if feasible (close fire doors).
4. Extinguish the fire if possible.

3.15: Electrical safety

Electrical cords should be checked regularly for fraying and replaced when necessary. All plugs should be the checked for electrical grounding and leakage at least annually. No extension cords should be used in the laboratory.

3.16: Handling of compressed gases

Compressed gas cylinders (CO_2, anaerobic gas mixture contain pressurized gases and must be properly handled and secured. In cases where leaking cylinders have fallen, tanks have become missiles, resulting in loss of life and destruction of properties. Therefore, gas tanks should be properly chained and stored in well ventilated areas. The metal cap, which is removed when the regulator is installed, should always be in place when a gas cylinder is not in use. Cylinders should be transported chained to special dollies.

3.17 Biosafety

Individuals are exposed in various ways to laboratory-acquired infections in microbiology laboratories. These involve the following:

- Rubbing the eyes or nose with contaminated hands
- Inhaling aerosols produced during centrifugation, vortexing, or spills of liquid cultures
- Accidentally ingesting microorganisms by putting pens or fingers in the mouth
- Suffering percutaneous inoculation, that is, being punctured by a needlestick

Risks from a microbiology laboratory may extend to adjacent laboratories and to families of those who work in the microbiology laboratory. For example, Blaser and Feldman noted that 5 of 31 individuals who contracted typhoid fever from proficiency testing specimens did not work in a microbiology laboratory. Two patients were family members of a microbiologist who had worked with *salmonella typhi,* two were students whose afternoon class was in the laboratory where the organism had been culture that morning, and one worked in an adjacent chemistry laboratory.

In the clinical microbiology laboratory, *shigellosis, salmonellosis*, *tuberculosis, brucellosis*, and hepatitis are the five most frequently acquired laboratory infections. Viral agents transmitted through blood and body fluids cause most of the infections in non-microbiology laboratory workers and in health care workers in general. These include hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and the human immunodeficiency virus (HIV). Of interest, males and younger employees (17 to 24 years old) are involved in more laboratory-acquired infections than females and older employees (45 to 64 years old).

3.18 control plan

It is the legal responsibility of the laboratory directory and supervisor to ensure that an Exposure Control Plan has been implemented and that the mandate safety guidelines are followed. The plan identifies tasks that are hazardous to employees and promotes employee safety through use of following:

- Employee education and orientation
- Appropriate disposal of hazardous waste
- Standard (formerly universal) precautions
- Engineering controls and safe work practices, as well as appropriate waste disposal and use of BSCs
- Personal protective equipment (PPE) such as laboratory coats, shoe covers, gowns, gloves, and eye protection (goggles, face shields)
- Post-exposure plan involving the investigation of all accidents and a plan to prevent recurrences

3.19 Employee education and orientation

Each institution should have a safety manual that is reviewed by all employees and a safety officer who is knowledgeable about the risks associated with laboratory acquired infections. The safety officer should provide orientation of new employees, as well as quarterly continuing education updates for all personnel. Initial training and all retaining should be documented in writing. Hand wash should be emphasized for all laboratory personnel. The mechanical action of rubbing the hands together and soaping under the fingernail is the most important part of the process; in the laboratory, products containing anti-bacterial agents do not prove to be any more effective than ordinary soap, unlike the situation in areas of the hospital such as the operating room.

All employees should also be offered, at no charge, the HBV vaccine and annual skin tests for tuberculosis. For those employees whose skin tests are already positive or those who have previously been vaccinated with BCG (Bacillus Calmette-Guerin), the employer should offer chest radiographs on employment although annual chest x-ray studies thereafter are no longer recommended by the CDC.

3.20 Disposal of hazardous waste

All materials contaminated with potentially infectious agents must be decontaminated before disposal. These include unused portions of patient specimens, patient cultures, stock cultures or microorganisms, and disposable sharp instruments, such as scaples and syringes with needles. Infectious waste may be decontaminated by use of an autoclave, incinerator, or any one of several alternative waste treatment methods. Some states or local municipalities permit blood, serum, urine, feces, and other body fluids to be poured into a sanitary sewer. Infectious waste from microbiology laboratory is usually autoclave on-site or sent for incineration, however.

In 1986, the EPA published a guide to hazardous waste reduction to limit the amount of hazardous waste generated and released into the environment. These regulations call for the following:

- Substituting less hazardous chemicals when possible, for example, the substitution of ethyl acetate for ether in ova and parasite concentrations and Hemo-de in place of xylene for trichrome stains.
- Developing procedures that use less of a hazardous chemical, for example, the substitution of infrared technology for radioisotopes in blood culture instruments.
- Segregating infectious wastes from uncontaminated (paper) trash.
- Substituting miniaturized systems for identification and antimicrobial susceptibility testing of potential pathogens to reduce the volume of chemical reagents and infectious waste.

Recently, several alternative waste-treatment machines were developed to reduce the amount of waste buried in landfills. These systems combine mechanical shredding or compacting of the waste with either chemical (sodium hypochlorite, chlorine dioxide, peracetic acid), thermal (moist heat, dry heat), or ionizing radiation (microwaves, radio waves) decontamination. Most state regulations for these units require at least a six-fold reduction in vegetative bacteria, fungi mycobacteria, and liquid-containing viruses and at least a fourfold reduction in bacterial spores. Infectious waste (agar plates, tubes, reagent bottles should be placed into two leak-proof, plastic bags for sturdiness; this is known as double bagging in common Laboratory jargon. Pipettes, swabs and other glass objects should be placed into rigid cardboard containers before disposal. Broken glass is placed in thick boxes lined with

plastic biohazard bags; when full, the box is incinerated or autoclaved, sharp objects, including scapels and needles, are placed in sharps containers, which are autoclaved or incinerated when full.

3.21 : Standard precautions

In 1987, the CDC published guidelines known as the universal precautions, to reduce the risk of HBV transmission in clinical laboratories and blood banks. In 1996, these safety recommendations became known as standard precautions. These precautions required that blood and body fluids from every patient be treated as potentially infectious. The essentials of standard precautions and safe laboratory work practices are as follows:

- Do not eat, drink, smoke, or apply cosmetics (including lip balm).
- Do not insert or remove contact lenses.
- Do not bite nail or chew on pens.
- Do not mouth-pipette.
- Limit access to the laboratory to trained personnel only.
- Assume all patients are infectious of HIV or other blood-borne pathogens.
- Use appropriate barrier precautions to prevent skin and mucous membrane exposure, including wearing gloves at all times and masks, googles, gowns, or aprons if there is a risk of splashes or droplet formation
- Thoroughly wash hands and other skin surfaces after gloves are removed and immediately after any contamination.
- Take special care to avoid injuries with sharp objects such as needles and scalpels.

The CDC's standards precautions should be followed for handling blood and body fluids, including all secretions and excretions (e.g., serum, semen, all sterile body fluids, saliva from dental procedures and vaginal secretions) submitted to the microbiology laboratory. Standard precautions do not apply to feces, nasal secretions, saliva (except in dental procedures), sputum, sweat, tears, urine, or vomitus unless they are grossly bloody. The consistent practice of standard precaution by health care workers handling all patient material will lessen the risks associated with such specimens. Mouth pipetting is strictly prohibited. Mechanical devices must be used for drawing all liquids into pipettes. Eating, drinking, smoking, and applying cosmetics are strictly forbidden in work areas. Food and drink must be stored in refrigerators in areas separate from the work area. All personnel should wash their hands with soap and water after removing gloves, after handling

infectious material, and before leaving the laboratory area. In addition, it is good practice to store sera collected periodically from all health care workers so that, in the event of an accident, a seroconversion (acquisition of anti-bodies to an infectious agents) can be documented.

All health care workers should follow standard precautions while working inside or outside the laboratory. When collecting specimens outside the laboratory, individuals should follow these guidelines:

- Wear gloves and a laboratory coat.
- Deal carefully with needles and lancets.
- Discard sharps in an appropriate puncture resistant container.
- Never recap needled by hand; if necessary, special devices should be available for resheathing needles.

FIGURE 1.2.4: COURTESY NATIONAL HOSPITAL ABUJA MICROBIOLOGY DEPARTMENT INFECTIOUS CAUTION GUIDES PICTURES

3.22: Engineering controls

Laboratory Environment

The biohazard symbol should be prominently displayed on laboratory doors and any equipment (refrigerators, incubators, centrifuges) that contain infectious material. The air-landling of a microbiology laboratory should move air from lower to higher risk areas, never the reverse. Ideally, the microbiology laboratory should be under

negative pressure, and air should not be recirculated after passing through microbiology. The selected use of BSCs for procedures that generate infectious aerosols is critical to laboratory safety. Many infectious diseases, such as plague, tularemia, brucellosis, tuberculosis and *legionellosis*, may be contracted by inhaling infectious particles, often present in a droplet of liquid. Because blood is a primary specimen that may contain infectious virus particles, sub culturing blood cultures by puncturing the septum with a needle should be performed behind a barrier to protect the worker from droplets. Several other common procedures used to process specimens for culture, notably mincing, grinding, vortexing and preparing direct smears for microscopic examination, are known to produce aerosol droplets. These procedures must be performed in a BSC.

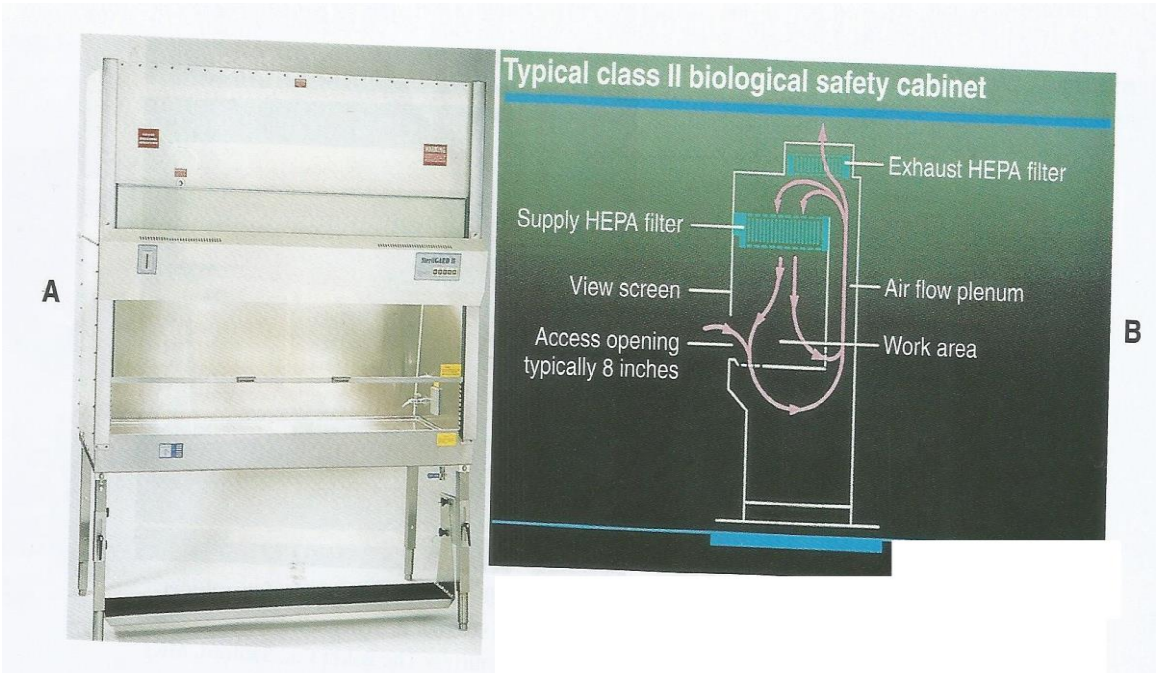
The microbiology laboratory poses many hazards to unsuspecting and untrained people; therefore, access should be limited to employees and other necessary personnel (biomedical engineers, housekeepers). Visitors, especially young children, should be discouraged. Certain areas of high risk, such as the mycobacteriology and virology laboratories, should be close to visitors. Custodial personnel should be trained to discriminate among waste containers, dealing only with those that contain noninfectious material. Care should be taken to prevent insects from infesting any laboratory area. Mites, for example, can crawl over the surface of media, carrying microorganisms from colonies on a plate to other areas. Houseplants can also serve as a source of insects and should be carefully observed for infestation, if they are not excluded altogether from the laboratory environment. A pest control program should be in place to control rodents and insects.

3.94: Biological Safety Cabinet

A BSC is a device that encloses a workspace in such a way as to protect worker from aerosol exposure to infectious disease agents. Air that contains the infectious material is sterilized, either by heat, ultraviolet light, or, most commonly, by passage through a HEPA filter that removes most particles larger than 0.3 µm in diameter. These cabinets are designated by class according to the degree of biologic containment they afford. Class 1 cabinets allow room (unsterilized) air to pass into the cabinet and around that area and material within, sterilizing only the air to be exhausted. They have negative pressure, are ventilated to the outside, and are usually operated with an open front. Class 2 cabinets sterilize air that flows over the infectious material, as well as air to be exhausted. The air flows in “sheets”, which serve as barriers to particles from outside the cabinet, and direct the flow of

contaminated air into the filters.

Such cabinets are called vertical laminar flow BSCs. Class II cabinets have a veriable sash opening through which the operator gains access to the work surface. Depending on their inlet flow of velocity and the percent of air that is HEPA- filtered and recirculated, class II cabinet are further differentiated into type A or B. The IIA cabinet is self-contained, and 70% of the air is recirculated. The exhaust air in class IIB cabinets is selected if radiosotopes, toxic chemicals, or carcinogens will be used. Because they are completely enclosed, with negative pressure, class III cabinets afford the most protection of the worker. Air coming into and going out and the infectious material within is handled with rubber gloves that are attached and sealed to the cabinet.



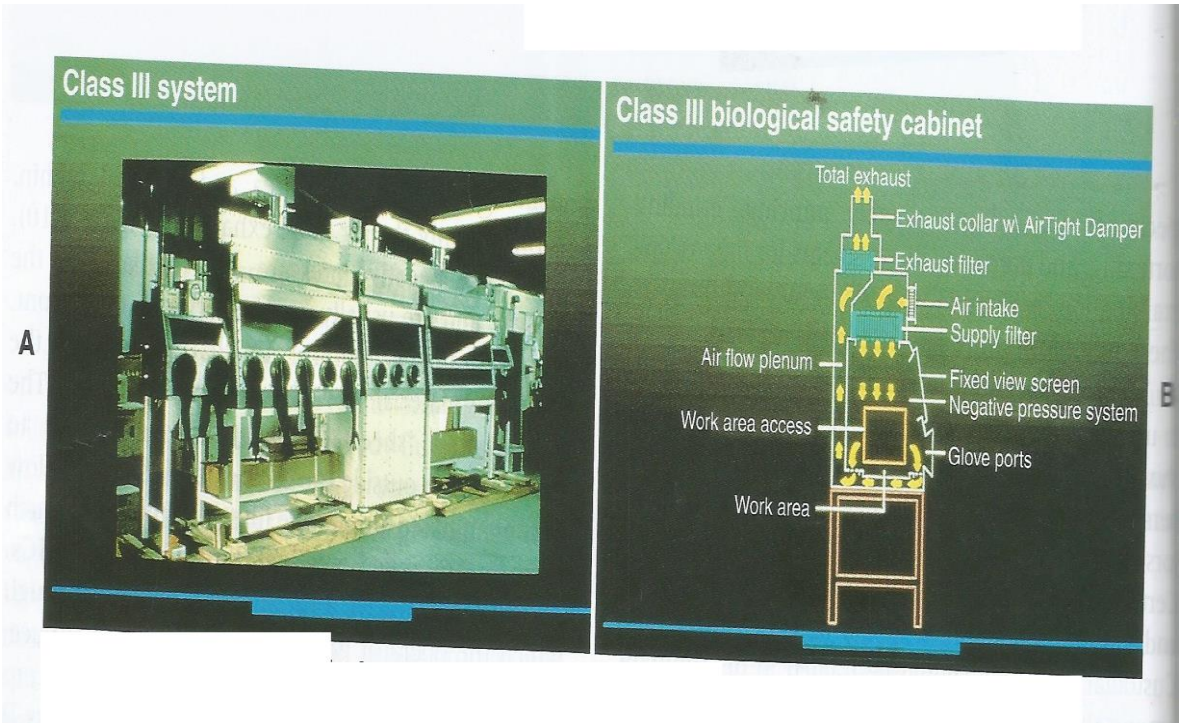


FIGURE 1.2.5 COURTESY NATIONAL HOSPITAL ABUJA MICROBIOLOGY DEPARTMENT INFECTIOUS CAUTION GUIDES PICTURES

Most hospital clinical microbiology laboratory technologists use class IIA cabinents. The routine inspection and documentation of adequate function of these cabinents are critical factors in an ongoing quality assurance program. Important to proper operation of laminar flow cabinets is maintenance of an open area for 3 feet (90 cm) from the cabinets during operation of the air-circulating system to ensure that infectious material is directed through the HEPA filter. BSCs must be certified initially, whenever moved more than 18 inches, and annually, thereafter.

Personal Protective Equipment

OSHA regulation require that health care facilities provide employees with all personal protective equipment necessary to protect them from hazards encounter during the course of work. This usually includes plastic shield or googles to protect workers from droplets, disposal containers for sharp objects, holders for glass bottles, tray in which to carry smaller hazardous items (e.g., blood culture bottles), handheld pipetting devices, impervious gowns, laboratory coats, disposable gloves masks, safety carriers for centrifuges (especially those used in AFB laboratory), and HEPA respirators.

HEPA respirators are required for all health care workers, phlebotomists, who enter rooms of patients with tuberculosis, and also to clean up spills of pathogenic

microorganisms. All respirators should be fit-tested for each individual so that each person is assured that his or hers is working properly. Males must shave their facial hair to achieve a tight fit.

Microbiologist should wear laboratory coats over their street clothes, and these coats should be removed before leaving the laboratory. Most exposure to blood-containing fluids occurs on the hands or forearm, so gowns with closed wrists or forearm covers and gloves that cover all potentially exposed skin on the arms are most beneficial. If the laboratory protective clothing becomes contaminated with body fluids or potential pathogens, it should be sterilized in an autoclave immediately and cleaned before reusing. The institution or a uniform agency should clean laboratory coats; it is no longer permissible for microbiologists to launder their own coats. Alternatively, disposable gowns may be used. Obviously, laboratory workers who plan to enter an area of the hospital where patients at special risk of acquiring infection are present (e.g., intensive care units, the nursery, operating rooms, or areas in which immunosuppressive therapy is being administered) should take every precaution to cover their street clothes with clean or sterile protective clothing appropriate to the area being visited. Special impervious protective clothing is advisable for certain activities, such as working with radioactive substances or caustic chemicals. Solid-front gowns are indicated for those working with specimens being culture for mycobacteria. Unless large-volume spills of potentially infectious material are anticipated, impervious laboratory gowns are not necessary in most microbiology laboratories.

Post-exposure control

All laboratory accidents and potential exposures must be reported to the supervisor and safety officer, who will immediately arrange to send the individual to employee health or an outside occupational health physician. Immediate medical care is of foremost importance; investigation of the accident should take place only after the employee has received appropriate care. If the accident is a needlestick injury, for example, the patient should be identified and the risk of the laboratorian acquiring a blood-borne infection should be assessed. The investigation helps the physician be assessed. The investigation helps the physician determine the need or prophyaxis, that is, HBIG (hepatitis B virus immunoglobulin) or an HBV booster immunization. The physician also is able to discuss the potential for disease transmission to family members following, for example, exposure to a patient with *neisseria meningitidis.* Follow-up treatment should also be assessed including, for example, drawing

additional sera at intervals of 6 weeks, 3 months, and 6 months for HIV testing. Finally, the safety committee or, at minimum, the laboratory director and safety officer should review the accident to determine whether it could have been prevented and to delineate measures to be taken to prevent future accidents. The investigation of the accident and corrective action should be documented in writing in an incident report.

3.23: CLASSIFICATION OF BIOLOGIC AGENTS BASED ON HAZARD

A CDC booklet titled *Classification of etiological agents on the basis of hazard* served as a reference for assessing the relative risks of working with various biologic agents until an update CDC/NIH document was published titled *Biosafety in microbiological and biomedical laboratories.* In general, patient specimens pose a greater risk to laboratory workers than do microorganisms in culture, because the nature of etiological agents in patient specimens is initially unknown. Bio-safety Level 1 agents include those that have no potential for infecting heathy people. These agents are used in laboratory teaching excercises for beginning-level students of microbiology. Level 1 agent includes *Bacillus subtilis* and *Mycobacterium gordonae.* Precautions for working with level 1 agent include standard good laboratory technique as described previously.

Biosafety Level 2 agents are those most commonly being sought in clinical specimens, and they include all the common agents of infectious disease, as well as HIV and several more unusual pathogens. For handling clinical specimens suspected of harboring any of these pathogens, biosafety Level 2 precautions are sufficient. This Level of safety includes the principles outlined previously plus limiting access to the laboratory during work procedures, training laboratory personnel in handling pathogenic agents, direction by competent supervisors, and performing aerosol-generating procedures in a BSC. Employers must offer hepatitis B vaccine to all employees determined to be at risk of exposure. *Bacillus anthracis* and *yersinia pestis*, two organisms mentioned as possible bioterrorism agents, are actually listed as BSL-2 organisms.

Biosafety Level 3 procedures have been recommended for the handling of material suspected of harboring viruses unlikely to be encountered in a routine clinical laboratory, and for cultures growing *mycobacterium tuberculosis,* the mold stages of systematic fungi, and for some other organisms when grown in quantities greater than that found in patient specimens. These precautions, in addition to those

undertaken for level 2, consist of laboratory design and engineering controls that contain potentially dangerous material by careful control of air movement and the requirement that personnel wear protective clothing and clothing for instance. Persons working with biosafety level 3 agents should have a baseline sera stored for comparison with acute sera that can be drawn in the event of unexplained illness. *Francisella tularensis* and *Brucella spp.* Isolated from naturally occurring infections or infections following bioterrorist event, are both BSL-3 pathogens. BSL-3 organisms are transmitted primarily by aerosols.

Biosafety level 4 agents, which include only certain viruses of the arbovirus, arenavirus, or filovirus groups, none of which are commonly found in united states, require the use of maximum containment facilities. Personnel and all materials must be decontaminated before leaving the facility, and all procedures are performed under maximum containment (special protective clothing, Class III BSCs). Most of these facilities are public health or research laboratories. Potential bioterrorists agents such as smallpox require BSL-4 facilities. BSL-4 agents pose life threatening risks, are transmitted via aerosols and do not have an available vaccine or therapy.

3.24: Mailing Biohazardous Materials

In march 2005 the requirements for packaging and shipping biologic material were significantly revised in response to the international community's desire to ensure safe and trouble-free shipment if infectious material while attempting to be more reasonable. Before this date, clinical specimens submitted for infectious disease diagnosis as well as isolates of any microorganisms were considered an infectious substance and packaged and labelled under UN6.2 dangerous goods regulations.

Training in the proper packing and shipping of infectious material is a key feature of the regulations. Every institution that ships infectious materials, whether a hospital or POL (physician office laboratory), is required to have appropriately trained individuals; training may be obtained through carriers, package manufacturers, and special safety training organizations. The shipper is the individual (institution) who is ultimately responsible for safe and appropriate packaging. Any fines or penalty will be the shipper's responsibility.

Infectious specimens or isolates should be wrapped with absorbent material and

placed within a plastic biohazard bag, called a primary receptacle. The primary receptacle is then inserted into a secondary container, most often a watertight hard plastic mailer. After the secondary container is capped, it is placed in an outer tertiary container constructed of fiberboard.

Shippers should note that some carriers have additional requirements for coolants materials such as ice, dry ice, or liquid nitrogen. Because the shipper is liable for appropriate packaging, it is best to check with individual carriers in special substances and update your instructions yearly when the new IATA Dangerous Goods regulations are published.

Specimen labeling

Specimen should be labeled at the very least with the patient's name, identifying number (hospital number) or date of birth, and source. Enough information must be provided on the specimen label so that the specimen can be matched with the requisition when it is received in the laboratory.

Specimen requisition

The specimen (or test) requisition is an order form that is sent to the laboratory along with a specimen. Often the requisition is a hard (paper) copy of the physician's orders and the patient demographic information (e.g, name and hospital number). Sometimes, however, if a hospital information system offers computerized order entry, the requisition is transported to the laboratory electronically. The requisition should contain as much information as possible regarding the patient history, diagnosis, and immunization record. This information helps the microbiologist to work up the specimen and determine which organisms are significant in the culture. A complete requisition should include the following:

- The patient's name
- Hospital number
- Age or date of birth
- Sex
- Collection date and time
- Ordering physician (may be UPIN number)
- Exact nature and source of specimen

- Diagnosis (may be ICD-9-CM code)
- Immunization history
- Current antimicrobial therapy

3.25: Rejection of unacceptable specimens

Criteria for specimen rejection should be provided and distributed to all clinical practitioners. In general, specimens are unacceptable if any of the following conditions apply:

- The information on the label does not match the information on the requisition (patient name or source of specimen is different).
- The specimen has been transported at the improper temperature.
- The specimen has not been transported in the proper medium (e.g. specimens for anaerobic bacteria submitted in aerobic transports).
- The quantity of specimen is insufficient for testing (the specimen is considered QNS [quantity is not sufficient]).
- The specimen is leaking.
- The specimen transport time exceeds 2 hours post-collection and the specimen is not preserved.
- The specimen was received in a fixative (formalin) which, in essence, kills any micro-organism present.
- The specimen has been received for anaerobic culture from a site known to have anaerobes as part of the normal flora (vagina, mouth).
- The specimen is dried up.
- Processing the specimen would produce information of questionable medical value (e.g., Foley catheter tip).

It is an important rule to always talk to the requesting physician or another member of the health care team before discarding unacceptable specimens. In some cases, such as mislabeling of a specimen or requisition, the person who collected the specimen and filled out the paperwork can come to the laboratory and correct the problem; identification of a mislabeled or requisition should not be done over the

telephone. Frequently, it may be necessary to do the best possible job on a less than optimal specimen, if the specimen would be impossible to collect because the patient is taking antibiotics; the tissue was collected at surgery, or the patient would have to undergo a second invasive procedure (bone marrow or spinal tap). A notation regarding improper collection should be added to the final report in this instance, because only the primary caregiver is able to determine the validity of the results.

3.26: Specimen processing

Depending on the site of testing (hospital, independent lab, Physician Office Laboratory) and how the specimens are transported to the laboratory (in-house courier or driver), microbiology samples may arrive in the laboratory as single test. Although batch processing may be possible in large independent laboratories, most often hospital testing is performed as specimens arrive. When multiple specimens arrive at the same time, priority should be given to those that are most critical such as cerebrospinal fluids (CSF), tissue, blood and sterile fluids. Urine, throat, sputa, stool, or wound drainage specimens can be saved for later. Acid-fast, viral and fungal specimens are usually batched for processing at one time. When a specimen is received with multiple requests but the amount of specimen is insufficient to do all of them, the microbiologist should call the clinician to prioritize the testing. On arrival in the laboratory, the time and date received should be recorded.

Gross examination of specimen

All processing should begin with a gross examination of the specimen. Areas with blood and mucus should be located and sampled for culture and direct examination. Stool should be examined for evidence of barium (i.e. chalky white color), which would preclude Oδ P examination. Notations should be made on the handwritten or electronic workcard regarding the status of the specimen (e.g., bloody, cloudy, and clotted) so that if more than one person works on the sample, all micro biologists working it up will know the result of the gross examination.

Direct microscopic examination

All appropriate specimens should have a direct microscopic examination. The direct examination serves several purposes. First, the quality of the specimen can be assessed; for example, sputa can be rejected that represents saliva and not respiratory tract secretions by quantitation of white blood cells or squamous epithelial cells present in the specimen. Second, the microbiologist and clinician can be given an indication of what may be wrong with the patient (e.g. 4+ gram-positive

cocci in clusters in an exudate). Third, the workup of the specimen can be guided by comparing what grows in culture to what was seen on the smear. A situation in which three different morphotypes (cellular types) are seen on direct gram stain but only two grow out in culture, for example, alerts the microbiologist to the fact that the third organism may be an anaerobic bacterium.

Direct examinations are usually not performed on throat, nasopharyngeal, or stool specimens but are indicated from most other sources.

The most common stain in bacteriology is the gram stain, which helps to visualize rods, cocci, white blood cells, red blood cells, or squamous epithelial cells present in sample. The most common stain is KOH (potassium hydroxide), PAS (periodic-acid Schiff), and calcofluor white. The most common direct acid-fast stains are AR (auramine rhodamine), ZN (Ziehl-Neelsen), and Kinyoun.

Selection of culture media

Primary culture media are divided into several categories. The first are nutritive media, such as blood or chocolate agars. Nutritive media support the growth of a wide range of microorganisms and are considered non-selective because, theoretically, the growth of most organisms is supported. Nutritive media can also be differential, in that, micro-organisms can be distinguished on the basis of certain growth characteristics evident on the medium. Blood agar is considered both a nutritive and differential medium because it differentiates organisms based on whether they are alpha (α)-, beta (β)-, or gamma (ϒ) - hemolytic. Selective media support the growth of one group of organisms, but not another, by adding antimicrobials, dyes, or alcohol to a particular medium. MacConkey agar, for example, contains the dye crystal violet, which inhibits gram-positive organisms. Columbia agar with colistin and nalidixic acid inhibit (CNA) is a selective medium for gram-negative organisms. Selective media can also be differential media if, in addition to their inhibitory activity, they differentiate between groups of organisms. MacConkey agar, for example, differentiates between lactose-fermenting and non-fermenting gram-negative rods by the color of the colonial growth (pink or clear, respectively); In some cases (sterile body fluids, tissues or deep abscesses in a patient on antimicrobial therapy), backup broth (also called supplemental or enrichment broth) medium is inoculated, along with primary solid (agar) media, so small numbers of organisms may be detected; this allows detection of anaerobes in aerobic cultures and organisms that may be damaged by either previous or concurrent antimicrobial therapy. Thioglycollate (thio) broth, brain-heart infusion broth (BHIB), and tryptic soy broth (TSB) are common backup broths.

Selection of media to inoculate for any given specimen is usually based on the organisms most likely to be involved in the disease process. For example, in determining what to set up for a CSF specimen, one considers the most likely pathogens that cause meningitis (*streptococcus pneumoniae, haemophilus influenzae, Neisseria meningitidis, Escherichia coli,* group B *streptococcus)* and selects media that will support the growth of these organisms (blood and chocolate agar at a minimum). Likewise, if a specimen is collected from a source likely to be contaminated with normal flora, for example, an anal fistula (an opening of the surface of the skin near the anus that may communicate with the rectum), one might want to add a selective medium, such as CNA, to suppress gram-negative organisms and allow gram-positive organisms and yeast to be recovered.

Specimen preparation

Many specimens require some form of initial treatment before inoculation onto primary plating media. Such procedures include homogenization (grinding) of tissue; concentration by centrifugation or filtration of large volumes of sterile fluids, such as ascites (peritoneal) or pleural (lung) fluids; or decontamination of specimens, such as those for legionellae or mycobacteria. Swab specimens are often vortexed (mixed) in 0.5 to 1 mL of saline or broth for 10 to 20 seconds to dislodge material from the fibers.

Inoculation of solid media

Specimens can be inoculated (plated) onto solid media either quantitatively by a dilution procedure or by means of a quantitative loop, or semi-quantitatively using an ordinary inoculating loop. Urine cultures and tissues from burn victims are plated quantitatively; everything else is usually plated semi-quantitatively. Plates inoculated for quantitation are usually streaked with a 1:100 or 1:1000 loop. The original streak line is cross-struck with an ordinary inoculating loop to produce isolated, countable colonies. Plates inoculated for semi-quantitation are usually struck out in four quadrants. the inoculum is applied by swabbing a dime-sized area or placing a drop of liquid specimen on the plate. The original inoculum is then cross-struck with an ordinary reusable nichrome inoculating loop (quadrant one) or sterile plastic single-use loop. The loop is then flipped over or flamed and quadrant two is struck by pulling the loop through quadrant one a few times and streaking the rest of the area. Finally, quadrant four is streaked by pulling the loop over the rest of the agar without

further flaming. This process is called streaking for isolation, because the microorganisms present in the specimen are successively diluted out as each quadrant is streaked until finally each morphotype is present as a single colony. Number of organisms present can subsequently be graded as 4+ (many, heavy growth) if growth is out to the fourth quadrant, 3+ (moderate growth) if growth is out to the third quadrant, 2+ (few or light growth) if growth is in the second quadrant, and 1+ (rare) if growth is in the first quadrant. This tells the clinician the relative numbers of different organisms present in the specimen; such semi-quantitative information is usually sufficient for the physician to be able to treat the patient.

Incubation conditions

Inoculated media are incubated under various temperatures and environmental conditions, depending on the organism been sought, for example, 28^{o} to 37^{o} C for most bacteria, viruses and acid-fast bacillus. A number of different environmental conditions exist. Aerobes grow in ambient air, which contains 21% oxygen (O_2) and a small amount (0.03%) of carbon dioxide (CO_2). Anaerobes usually cannot grow in the presence of O_2 and the atmosphere in anaerobic jars, bags, or chambers is composed of 5% to 10% hydrogen (H_2), 5% to 10% CO_2, 80% to 90% nitrogen (N_2), and 0% O_2. Capnophiles, such as *Haemophilus influenzae* and *Neisseria gonorrhoeae,* require increased concentrations of CO_2 (5% to 10%) and approximately 15% O_2. This atmosphere can be achieved by a candle jar (3% CO_2) or a CO_2 incubator, jar, or bag. Microaerophiles *(Campylobacter jejuni, Helicobacter pylori)* grow under reduced O_2 (5% to 10%) and increased CO_2 (8% to 10%). This environment can also be obtained in special designed jars or bags.

Specimen workup

One of the most important functions that a microbiologist performs is to decide what is clinically relevant regarding specimen workup. Considerable judgment is required to decide what organisms to look for and report. It is essential to recognize what constitutes indigenous (normal) flora and what constitutes a potential pathogen. Indiscriminate reporting of normal flora can contribute to unnecessary use of antibiotics and potential emergence of resistant organisms. Because organisms that are clinically relevant to identify and report vary by source, the microbiologist should know which ones cause disease at various sites.

3.27: Scope of Identification Required

As health care continues to change, one of the most problematic issues for microbiologist is the extent of culture workup. Microbiologists still rely heavily on definitive identification, although shortcuts, including the use of limited identification procedures in some cases, are becoming commonplace in most clinical laboratories. Careful application of knowledge of the significance of various organisms in specific situations and thoughtful use of limited approaches will keep microbiology testing cost effective and the laboratory's workload manageable, while providing for optimum patient care.

Complete identification of a blood culture isolate, such as *clostridium septicum* as opposed to a genus identification of *Clostridium* ssp., will alert the clinician to the possibility of malignancy or other disease of the colon. At the same time, a presumptive identification of *Escherichia coli* if a gram-negative, spot indole-positive rod is recovered with appropriate colony morphology on MacConkey agar (flat, lactose-fermenting colony that is precipitating bile salts) is probably permissible from an uncomplicated urinary tract infection. In the final analysis, culture results should always be compared with the suspected diagnosis. The clinician should be encouraged to supply the microbiologist with all pertinent information (e.g., recent travel history, pet exposure, pertinent radiograph findings) so that the microbiologist can use the information to interpret culture results and plan appropriate strategies for workup.

3.28: Communication of laboratory findings

To fufill their professional obligation to the patient, microbiologists must communicate their findings to those health care professionals responsible for treating the patient. This task is not as easy as it may seem. This is nicely illustrated in a study in which a group of physicians was asked whether they would treat a patient with a sore throat given two separate laboratory reports, that is, one that stated, "many group A *Streptococcus (streptococcus pyoogenes)* is considered significant in any numbers in a symptomatic individual, the physicians said that they would treat the patient with many organisms but not the one with few organisms. Thus, although a pathogen (group A *Streptococcus*) was isolated in both cases; one word on the report (either "many" or "few") made a difference in how a patient would be

handled.

In communicating with the physician, the microbiologist should avoid confusion and misunderstanding by not using jargon or abbreviations and by providing reports with clear-cut conclusions. The microbiologist should not assume that the clinician is fully familiar with laboratory procedures or the latest microbial taxonomic schemes. Thus, when appropriate, interpretive statements should be included in the written report along with the specific results. One such example would be the addition of a statement, such as “suggests contamination at collection,” when more than three organisms are isolated from a clean-voided midstream urine specimen.

Laboratory newsletters should be used to provide physicians with material such as details of new procedures, periodic education /updates seminars, nomenclatures changes, and changes in usual antimicrobial susceptibility patterns of frequent isolated organisms. Empiric therapy is based on the physician determining the most likely organism causing a patient’s clinical symptoms and then selecting an antimicrobial that, in the past, has worked against that organism in a particular hospital or geographic area. Empiric therapy is used to start patients on treatment before the results of the patient’s culture are known and may be critical to the patient’s well-being in cases of life-threatening illnesses.

Positive findings should be telephoned to the clinician, and all verbal reports should be followed by written confirmation of results. Results should be legibly handwritten or generated electronically in the laboratory information system (LIS).

Critical (panic) values

Certain critical results must be communicated to the clinician immediately. Each clinical microbiology laboratory, in consultation with its medical staff, should prepare a list of these so called panic findings . Common panic values include:

- Positive blood cultures
- Positive spinal fluid Gram stain or culture
- *Streptococcus pyogenes* (group A *Streptococcus*) in surgical wound
- Gram stain suggestive of a gas gangrene (large box-car sharp gram-positive rods)
- Blood smear positive for malaria

- Positive cryptococcal antigen test
- Positive acid-fast stain
- Detection of a significant pathogen (e.g., *Legionella, Brucella,* vancomycin-resistant *Stapylococcus aureus).*

Expediting results reporting-computerization

Before widespread computerization of clinical microbiology laboratories, result reporting was accomplished by handwritten reports and having couriers delivered hard copies that were pasted into the patient's chart/case notes. Today, microbiology computer software is available that simplifies and speeds up this task.

CPUs (central processing units, disks, tape drives, controllers, printers, video terminals, communication ports, modems, and other types of hardware support running the software. The hardware and the software together make up the complete LIS. Many LIS systems are, in turn, hooked up with a hospital information system (HIS). Between the HIS and LIS, most functions involved in ordering and reporting laboratory tests can be handled electronically. Order entry, patient identification, and specimen identification can be handled using the same type of bar coding that is commonly used in supermarkets. The LIS also takes care of the results reporting and supervisory verification of results, stores quality control data, allows easy test inquiries, and assists in test management reporting by storing, for example, the number of positive, negative, and unsatisfactory specimens. Most large systems also are capable of interfacing (communicating) with microbiology instruments to automatically download (transfer) and store data regarding positive cultures or antimicrobial susceptibility results. Results of individual organism anti-biograms (patterns) can then be retrieved monthly so hospital-wide susceptibility patterns can be studied for emergence of resistant organisms or other epidemiologic information. Many vendors of laboratory information systems are now writing software for microbiology to adapt to personal computers (PCs) so that large CPUs may no longer be needed. This brings down the cost of microbiology systems so that even smaller laboratories are able to afford them. Today, small systems can be interfaced with printers or electronic facsimile machines (faxes) for quick and easy reporting and information retrieval. This further improves the quality of patient care.

4.0 : Conclusion

In this unit we have seen that sterilization is a process whereby all forms of microbial life, including bacterial spores, are killed. Sterilization and disinfection may be accomplished by different means as already discussed. Likewise, various laboratory safety, engineering control and specimen handling methods for successful microbiology reporting and hints on interpretation were comprehensively covered.

5.0 Summary

Going through this unit we have considered the following:

- **Methods of sterilization**
- **Methods of disinfection**
- **General Safety Methods**
- **Engineering controls in microbiology laboratory**
- **Classification of Biologic Agents Based on Hazard**
- **Rejection of unacceptable specimens and Specimen processing**
- **Communication of laboratory findings**

6.0 Tutor-Marked Assignment

Ques. a) Write comprehensive essays on each of the following:

i) Sterilization
ii) Disinfection
iii) General safety measures in a microbiology laboratory
iv) Engineering control methods in microbiology laboratory

Ques.b) What are the criteria for the rejection of specimens for microbiological processing during a particular clinical or public health investigation?

Ques. c) How would you effectively communicate microbiology laboratory findings to the concerned stake holders?

7.0 References/Further Reading

Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby**.**

Sterilisation and Disinfection by T. D. Whitter, W. B. Hugo, G. R. Wilkinson and J. B. Stenlake

University of Sothern Carolina: Online Microbiology Teaching Aids and Resources, 2013.

Unit 3: Microscopy

1.0 : Introduction to Microscopy

2.0: Objective

3.0 : Content

3.1Types of Microscopy

3.11 Bright field Microscopy/ Light Microscopy

3.12 Fluorescence Microscopy

3.13 Phase Contrast Microscopy

3.14 Dark Field Microscopy

3.15 Electron Microscopy

3.2 Applications of Microscopy in Diagnostic Public Health Microbiology

1.0: Introduction to Microscopy

In the diagnosis of infectious diseases of public health importance, microscopy is a key component of the procedures involved. Microscopy simply entails the use of microscope to visually enlarge objects too small to visualize with the unaided eye. For this reason it plays a key role in microbiology.

Normally there are different types of micro-organisms to be identified, and the extent to which such needs to be done. This means you shall need to know the different types of microscopes and what we refer to as their levels of resolutions and other terminologies as applicable to microscopy;

2.0: **Objective**

At the end of this unit you should be able to describe the following as far as microscopy is concerned:

- Types of Microscopy
- Applications of Microscopy in Diagnostic Public Health

Microbiology

- Control, Care and Maintenance of a Microscope

3.0 Contents

3.1 Types of Microscopy

- Bright field Microscopy/ Light Microscopy
- Fluorescence Microscopy
- Phase Contrast Microscopy
- Dark Field Microscopy
- Electron Microscopy

We shall briefly consider each of these types of microscopy their underlying principles and terminologies frequently used with respect to a particular form of microscopy.

3.11: Bright field Microscopy/ Light Microscopy

Principles

In bright field microscopy otherwise referred to as light microscopy the basic principle relies on magnification that takes place when light passes through a particular microbiological specimen and then through a series of lenses that refract (bend) the light in such a form that leads to enlargement of the organisms present in the prepared microbiological specimen.

Magnification (Enlargement)

By definition, refers to the ratio of the height of the image to that of the object.

Magnification = Height of Image/Height of object

or

Distance of Image from the lens/ Distance of object from the lens

Normally the objective lens, which is always closest to the specimen, enlarges the object or the organism in the specimen one hundred times (100x).

The ocular lens very close to the eye enlarges it only about ten times. This gives a total enlargement of 10 x 100 which is 1000x the object's actual size when a light microscope is viewed through the ocular lens.

It means that for objective lens of lower magnification, 10x, 20x and 40x , : we can achieve a lower magnification of

10x10 = 100x

20 x10 = 200x

40 x 10 = 400x

At these magnifications, Fungi, Yeast, parasites and bacteria can be identified but not viruses which require magnification of at least x100,000.

Resolution

This refers to the extent at which details in the magnified object is maintained and visualized.

Resolving Power

This is the closest distance between two objects that when magnified still allows the two objects to distinguished from each other. In most light microscopes, the resolving power allows bacterial cells to be distinguished from one another but usually does not allow bacterial structures, external or internal to be detected. The oil immersion used in 1000x magnification achieves higher resolution than lower magnifications we earlier mentioned above by preventing light rays from dispersing and changing wavelength after passing through the specimen.

Contrast

This is needed to make objects stand out from the background. As a result of the high water content of microorganism in public health or clinical specimens they are very transparent. For this reason, they cannot be easily detectable unless stained, this allows them to be detected and differentiated from one another and from their background. We shall discuss staining very soon. (Most especially Gram Staining technique in microbiology).

3.12: Fluorescence Microscopy

Principle

Fluorochrome, which are certain dyes, when they absorb ultra violet light are raised to higher level of energy. As the molecules of the dye return to normal, lower energy level they release energy in the form of visible (fluorescent) light. This is called fluorescence. Microscopic method developed with this regard is referred to fluorescent microscopy.

3.13: Phase Contrast Microscopy

Principle

Instead of using staining to provide contrast, the microorganisms of different densities or thicknesses in specimen provide such contrast. When beams of light pass

through such specimen differential refractive indices are produced. The greater the refractive index of an object, the more the beam of light is slowed which results in decreased light intensity. On the other hand, the lesser the refractive index of another object, the beam of light becomes faster which results in increased light intensity. These differences in light intensity translate into differences that provide contrast.

3.14: Dark Field Microscopy

Principle

Just like the phase contrast microscopy we just described above, simple change in microscopic technique instead of using dyes to provide contrast is employed. Here, the condenser blocks light beam from passing through it directly in such a manner that it only allows the light to hit the specimen only at oblique angle. For this reason, only light that hit the object in the specimen would be deflected upwards into the objective lens for visualization. All other light that passes through the specimen will miss the objective, thus making the background a dark field.

3.15: Electron Microscopy

Principle

In electron microscope, electrons are used instead of light to visualize small objects. As opposed to other microscopes, lenses are replaced with electromagnetic fields which are used in focusing the electrons. Due to the marked increase in resolution, magnification of a million fold could be achieved. (1000000x). There are two types of Electron microscopes:

Transmission Electron Microscope (TEM): This allows internal structures of objects to be visualized as it passes the electron beam through the object.

Scanning Electron Microscope (SEM) : Allows the three dimensional view of surface structures to be visualized as it passes electron beam to scan surface structures of objects in public health or clinical specimens.

3.2: APPLICATIONS OF MICROSCOPY IN DIAGNOSTIC MICROBIOLOGY

- Rapid preliminary identification by direct visualization in patient's specimens
- Rapid final identification of certain organisms by direct visualization in patient's samples Detection of various organisms present in the same sample
- Detection of organisms not easily cultivated in the laboratory
- Evaluation of patients samples /specimens for the presence of cells indicative of inflammation (i.e. phagocytes) or contamination (squamous epithelial cells)
- Determination of organisms' clinical significance. Bacteria contaminants usually are not present in patients specimens at sufficiently high numbers ($x10^5$ cells /ml) to be seen by light microscopy
- Provide pre-culture information about which organisms might be expected to grow so that appropriate cultivation techniques are used.
- Determine which tests and methods should be used for identification and characterization of cultivated organisms
- Provide a method for investigating unusual or unexpected laboratory test results
- Assists in knowing the fine ultrastructure of pathogens of public health or clinical interest, hence aid scientists in advance research concerning the pathophysiological/ morphological details of infectious micro-organism

3.3: Cleaning, Care, and Maintenance of Microscopes

Microscopes often represent a significant investment of funds and are sophisticated optical instruments that require periodic maintenance and cleaning to guarantee production of high-contrast images equal to the quality of the optical, electronic, and mechanical components. When neglected by exposure to dust, lint, pollen, and dirt, failure to remove immersion oil in a timely manner, or when expensive objectives are abused, optical performance can experience a serious decline that increases over time.

FIGURE 1.3.1 COURTESY NATIONAL HOSPITAL ABUJA MICROBIOLOGY DEPARTMENT INFECTIOUS CAUTION GUIDES PICTURES

A microscope that remains unused for a lengthy period of time can accumulate dust and debris from the air (a condition that is only aggravated by leaving the instrument uncovered), which can lead to deterioration of image quality even though the instrument may be practically new. Proper use and regular maintenance of the microscope's mechanical components are equally important to prevent impairment of operation and eventual damage to the entire mechanical integrity of the instrument. The best instrument covers are designed to provide maximum protection from airborne contaminants for specific microscope types, as they are typically configured with their common attachments (see Figure 1.3.1). Even when carefully covered for protection during periods of inactivity, microscopes that are used regularly are subject to build up of contaminants. Some of these are unavoidably introduced from the environment and others by the microscopists themselves, especially in areas where the hands, eyelashes, and even moisture from breathing contact the instrument over time.

Blemishes such as dust, lint, and smudges on the optical components, as well as scratches, pinholes, and striae in the lenses, filters, prisms, mirrors, and faceplate of the image sensor, tend to degrade overall microscope performance. The objective front element illustrated in Figure 1.3.2 exhibits a variety of particulate contamination, as well as severe scratches that seriously degrade its performance. When the optical elements at or near the conjugate field (image) planes are dirty, damaged, or defective, artefacts are likely to appear in sharp focus superimposed on the specimen image. Ironically, the higher the quality of optical components, such as the condenser and collecting and relay lenses, the more these blemishes interfere and contribute to optical noise.

After a source of optical noise is localized to a given component (by turning or shifting the suspected components in turn), the dirt may be removed by a variety of procedures discussed in detail in subsequent paragraphs. The utilization of

immersion oil is essential in maximizing microscope optical performance, but its improper use or the failure to immediately remove the oil after each use constitutes the most serious contaminant that must be dealt with in instrument maintenance. Because immersion oil is a known substance intentionally applied to the microscope to enhance optical performance, its clean-up is discussed separately from the removal of other debris that inadvertently accumulates on either the mechanical or optical microscope components.

Routine Removal of Loose Particulate Matter

If the microscope has been idle and uncovered for a lengthy period of time, a significant amount of debris accumulation has probably occurred. In the typical laboratory environment, a surprising amount of particulate material can be seen to accumulate on an objective or other component that is left uncovered on a bench for even a short period, such as overnight. Because such debris is often highly abrasive, it must be removed from the microscope frame and mechanical parts with care, using a small vacuum cleaner or by dabbing with a moist paper towel. Dirt that is non-adherent may be removed from less delicate lens surfaces by gentle brushing with a clean camelhair brush. Figure 1.3.3 illustrates two of the basic cleaning tools commonly used in microscopy. Alternatively, an air blower or compressed gas duster can be employed, but it must be assured that no oil or similar spray is released from the compressed gas can.

Several manufacturers produce oil-free compressed gas cylinders that are ideal for dusting glass surfaces if appropriate precautions are followed. The common small portable cans of compressed gas must absolutely not be tipped or shaken while spraying in order to avoid release of cold liquid propellant. Although it is difficult to resist the almost reflexive tendency to blow away dust by mouth when it is noticed on lenses and other areas of the microscope, this should be avoided. One should never attempt to blow the dust off lens surfaces with a strong breath because doing so risks spraying the lens surface with droplets of saliva that can mix with dirt to produce an abrasive slurry. A deliberate and systematic cleaning protocol is recommended for thorough contamination removal, and appropriate techniques are detailed in the following sections. While it is often suggested that a regular maintenance schedule be followed at periodic intervals, the necessity for cleaning is dictated by the use of the instrument and by the effectiveness of preventive measures taken to avoid build up of debris. Delicate components should only be cleaned when necessary, as most scratches and other damage to optical surfaces result from improper attempts to clean them.

Proper Use and Removal of Immersion Oil

Following proper procedures in the use of immersion oil will significantly ease the task of removing the oil from microscope components before it causes damage. It is important to recognize that immersion oils are not inert with respect to either optical or mechanical microscope components, and if left in contact with the instrument, oil will penetrate into gears and sliding mechanisms and into crevices between lens elements and their mounting structures, with the potential to cause irreversible damage. Even when employed properly, immersion oil must be removed immediately after use to prevent its accumulation in unwanted areas of the microscope, as well as to avoid optical degradation from dried oil residue on the objective. Oil that has been stored for more than one or two years may not perform optically the same as fresh oil, and a potential increase in viscosity often makes it more difficult to remove. Consequently, containers of immersion oil should be labelled with the date received, and discarded when necessary.

Severely Damaged Microscope Objective

FIGURE 1.3.2

COURTESY NATIONAL HOSPITAL ABUJA MICROBIOLOGY DEPARTMENT INFECTIOUS CAUTION GUIDES PICTURES

The full utilization of the microscope optical system numerical aperture when immersion objectives are used requires a double oiling technique in which immersion oil is applied to both the top and lower surfaces of the specimen slide. Placing immersion oil in the gaps between the objective and slide and between the condenser and slide provides a homogeneous optical medium from the condenser, through the specimen (in an appropriate mounting medium), and into the objective. Although the viscosity of immersion oil minimizes any immediate migration into unintended locations, if it is not removed promptly and is allowed to accumulate, the effects of gravity and capillary forces will ultimately result in the oil moving into parts of the sub stage mechanism and microscope stand, and perhaps even into the objective. This accumulation may not be readily visible, and can go unnoticed until

mechanical or optical problems become severe enough to require service by a microscope repair facility.

Correct utilization of immersion oil requires placing a single drop on the top lens surface of the sub stage condenser and another single drop on the top of the specimen slide. The condenser is then raised just to the point that the oil drop contacts the lower surface of the slide, and the objective front lens is brought into contact with the oil drop on top of the slide. It should be stressed that the oil immersion technique is only to be used with a condenser equipped with an immersion-type top lens, and with immersion objectives. Any attempt to improve the performance of a dry objective by application of immersion oil will likely result in its destruction, as such objectives are optimized optically for use in air, and are not sealed against the intrusion of fluids into the lens barrel.

After each specimen has been studied, the immersion oil should be completely removed, even if additional slides are going to be examined. While it seems expedient to simply add additional drops of oil when changing to the next specimen, this practice results in excess oil accumulating on the microscope, which will eventually find its way into damaging locations in the sub stage assembly and even the microscope stand. Only a single drop of oil at each specimen-optical interface can be accommodated without producing contamination that may be impossible to remove without complex disassembly or factory servicing of the instrument.

Immersion oil is most safely removed using only lens tissue, without employing any solvents. After moving the stage away from the objective, and lowering the condenser away from the slide, the slide can be removed from the stage and set aside for subsequent cleaning. With most microscopes the objective that requires cleaning is most easily accessed by swinging the lens turret to position the objective toward the front of the microscope. Lens cleaning paper that is specifically for use on high quality optics must be employed, and it should be stored in a covered container to prevent contamination with airborne particulates. A folded piece of lens tissue is drawn across the objective front lens to absorb the oil, and repeated with a new area of the tissue. This gentle wiping of the lens surface should be repeated, with as many tissues as required, until no oil streaks are seen on the tissue, and each tissue discarded immediately to avoid inadvertently reusing contaminated tissues on the objective. The folded tissues can be held under light tension with two hands while wiping, or pulled across the lens like a paper swab.
Direct pressure from the fingers should never be applied to the glass lens surface through the paper in order to minimize the possibility of scratching the lens if any

particulates are present on the tissue. It should be emphasized that using a number of fresh lens tissues is essential to the success of this procedure, and the natural tendency to minimize "waste" is definitely misdirected economy considering the relative cost of lens tissue compared to the potential of damaging an expensive objective. If 20 tissues are required to clean an optical component, then that many should be used and discarded without hesitation.

FIGURE 1.3.3 COURTESY NATIONAL HOSPITAL ABUJA MICROBIOLOGY DEPARTMENT INFECTIOUS CAUTION GUIDES PICTURES

When no residual traces of immersion oil are apparent on the final tissue paper, another tissue should be employed to wipe the lens with moisture from the breath. As cautioned previously, one must not blow through closed lips onto the lens, but should breath gently on it with the mouth open, so that no saliva droplets are expelled. If possible, the mouth should be positioned beneath the level of the objective to further reduce any possibility of droplets landing on the lens. With moisture condensed from the breath as a lubricant and solvent, a fresh piece of lens tissue is used to wipe the lens surface in a circular motion. An effective method of preparing lens paper for this cleaning is to fold all four corners of a piece of tissue together, leaving the untouched center of the tissue bulging out. The corners can be twisted together slightly to form a stem for handling the tissue. When the tissue is held by this stem, and wiping performed with the puffed-out tissue center, the force that can be applied to the objective is limited by the springiness of the tissue. Circular wiping motion can be applied in this manner, with very little direct force on the lens surface.

The procedure of breathing on and wiping the objective front lens should be repeated several times with a new tissue each time. With high-magnification objectives, having very small front lens elements, the lens paper can be twisted into a sharper point if necessary, taking care not to touch the portion of tissue applied to the lens. The spring effect of the paper can still be exploited to limit the force that can be applied to the lens surface when cleaning. Removal of immersion oil without

removing the objective from the microscope assumes that the structure of the instrument does not restrict access to the objectives. In the latter case, the objective must be carefully removed from the nosepiece and placed on a suitable protected surface on the lab bench for cleaning. In any instance, objectives that are regularly used should be removed (one at a time) for a thorough cleaning at periodic intervals. This allows each to be more carefully inspected, as described in a following section, for signs of any type of accumulated contamination. Figure 1.3.5 demonstrates cleaning and inspection of an objective that has been removed from the microscope. The small front element of the objective can be effectively cleaned with a tissue formed into a point (Figure 1.3.5(a)), and the effectiveness of the cleaning evaluated under magnification using a loupe or inverted ocular (Figure 1.3.5(b)).

Ideally, the removal of immersion oil from the objective is successfully accomplished only through the mechanical application of lens tissue, and a similar procedure is then applied to the condenser top lens. It may be advantageous to remove the top lens of the condenser to facilitate cleaning, especially if removing it minimizes the likelihood of dispersing oil into other parts of the condenser body. The procedure described for cleaning the front of the objective should be repeated with the condenser lens that was oiled, and the body of the condenser inspected for any stray oil, which must be removed. Following cleaning of the optics, immersion oil should be cleaned from both surfaces of the specimen slide using laboratory tissues (brand names such as Kim wipes or Micro-Wipes). It is not necessary to utilize lens tissue for removing oil from larger areas such as specimen slides, or from other portions of the microscope base or stand. All such areas on the instrument should be routinely checked for any traces of immersion oil, which if found, may be removed with laboratory towels or soft cotton cloth.

Inverted (tissue culture) microscopes present special problems with regard to the use of oil-immersion objectives because spilled or migrating oil can very easily intrude into the interior of the objective at the juncture between the body and the telescoping spring-mounted front lens barrel. If oil is allowed to accumulate, it can conceivably flow, under the force of gravity, even into the objective turret or nosepiece. Specially designed higher-viscosity immersion oils are available for use with inverted microscopes, and should be employed to prevent migration of oil from the objective front element.

Hazards of Solvent Cleaning

Numerous publications by respected authorities in microscopy, including several microscope manufacturers, recommend the use of various solvents as aids in

removing immersion oil from objectives and other optics, as well as for routine removal of other contaminants. While this may simplify and accelerate the cleaning process, the variations in lens construction and the materials used in other microscope components, as well as the health and safety hazards presented in using most of the applicable solvents, make it inadvisable to recommend their general use. Extreme care must be exercised in applying solvents to components that may be irreparably damaged if solvent migrates into internal areas or if it is applied in excess and remains in contact with the surface for too long before evaporating. Many cleaning procedures that have been used successfully for decades have become unacceptable today for a variety of reasons, including additional knowledge of health and safety hazards associated with the solvents for organic non-polar compounds used in immersion oils. The issue of the use of solvents is complicated, and is confused by contradictory recommendations in the scientific literature, as well as by differences in manufacturers' technical publications. Some of the considerations relevant to solvent cleaning are discussed in more detail in the following sections.

FIGURE 1.3.4 COURTESY NATIONAL HOSPITAL ABUJA MICROBIOLOGY DEPARTMENT INFECTIOUS CAUTION GUIDES PICTURES

In the past, solvents have been routinely employed for nearly any cleaning task in microscopy, and particularly for removal of immersion oil. Potential problems associated with solvent cleaning are sufficiently serious that the best current approach in cleaning the microscope is to use solvents only when absolutely necessary, essentially as a last resort rather than a first step. Information provided in instruction manuals of microscope manufacturers exemplifies the difficulty in selecting a cleaning solvent when one is required. Some manufacturers have for years warned specifically against the use of alcohol as a lens-cleaning solvent, while others recommended ethanol and mixtures of ethanol with other solvents. An ideal solvent would be miscible with organic non-polar compounds, not highly flammable,

sufficiently volatile to evaporate quickly leaving no residue, and be non-hygroscopic and non-toxic. Most solvents that have been routinely used historically fail one or more of these criteria. With optics allowing use of alcohols, a mixture of ether and ethanol (50:50 by volume) is effective, as is the modified mixture of ether, ethanol, and chloroform (48:48:4 by volume), but both are dangerously flammable or explosive, and produce toxic vapours.

One of the most significant dangers with many of the solvents proven effective for cleaning microscope optics is that they have the potential to dissolve the cements utilized in lens assembly (as do the immersion oils themselves if allowed to remain on the optics). In the past, benzene was regarded as a highly effective lens cleaning solvent, but always required great caution to limit contact with the lens for no more than a second or two, due to the high solubility in benzene of balsam and some other cements used for lens mounting (and for mounting coverslips on specimen slides). The high volatility of benzene is an advantage in this regard, but the material is also highly flammable and toxic. It is now known that benzene is readily absorbed through the skin, and this as well as inhalation of the vapours can cause liver damage. As a consequence of the numerous hazards, benzene should never be used for cleaning. Xylene has been widely utilized for years, and is considered a less aggressive solvent than benzene, but because of its lower evaporation rate, residual liquid may be more likely to penetrate and damage a lens unless the xylene is used very sparingly. Xylene is, however, highly flammable, toxic and carcinogenic, and may cause skin contact sensitivity. Although alcohol and xylene are widely recommended as lens cleaning solvents, they are also named as being harmful to both the mechanical and optical components of many microscopes. The finish on portions of the microscope stand and the materials used in a number of the parts themselves can be severely damaged by exposure to either material.

Because of the variation in solvent recommendations, and the likelihood that some of the materials used in the instrument components are not known to the user, it is prudent to restrict use of any solvent to an absolute minimum. Optical components should not be immersed in any solvent, and cleaning tissues should only be moistened, never saturated, with a cleaning solution. Minute gaps commonly exist at the glass-metal junctures of an objective front element, allowing the possibility of solvent migration into the interior of the optical component if excessive solvent is applied. Depending upon its composition, the optical cement used to join lens element combinations in objectives is commonly soluble in one or more of the solvents, alcohol, xylene, and acetone. The result of solvent penetration between lens elements is illustrated in Figure 1.3.6, in which the partial separation of

cemented lens groups has occurred. Although most modern optical cements are not readily affected by xylene, some older objectives utilize cements that are totally soluble in xylene.

Alternative Cleaning Materials

Several alternatives to hazardous solvents have been found to be effective in microscope cleaning, and a variety of cleaning agents, as well as cleaning materials, are recommended by different microscopists and manufacturers (see Figure 1.3.7 for examples). Safer alternatives to xylene have been widely pursued, in part because that solvent is commonly used in histopathology and cytology laboratories as a deparaffinising and clearing agent. The proprietary solvents Histolene and Histoclear are gaining popularity as replacements for xylene in microscopy laboratories, and have been found to be effective for instrument cleaning as well. These solvents are based on the naturally-occurring compound d-limonene, which is the major constituent of citrus peel oils and other ethereal oils, and which has been used extensively in the food and cosmetics industries for years. Although the limonene-based solvents require adequate ventilation and skin protection, they currently are thought to be safer overall than xylene. Pure distilled water is the safest cleaning fluid for any contamination that is water soluble; if that is inadequate, commercial photographic lens cleaning liquids are very effective and are safe for precision optics when used sparingly. This type of cleaning agent consists primarily of water to which is added a small percentage of surfactant and alcohol. Commercial window cleaning products (such as Windex and Sparkle) are used by some microscopists, with no reported damage to optical components, and isopropyl alcohol is employed successfully by others.

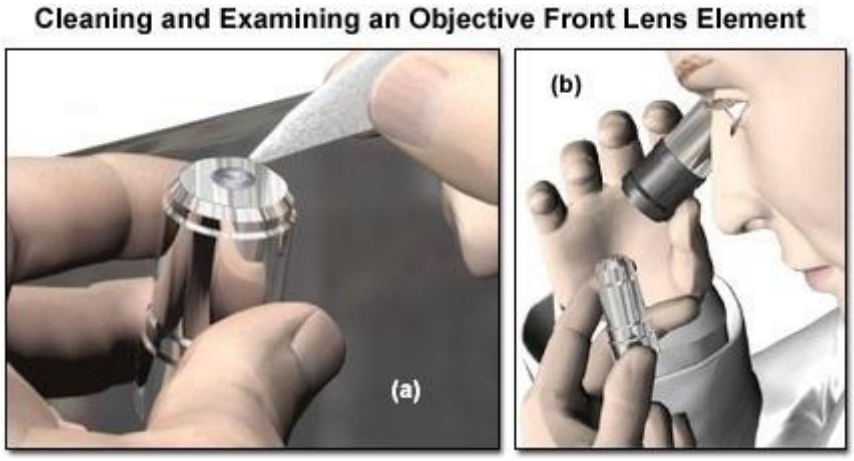


FIGURE 1.3.5. CLEANING AND EXAMINING AN OBJECTIVE FRONT LENS ELEMENT

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Great care must be taken in choosing materials for applying water or other cleaning liquids to precision optical components. Although many products are marketed as being suitable for lens cleaning, and other materials give the subjective impression that they would not be harmful, the suitability of specific materials for delicate optics is not always obvious. As an example, the laboratory tissues marketed under the name Kimwipes have been shown to be suitable for lens cleaning, although they feel quite coarse to the touch. In contrast, typical facial tissues are processed to feel soft to the skin, but contain hard particulates that are definitely harmful to optical surfaces. Lens tissues are available in varieties that feel relatively stiff and hard-surfaced, with tight fibrous texture, and others that are loosely textured and very flexible. The softer type is generally preferable for delicate optics, even though these tissues tend to leave residual loose fibers following cleaning, which must be blown off with air. Freshly laundered pure cotton or linen fabric is recommended by some microscopists for lens cleaning, but with any material that is reused, it is essential that no detergent residues or particulates remain after washing. Not only is this not a trivial requirement to meet, it is also important to ensure that if manufactured cloths such as handkerchiefs are used, they are not hemmed or otherwise sewn with polyester or other abrasive thread.

A common recommendation in the past for performing lens cleaning was to wrap small portions of cotton wool around the tip of an orangewood stick (an oil-free wood) for use as a cleaning swab. This is no longer advisable, due to the fact that cotton wool such as that now sold by pharmacies in rolls typically contains some proportion of synthetic fibers, and is not as suitable for delicate surfaces as is 100-percent cotton wool. Cotton swabs that are untreated are still considered to be suitable, although these are wound into very tight buds at the factory, and before use it is wise to loosen some of the cotton at the tip of the swab with clean forceps (not the fingers, which will deposit skin oils) so that less force is applied to the surface being cleaned. Applicators made by attaching small pieces of clean chamois to orangewood sticks are commonly used by optical technicians, and these are commercially available or can be made-up in special sizes, as desired.

Basic Cleaning Procedures of Mechanical Components

The primary concern in maintenance of the mechanical components of the microscope are areas of the instrument which are unavoidably exposed to skin oils from the hands and moisture from breathing, and the stage area, which is subjected to a variety of contaminants during imaging sessions. In addition to the stage, other components to be cleaned include controls such as knobs, levers, and movable

control rods, the body tube, and the stand. Because many of the microscope controls, such as focusing knobs, are ribbed or milled in a fine crosshatch pattern, skin oils tend to collect in these areas and attract dust, which can become tightly bound to the control. Cleaning may be required frequently on microscopes that are heavily used. An effective cleaning liquid may be prepared by adding approximately 10 percent alcohol, by volume, to a commercial glass and surface cleaning product. A piece of terry cloth towelling moistened with the cleaner should be used to remove contamination from the ridges of every control by wiping in the direction of the ridges, or in multiple directions on milled surfaces. Pre-packaged moistened wipes for optical components provide an alternative method of applying a controlled amount of cleaning fluid, which may be effective for cleaning many microscope surfaces (see Figure 1.3.8). Each cleaned control surface should be dried with a clean piece of towelling.

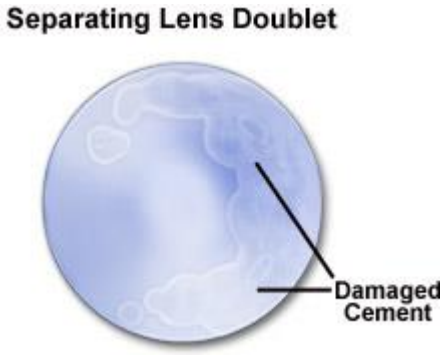

FIGURE 1.3.6 SEPERATING LENS DOUBLET

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As a microscopists works with the eyes adjacent to the oculars, the close proximity of the facial areas around the eyes and nose to the cooler surfaces of the body tube result in vaporized moisture and skin oils condensing on these microscope surfaces, leading to a significant amount of contamination. Additionally, the breath impinges on both the body tube and objective nosepiece, contributing further to the collection of airborne contaminants on the moist surfaces that result. The use of an air deflection shield, commonly referred to as a breath shield, on the microscope is effective in reducing this source of contamination by diverting the breath away from the nosepiece and microscope stand. The body tube and other parts of the instrument stand can be cleaned with soft cotton cloth lightly moistened with the surface cleaner referred to previously. It is especially important to clean the area around the eyepiece interocular distance adjustment mechanism, which is particularly prone to the build-up of contamination. In order to avoid getting any moisture inside the eyepiece tubes, they should not be wiped with the moistened

cloth near the top at the mating surface where the ocular rests. After cleaning the body tube, it should be dried with another piece of cotton cloth, and this dry cloth can be used to clean the top portion of the eyepiece tube and the outer rims of the oculars, taking care to avoid touching the glass lens surfaces.

The microscope stage is cleaned in a similar manner to the body tube, first with a moistened cloth, then with a dry one. Because of the variety of contaminants that may be deposited on the stage from specimens and from constant handling and manipulation, it should be cleaned after every use of the microscope. Care must be exercised in cleaning around the edge of the center opening in the stage, and contact should not be made with the underside of the stage where there may be exposed grease from bearing surfaces. Any cloth contaminated with the special grease used on the instrument stage should be discarded to avoid transferring it to other parts of the microscope, as it may be virtually impossible to remove.

The remainder of the microscope stand should be cleaned carefully with the same procedure of a moistened cotton cloth followed by a dry cloth, taking care to avoid optical surfaces or any area that might be subject to moisture penetration that could damage internal mechanisms or electronic circuitry. Following complete cleaning of the mechanical components as described, and carefully wiping up any liquid spills in the vicinity of the instrument, a small vacuum cleaner (see Figure 1.3.3), with a flexible hose and soft brush attachment, can be employed to vacuum up any loose material on the stand and table area around it. Extreme care should be taken to avoid touching any optical surfaces with the vacuum brush.

Basic Cleaning of Optical Components

A systematic protocol for inspection and cleaning of microscope optical components is essential for several reasons. Not only are the optics the most crucial components in image formation and recording, they are the most expensive, as well as the most delicate and most subject to damage. Inspection of optical surfaces with magnification, provided by a loupe or an inverted ocular, is an important first step in cleaning. Evaluating whether contamination is present and determining the type of material is important both because unnecessary cleaning is counterproductive and because certain types of contamination are not obvious without careful inspection. In particular, the front elements of the objective and condenser should be regularly inspected with a magnifier under reflected light by carefully positioning a light source at an angle to the surface being examined so that any debris can be seen. In troubleshooting a blurry or low contrast microscope image, it can be assumed that the most likely cause is a dirty front objective element, debris on glass surfaces near

the imaging sensor, or a dirty coverslip. High-magnification objectives, with very short working distances, are especially vulnerable to contamination, and require frequent inspection.

The presence of even minor dirt or smudging on an objective, no matter what the nature of the material, produces the same effect, which is a reduction of image sharpness. This is true for particulate material and for contamination with perfectly transparent material such as immersion oil. Oil traces, including greasy fingerprints on a dry objective front element, interfere with the transmission of light rays through the objective in the same manner as would a damaged lens or one having an optical manufacturing defect. Inspection of the front objective element is the best way to determine whether contamination is present, and if so, what course of action is required for its removal.

FIGURE 1.3.7 LENS CLEANING PRODUCTS

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The cleaning procedures described below apply only to exposed surfaces of the various optical components of the microscope. No attempt should ever be made to clean internal optical surfaces of most microscope components, and cautions are given in each of the following sections pertaining to specific components in order to emphasize potential damage that can be caused by not strictly following this advice. The basic cleaning protocol for optical surfaces, which is generally followed for all optical components, should be undertaken in steps, as follows:

- **Inspection of the Lens Surface** - The optical component to be evaluated is removed from the microscope and placed on a laboratory towel or similar protective surface on the instrument table. Before any cleaning is attempted, the optical surface should be inspected with magnification under reflected light to determine the condition of the component. Particular attention should

be given to the presence of any particulate material, which must be assumed to be abrasive, and removed before any other cleaning is done. Additionally, the presence of any films, smudges, or stains should be noted. A magnifying lens of 2-3x is appropriate for examining larger optics such as oculars and condensers, while the smaller lens elements of objectives require approximately 5x to 10x magnification for proper inspection. It is crucial that particulate matter be removed from a lens surface as the first step in cleaning, because any particle can be abrasive and result in scratches if it is moved across the surface with even the gentlest lens tissue.

- **Removal of Non-attached Particles** - If any dust, fibers, or other particles are observed on the lens surface, an attempt should be made to remove them in the least aggressive manner possible, which is by gently blowing air across (not perpendicular to) the lens surface. The safest method of air dusting is to use a rubber bulb or balloon, such as the ones intended for use as ear and enema syringes for infants. The larger enema syringe is appropriate for larger optics such as eyepieces, condensers, and prisms; the smaller ear syringe is better for small objective lens surfaces. The reason for not blowing directly toward the particle and surface is that this can force abrasive particles into delicate lens coatings, and possibly make them more difficult to remove, ultimately damaging the surface in the process. The cumulative effect of repeated abrasions of this type, though minor, can degrade the performance of the optic. Care is required to avoid touching the tip of the syringe to the lens surface. The best advice is to avoid any use of compressed air cans for lens cleaning. It is difficult with these to control the pressure of air impinging on the surface being cleaned, and there is always the risk of either extremely cold air or freezing liquid being expelled onto a lens surface and causing irreparable damage. Neither lens coatings, nor optical cement between lens elements can withstand localized freezing without damage. If, for any reason, a canned-air duster must be used, it should be stabilized in an upright position to avoid tilting (which will expel cold liquid), and fitted with a length of flexible plastic tubing to allow air to be directed in the desired direction onto the optical surface. It is far preferable to utilize the manual air bulb type duster to completely eliminate this risk.
- **Reinspection of the Lens Surface** - Inspection of the lens again with a magnifier will reveal whether all particles have been removed, and if so, any contaminating films should be noted for subsequent removal. If particles remain after the initial air dusting, another attempt should be made to remove

them with air alone. Any that are still present on another examination are most likely attached through direct interfacial tension between the particle and surface, or due to an intervening film of some type, and these must be removed before further cleaning of film contaminants can proceed.

- **Removal of Attached Particles** - Particulate material that resists removal by air dusting alone is most likely held by a surface film through a minute contact area, and can be dislodged by delicately applying slight lateral force to the side of the particle. This procedure requires practice, and definitely must be done with adequate magnification to ensure that no damage is done to the lens. To devise a tool for nudging attached particles from their positions, a thin bamboo skewer or wooden toothpick can be cut off to a very fine square point with a razor blade. After breathing very gently onto the lens (with mouth open wide) to produce condensed moisture, which should loosen the particle from its adherent film, the point of the wooden tool is brought into contact with the side of the particle. It is gently nudged sideways, taking care not to touch the lens surface with the tool. This process is repeated for any other attached particles, and the small ear syringe is employed to blow across the lens surface to remove the freed material.
- **Reinspection of the Lens Surface** - The lens is inspected again under magnification to determine if all particles have been removed. If any remain, the removal procedure is repeated, and the lens inspected again. When all particulates have been removed, if no additional contamination is present, the component can be reinstalled on the microscope. If any other film, streaking, fingerprints, droplets, or other contaminants are present on the optical surface, the following steps are performed.
- **Removal of Water-Soluble Films** - Water-soluble materials can be removed from a lens surface using lens tissue and moisture produced by slowly breathing onto the lens. The tissue should be utilized in the manner previously described to limit the force applied to the lens, and never just rubbed on the surface directly with finger pressure. In addition to the puffed-out tissue technique, several other methods are suitable for limiting the force applied by the lens tissue. One that is effective for relatively small lens surfaces is to roll a folded lens tissue into a tight tube, and then to tear it in half forming two shorter tubes each having a frayed end. The frayed end of each tube is used to clean the lens surface. The tearing action not only should dislodge any particles on the paper in that area, but the torn end minimizes the force that can be

applied to the lens. After gently and slowly breathing on the lens with the mouth opened wide to provide moisture, the lens is cleaned with a frayed tissue tube in a circular motion starting at the lens center and working outward toward the periphery. The tissue is discarded and the process repeated with additional torn pieces, until the lens appears clean or no more improvement is noted. The lens may not become completely clean if any contaminants are present that are not water-soluble.

- **Inspection of Lens Surface** - The lens is again inspected using magnification, and if it is completely clean, the component can be returned to service on the instrument. If any film-like deposits or smudges remain on the lens, it is most likely a non-water soluble material, which must be removed with the following additional cleaning step.
- **Removal of Non-Water Soluble Films** - Contaminants on an optical surface that are not readily removed with water (other than immersion oil, discussed previously) require an additional cleaning component. One of the safest materials that is effective on deposits of this type is one of the commercial lens cleaning fluids for precision optics, which are usually composed of distilled water to which small proportions of a surfactant and alcohol are added (see examples in Figure 1.3.7). A very limited amount of fluid should be utilized, and it should never be applied directly to the lens surface. An effective means of controlling the amount of fluid allowed to contact the lens is to use a cotton swab to which is applied a very small drop of cleaning solution. The tip of the cotton bud should be inspected for any particulates that are present, and as described previously, the tightly wound cotton can be loosened slightly by pulling the tip with clean tweezers or teasing out some of the cotton with a needle. The lens can be cleaned by lightly applying the swab in a circular motion starting at the lens center and moving out. As an alternative to the cotton swab, a soft lens tissue may be twisted into a point, being careful to not touch it at the end, and used as discussed regarding removal of immersion oil. When employing a cotton swab in this manner, extreme care must be taken to limit the force applied to the lens surface, and this technique should never be employed except as a final cleaning step immediately after complete removal of particulate materials.

Still another effective method of utilizing a lens cleaning fluid so that very little force is applied to a small lens, such as an objective front element. With the component resting on a soft surface on the table, a single drop of cleaning fluid is placed on a

folded tissue, and while supporting the tissue with both hands, the drop is brought into contact with the lens surface. The tissue is then drawn horizontally over the lens surface, which will leave a streak of fluid on the tissue. There should be no attempt to force the tissue into contact with the lens; in fact the surface tension between the lens and drop of fluid may make it possible to slightly pull the tissue away from the lens while moving it across, if this is not done with so much force that the tissue loses contact with the lens surface. The process should be repeated several times with a fresh drop of fluid and a new tissue each time. After cleaning with the moistened swab or tissue, the lens surface should be dried by repeated application of several torn lens tissue tubes, discarding each after use. An indication of the success of the cleaning can be obtained by breathing slowly on the lens to moisten it, noting whether the moisture film is even and without disruption. As a final step, the moisture is removed by wiping in a circular motion with a lens tissue tube.

- **Final Evaluation of Lens Surface** - Inspecting the lens surface with magnification is the final step in determining whether the component is completely clean before replacing it on the microscope.

Notes and Cautions on Cleaning Specific Components

Modern, highly-corrected objectives may contain over 15 individual lens elements, some joined by optical cement into compound lens groups, which are assembled at precise separation distances within the objective barrel. Objectives should never be disassembled in an attempt to clean internal lens surfaces, or for any other reason. The component lens elements are precisely cantered optically, and assembled with a precision that cannot be duplicated outside of the manufacturer's factory setting, and any attempt at disassembly will undoubtedly result in a damaged objective. Even if access to internal surfaces were possible, they could not be successfully cleaned without damage, due to the fragility of the anti-reflection and other lens coatings that are commonly utilized. Most precision lens surfaces employ one or more interference-film coatings that may be only a few atomic layers thick. These coatings are protected by hardened protective layers on external lens surfaces, to enable them to tolerate normal cleaning procedures, but the coatings on internal surfaces are much softer and very easily damaged.

Under no circumstances should the rear objective lens element be cleaned, other than to blow off dust that settles there with the ear-syringe blower. Due to the construction of the objective, which makes access to the rear element difficult, attempts to clean the rear element risk introducing tissue fibers or other contamination into the interior of the assembly. The interior can be checked for

contamination by looking through the objective from the front, with a light source (such as a bare lamp) positioned close to the rear element. Unfortunately, if internal contamination is present, it can only be removed by qualified service centres.

FIGURE 1.3.8 COURTESY NATIONAL HOSPITAL ABUJA MICROBIOLOGY DEPARTMENT INFECTIOUS CAUTION GUIDES PICTURES

One previous caution that bears repeating is the importance of not applying excessive pressure to the front lens surface of an objective. The front element of higher-magnification objectives is a very small, partially hemispherical lens that is held in place by minimal contact with the lens assembly. The large amount of metal surrounding the small glass element gives these objectives a robust appearance that is deceptive, as the front element can be easily moved out of alignment by excessive pressure, resulting in a damaged objective. Furthermore, even if the element is not forced out of alignment, applying too much pressure during cleaning or through accidental contact can produce minute gaps at the juncture between the lens and the surrounding metal barrel, causing oil or cleaning fluids to be drawn by capillary force into the objective interior, destroying the objective.

The top lens of most condensers is removable, and cleaning involves application of the basic optical cleaning procedure to the top and bottom surfaces of the top lens, as well as to the top lens surface of the middle assembly. The component parts of the condenser body should absolutely not be disassembled. They are assembled with similar precision to objectives, and cannot be realigned outside of the manufacturers' facilities. The same is true for phase contrast elements and differential interference contrast prisms, as well as for polarizing devices that are components of some condensers. These elements must be realigned at the factory if disturbed, and should never be removed or disassembled. Because of its location, the sub stage condenser

collects a variety of contaminants, and must be cleaned more frequently than other components. Due to its relative inaccessibility on most microscopes, the condenser usually requires removal for proper cleaning, and should be handled with the same care given to objectives.

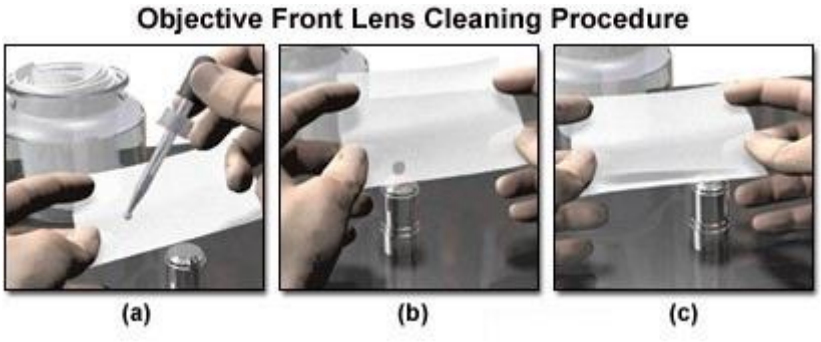

FIGURE 1.3.9 COURTESY NATIONAL HOSPITAL ABUJA MICROBIOLOGY DEPARTMENT INFECTIOUS CAUTION GUIDES PICTURES

After removal of the condenser from the microscope, the top element can usually be removed by unscrewing. If a filter carrier is present beneath the condenser, it should be swung away from the condenser and any filters removed for subsequent cleaning. The surface of the top element may be contaminated with both particulate and film deposits, and is cleaned following the basic protocol of first removing particles, and then films or smudges. The lower surface of this lens will typically only have particulate debris, but should be inspected with a magnifier to confirm this, and cleaned accordingly. The next step is inspection and cleaning of the upper surface of the middle optical section of the condenser that is exposed when the top element is removed.

The condenser should next be turned over so that the bottom surface of the lower lens assembly can be inspected and cleaned if necessary. The primary caution at this stage is to avoid damaging the iris diaphragm if one is utilized beneath the lower optical combination of the condenser. If present, the diaphragm must be opened completely to allow access to the bottom lens surface and to protect the blades of the diaphragm. These blades are extremely fragile and should not be cleaned, touched, or exposed to any liquid. If opening the diaphragm does not retract the blades completely into the rim of the assembly, do not attempt to clean the lower lens by reaching through the iris opening, as damage to the diaphragm is likely. Cleaning of any filter removed previously must be done with the same care as exercised with other optical components. Interference filters are constructed utilizing very thin vacuum-deposited films similar to anti-reflection lens coatings, and filters of this type are commonly utilized in the condenser assembly and elsewhere in the microscope optical path. Particular caution must be used in handling and cleaning of

such filters to prevent damage to the thin coatings.

The optical trains of modern microscopes contain a number of precision prisms and front-surface mirrors, most of which are housed within the microscope base and stand. As a general rule, none of these components should be cleaned unless they are accessible without disassembly of any part of the instrument. When internal components of this type are dirty, they require factory service to be cleaned without damage. The only exceptions are three external prism surfaces that are accessible in the body tube, and a mirror that is exposed (without any disassembly) in the base of some microscopes. Front-surface mirrors employ an unprotected reflective coating (usually silver) on the front of a glass base, and are very easily damaged. Removal of dust and fibers can be accomplished with gentle air dusting, followed by very gentle cleaning with lens fluid only when absolutely necessary. Cleaning with tissue should be done employing every effort to limit friction on the reflective mirror surface, which is easily abraded.

Binocular body tubes contain prisms for the right-eye and left-eye light paths that are precisely aligned using special collimating equipment, and no disassembly for cleaning should be attempted except by factory service facilities. Because the initial assembly is done under clean-room conditions to ensure a minimum of particulate contamination, any internal cleaning efforts would probably only introduce additional debris. The external prism surfaces that are visible when the eyepieces are removed from the body tube can be carefully cleaned by blowing off particulates, followed by use of cotton swabs that are softened on their tips as described previously for objective cleaning. Dust should be blown off with a large infant syringe after inverting the body tube so that dust falls away from the prism surfaces and out of the body tube. If further cleaning to remove smudges or films is required, it may be necessary to provide moisture for this procedure by breathing on the cotton swab tip instead of the prism itself, because of the recessed location of the prisms within the body tube. When the body tube is turned upside down, the lower opening reveals the third prism surface or an optical flat covering it, and this surface should be examined for signs of contamination and cleaned carefully if necessary.

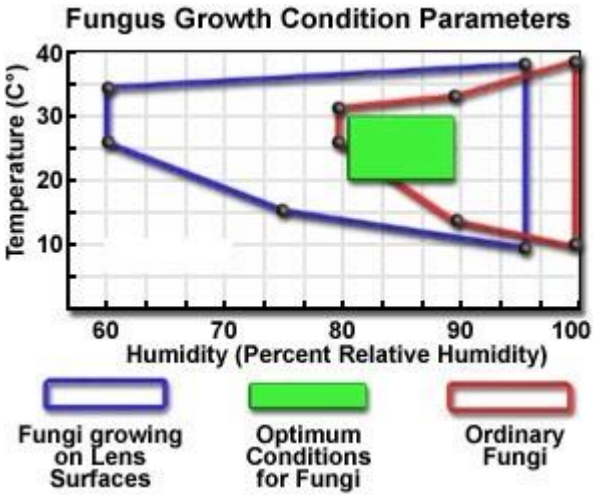

FIGURE 1.3.10 FUNGUS GROWTH CONDITION PARAMETERS

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Eyepieces require fairly frequent cleaning of their external optical surfaces, but do not generally become contaminated internally. The eye lens top surface is vulnerable to many types of contamination due to its proximity to the microscopist and its likelihood of collecting airborne particulates. Because the eyepieces are frequently removed for various reasons during use of the instrument, the lower field lens surface can become soiled and should also be examined for debris. Both of these lens surfaces should be cleaned as required following the basic protocol for optical components. In the rare circumstance that dust or fibers are seen in the interior of an eyepiece when it is inspected following external lens cleaning, it is possible in some cases to remove the eye lens in its mount and the field lens in it mount, and to clean the tube interior and the inner surfaces of the two lens components. They must be very carefully reassembled in their exact original configurations or the eyepiece will not perform properly. Particular care must be exercised with the finely threaded lens cells to avoid cross-threading the components upon reassembly. Note however, that under no circumstances should a Filar micrometer eyepiece, a measuring eyepiece containing an internal reticle, or any type of digital-readout eyepiece be opened or disassembled for any reason. Doing so will destroy the calibration, and require factory restoration.

Microscopes that are equipped with digital cameras may develop a degradation in captured image quality or exhibit image artefacts caused by accumulation of contamination either on filter elements that are sometimes utilized in the camera adapter or on the optical glass window that may be incorporated to seal the camera housing and protect the CCD or CMOS image sensor. In practice, if dark specks or similar in-focus artefacts are observed in digital images, and they are not in the

specimen plane, their most likely cause is particulate contamination on the image sensor or an associated filter surface. Some digital cameras incorporate removable infrared filters in the camera system, while in others the required filtration is an integral part of the sensor window. Because of the variety of configurations encountered in scientific digital cameras, the manufacturer's recommendations regarding cleaning should always be followed. Some cameras, particularly those in which the sensor is cooled, are hermetically sealed, and the sensor is not directly accessible.

In general, the optical glass surfaces on sealed cameras should be inspected and cleaned, if necessary, following the standard cleaning methods for lens surfaces, always removing particulate debris before gently cleaning the glass surface with moisture from the breath, followed by lens tissue moistened with lens cleaning fluid for non-water soluble contamination. If the window is difficult to access with lens tissue (such as with torn tissue tubes), cotton swabs can be used provided that care is taken to limit pressure on the window surface. In some cameras, the sensor surface is directly exposed within the camera body, and is highly likely to attract dust and other debris. Special techniques may be required in cleaning the sensor to avoid static charge damage to the device, and the manufacturer's service personnel should be consulted for guidance on proper procedures.

Fungal Growth on Optical Surfaces

An especially serious problem that may plague microscope optical components is the development of fungal damage. Formation and growth of fungal colonies may occur rapidly in some climates, and when established on glass surfaces, it is unlikely that they can be removed before damage has been done to the surface. Unfortunately, fungal growth commonly occurs in the interior of optical components, and may be quite advanced before it is even noticed. At least one microscope manufacturer states that over 50 percent of deterioration in optical performance is attributable to cloudiness caused by certain fungus types. Although there are over 100,000 fungus species, two members of the genus Aspergillus are believed responsible for most lens deterioration. Optimum growth conditions for these fungi are relatively high temperature and high humidity, but they also are more adaptable to lower humidity levels than most other fungi. Figure 1.3.10 illustrates both the optimum and tolerable growth conditions for these fungi growing on lens surfaces, in comparison to the most favourable conditions for other common fungus species. Unfortunately, the conditions most conducive to proliferation of the lens-damaging fungi match very closely the most suitable environment for humans. This greatly complicates attempts

to eliminate or inhibit growth of the fungi on optical components.

Fungi growing on lens surfaces reduce lens performance due to the lowered transmittance caused by the cloudiness, as well as by light dispersion from the thread-like filaments (hyphae) of the fungal colonies. Fungi growing on glass surfaces are not attached by roots and can be wiped off, but unfortunately, residual corrosion marks remain and the original lens performance cannot be recovered. The corrosion is a form of surface etching occurring when an organic acid produced by the fungus mixes with water vapour from the air that accumulates on fungus hyphae. Lenses with significant fungal growth usually must be replaced, since the only effective means to avoid fungal damage to optical components is to prevent its growth in the first place.

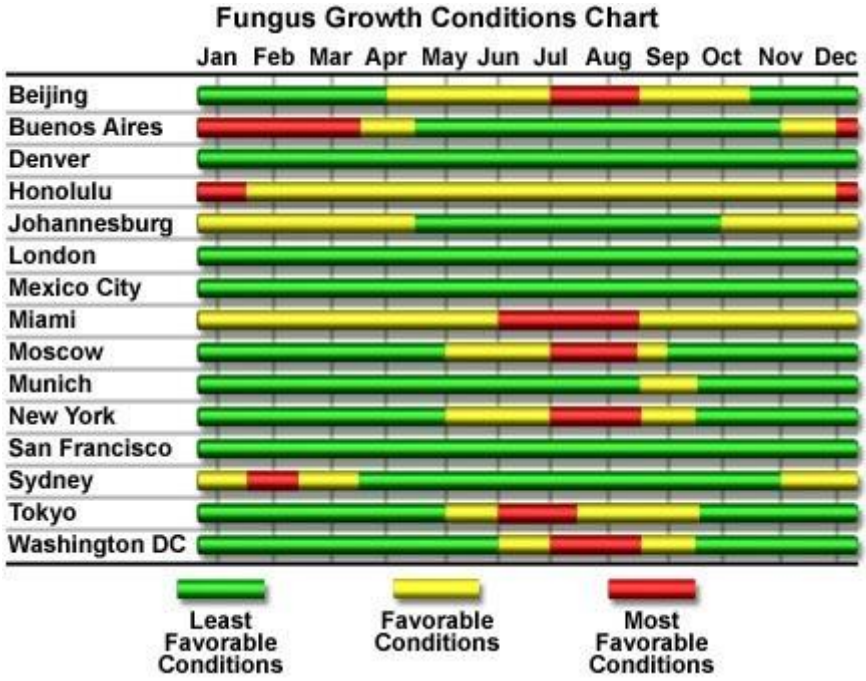

FIGURE 1.3.11 ; FUNGUS GROWTH CONDITION CHART

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Favourable conditions for limiting the occurrence of fungal growth on surfaces such as lenses include a low-humidity environment, sufficiently low temperatures, good ventilation, and occasional exposure of the surface to sunlight. Climatic factors cannot be controlled completely, and the use of air-conditioning systems and dehumidifiers in warm and humid climates is beneficial and necessary, but does not eliminate the growth of the highly resilient fungus types, which can adapt to a wide range of conditions (see Figure 1.3.10). The strategy of storing optical components under desiccated conditions is sometimes suggested, but this is not an advisable practice, because extremely low (0 percent humidity) moisture levels can accelerate

the breakdown of cements used to join optical elements. The geographical area in which the microscope is located determines, to a large extent, the seriousness of fungal growth as a factor in instrument care.

Figure 1.3.11 presents, in chart form, the seasonal variability of fungus growth conditions for a number of worldwide cities. It can be inferred from the chart that fungal growth is least likely in regions having consistently low humidity, or in regions that have relatively low average temperatures during periods of high humidity. In some climates, it is virtually impossible to inhibit fungal growth unless the microscope can be placed in a sterile environment. The major microscope manufacturers produce special versions of some equipment for use in tropical or other fungus-prone environments. Among preventive measures that have been developed are to enclose an antifungal chemical substance inside objectives, eyepieces, and the microscope base, and to improve the effectiveness of seals on any moving parts to minimize the entry of dust and fungal spores from the environment. The chemical is a solid substance designed to slowly sublimate and produce an antifungal vapour that is harmless to the microscope optical and mechanical components. The antifungal activity can be maintained over long periods of time by encasing the chemical in a material with only slight air-permeability, thereby strictly controlling the sublimation rate.

Benefits of Preventive Maintenance

The ideal microscopy room would be designed specifically for that purpose, and incorporate every mechanism available for limiting contamination by dust, chemical vapours, and other airborne contaminants, as well as isolating the instrument from acoustic and mechanical vibration and temperature variations. This ideal situation is seldom realized, and most microscopes are located in areas subject to a considerable number of environmental deficiencies. Some contamination is unavoidable, due to the rigors of daily use, but at the very least, the microscope should be protected as well as possible during periods of non-use by covering the entire instrument with a suitable cover. Instrument manufacturers and aftermarket suppliers offer a variety of specially designed dust covers (see examples in Figure 1.3.1). Of several types of plastic cover, those made of softer more flexible material are probably less prone to attraction of dust. Lint-free fabric covers are also available, and provide an effective dust barrier that can minimize the need for cleaning.

While the cost of a modern research grade microscope can range from approximately a few tens of thousands to several hundred thousand dollars, if properly used and maintained, the basic optical and mechanical components of the instrument can

easily outlive several generations of microscopists. Only if the instrument is used correctly and maintained regularly is it capable of producing the best image data possible. Careless, incorrect operation and maintenance techniques not only result in unreliable and poor quality images, but cause productivity at the microscope to suffer, and the instrument's useful lifetime to be greatly reduced.

4.0: CONCLUSION

Microscopy is a key component of the procedures involved in the diagnosis of infectious diseases of public health importance. Microscopy simply entails the use of microscope to visually enlarge objects too small to visualize with the unaided eye. There are different types of microscopes with different operating principles and terminologies as we saw above.

5.0 SUMMARY

So far in this unit we have learnt about the following:

- Types of Microscopy
- Applications of microscopy in Public Health Microbiology
- Cleaning, Care and Maintenance of Microscopes

6.0: Tutored Marked Assignment

Ques. A) Enumerate the various types of microscopes you have recently studied and briefly discuss their operating principles.

Ques. B) What are the general applications of Microscopes?

Ques. C) Write a comprehensive essay on the cleaning, care and maintenance of the microscopes

7.0: SUGGESTED FURTHER READING AND REFERENCE

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Thomas J. Fellers and Michael W. Davidson (2016): National High Magnetic Field Laboratory, 1800 East Paul Dirac Dr., The Florida State University, Tallah Florida,

UNIT 4 BACTERIA CELL NUTRITION, ENERGY AND GROWTH:

CONTENTS

1.0 INTRODUCTION

Bacterial requirements for growth include sources of energy, "organic" carbon (e.g. sugars and fatty acids) and metal ions (e.g. iron). Optimal temperature, pH and the need (or lack of need for oxygen) are important.

1.01 Oxygen Requirements

Obligate aerobes must grow in the presence of oxygen; they cannot carry out fermentation. Obligate anaerobes do not carry out oxidative phosphorylation. Furthermore, they are killed by oxygen; they lack certain enzymes such as catalase [which breaks down hydrogen peroxide, H_2O_2, to water and oxygen], peroxidase [by which NADH + H_2O_2 are converted to NAD and O_2] and superoxide dismutase [by which superoxide, $O_2^{\cdot}$, is converted to H_2O_2]. These enzymes detoxify peroxide and oxygen free radicals produced during metabolism in the presence of oxygen. Aero tolerant anaerobes are bacteria that respire anaerobically, but can survive in the presence of oxygen. Facultative anaerobes can perform both fermentation and aerobic respiration. In the presence of oxygen, anaerobic respiration is generally shut down and these organisms respire aerobically. Microaerophilic bacteria grow well in low concentrations of oxygen, but are killed by higher concentrations.

1.02 Nutrient Requirements

These include sources of organic carbon, nitrogen, phosphorus, sulphur and metal ions including iron. Bacteria secrete small molecules that bind iron (siderophores, e.g. enterobactin, mycobactin). Siderophores (which bound iron) are then internalized via receptors by the bacterial cell. The human host also has iron transport proteins (e.g. transferrin). Thus bacteria that ineffectively compete with the host for iron are poor pathogens.

1.03 Temperature

Bacteria may grow at a variety of temperatures from close to freezing to near to the boiling point of water. Those that grow best at the middle of this range are referred

to as mesophiles; which includes all human pathogens and opportunists. (Those having lower and higher temperature optima are respectively known as psychrophiles and thermophiles).

1.04 pH

Many bacteria grow best at neutral pH; however certain bacteria can survive and even grow in quite acid or alkaline conditions.

Measuring bacterial mass in liquid cultures of bacteria

Common methods include:

a) Turbidity (the cloudiness of a liquid culture of bacteria - a measure of total bacteria [live and dead] - This is usually quantitated with a spectrophotometer). Figure 1.3.1

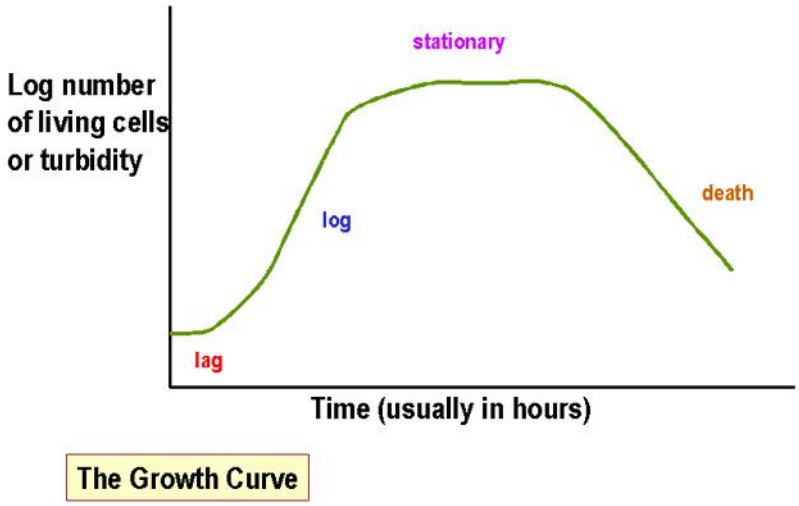

FIGURE 1.4.1 THE GROWTH CURVE

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b) The number of viable bacteria in a culture - usually assessed by counting the number of colonies that grow after streaking a known volume on a plate ("plate counting" of colony forming units). In either case plotting the log of turbidity or number of living cells versus time is referred to as the growth curve. The generation time is defined as the time required for bacterial mass to double.

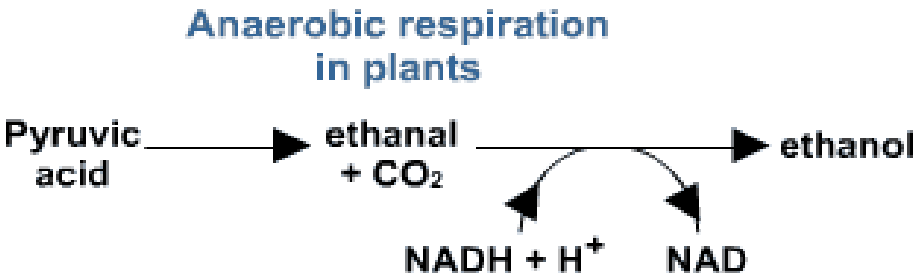
2.0 OBJECTIVES

At the end of this Unit, you will be able to

- Know anaerobic metabolism in bacteria
- Know aerobic metabolism in bacteria
- Describe metabolism of Fatty acids in bacteria
- Describe how sugars are metabolized in bacteria

3.1 Anaerobic Respiration

Anaerobic respiration includes glycolysis and fermentation. During the latter stages of this process NADH (generated during glycolysis) is converted back to NAD by losing hydrogen. The hydrogen is added to pyruvate and, depending on the bacterial species, a variety of metabolic end-products are produced.



3.2 Aerobic metabolism

By addition of CO_2 to pyruvate a C4 compound is produced. In this instance, additional molecules of C4 (a cycle component) are formed.

Thus if some of the cycle components are removed for use in other biosynthetic pathways, they can be replenished via this reaction.

3.3 METABOLISM OF FATTY ACIDS

Fatty acids are broken down to acetyl groups (C2) which feed into the Krebs Cycle by addition to a C4 intermediate to produce C6. During the cycle, the added C2 is lost as CO_2 C4 regenerated. Overall, no increase in the number of molecules of cycle intermediates occurs. Thus, if fatty acids are the sole carbon source, no Krebs cycle intermediates can be removed without shutting it down:

Instead, bacteria utilize the glyoxylate cycle (Figure 1.4.2) (a modified Krebs Cycle) in which the enzymatic steps in which two CO_2 molecules are removed from the C6 intermediate are by-passed. The C6 intermediate is converted to two C4 compounds (both components of the cycle).

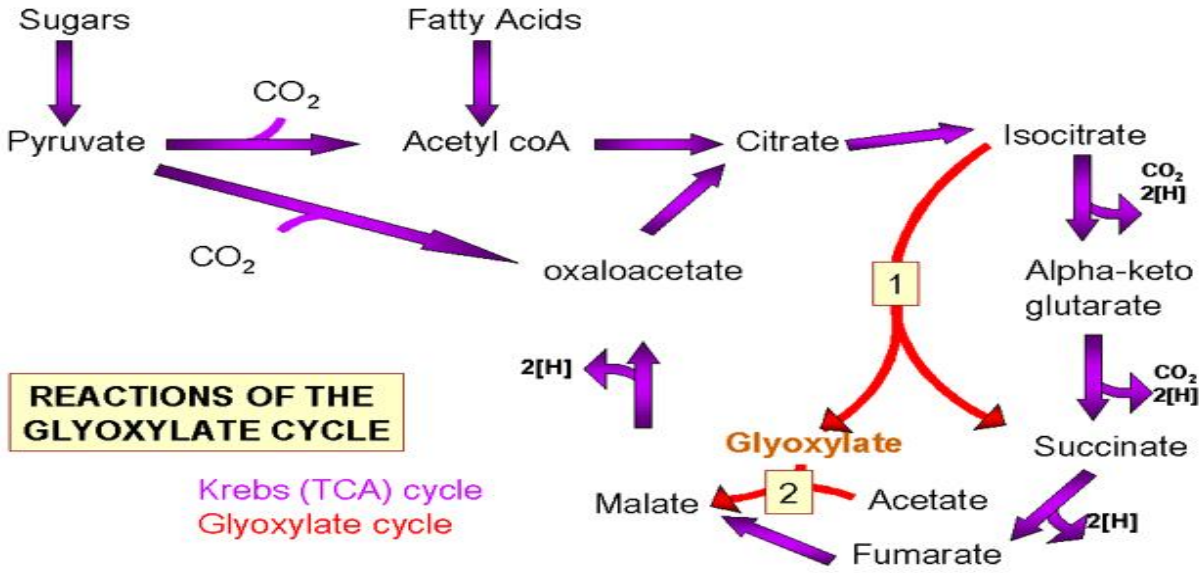


FIGURE 1.4.2 METABOLISM OF FATTY ACIDS

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Thus for every acetyl group (from fatty acids), an additional cycle intermediate can be produced. The glyoxylate pathway is not generally found in animal cells since pre-formed fatty acids, present in the food, are utilized.

In summary, the Krebs Cycle functions in a biosynthetic and energy producing fashion. However, if intermediates are to be removed for use in other metabolic pathways, they must be replenished. The replenishment process for utilization of sugars and fatty acids is different.

3.3 **Metabolism of sugars**

METABOLISM OF SUGARS (as an example of metabolic pathways)

Glycolysis(Emden, Meyerhof and Parnas [EMP] Pathway)

This is the most common pathway in bacteria for sugar catabolism (It is also found in most animal and plant cells). A series of enzymatic processes result in conversion of sugars into pyruvate, generating ATP (adenosine triphosphate) and NADH (nicotinamide adenine dinucleotide). Chemical energy needed for biosynthetic purposes is stored in the newly formed compounds (ATP and NADH)

There are alternatives to this pathway for catabolizing sugars in order to produce stored energy within ATP. These include the pentose phosphate pathway (hexose monophosphate shunt) which is found in most animal and plant cells. NADPH is generated using this pathway. Another pathway, the Entner Doudoroff pathway, is generally only found in certain bacterial cells

4.0 **CONCLUSION**

Bacterial requirements for growth include sources of energy, "organic" carbon (e.g. sugars and fatty acids) and metal ions (e.g. iron). Optimal temperature, pH and the need (or lack of need for oxygen) are important. Anaerobic respiration includes glycolysis and fermentation; while aerobic respiration utilizes oxygen through the Krebs cycle we earlier described above. Important biochemical intermediates generate several ATP molecules. Other important metabolic pathways include the pentose phosphate pathway (hexose monophosphate shunt) which is found in most animal and plant cells as well. NADPH is generated using this pathway. Another pathway, the Entner Doudoroff pathway, is generally only found in certain bacterial cells.

5.0 SUMMARY

- In summary, the Krebs Cycle functions in a biosynthetic and energy producing fashion.
- However, if intermediates are to be removed for use in other metabolic pathways, they must be replenished.
- The replenishment process for utilization of sugars and fatty acids is different

6.0 TUTOR-MARKED ASSIGNMENT

1. List bacteria requirements for growth
2. Briefly explain all the requirements you have listed
3. How would you measure bacteria mass in liquid cultures ?
4. What is glycolysis?
5. Between glycolysis and Krebs cycle which has higher efficiency in terms of energy production and utilization?
6. Fully describe the Tricarboxylic Acid cycle or the Krebs cycle.

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UNIT 5.0: BACTERIAL CELL ENVELOPE, SPORES AND MACROMOLECULAR BIOSYNTHESIS

CONTENTS

1.0 Introduction

In this unit, the structure of the Gram negative, Gram positive and acid fast cell envelopes will be discussed. The composition and function of unique cell envelope macromolecules and their biosynthesis will be described. In addition, endospores, which are unusual in many ways (including cell envelope structure), would be discussed.

2.0 OBJECTIVE

At the end of this Unit, you will be able to

- To describe the mode of action of antibacterial chemotherapeutic agents.
- To discuss antibiotic susceptibility testing.
- To review the mechanisms by which bacteria express resistance to Antibiotics.
- Confidently describe the bacterial Cell Envelope with special emphasis on-

a. Gram Positive Cell Envelope.

b. Gram negative cell envelope.

- Discuss Acid fast and related bacteria (*Mycobacteria, Nocardia and Corynebacteria*).
- Explain in clear terms the Synthesis of cell envelope of macromolecules.

3.0 Contents

3.1 Cell Envelope

The cell envelope may be defined as the cell membrane and cell wall plus an outer membrane if one is present. The cell wall consists of the peptidoglycan layer and attached structures. Most bacterial cell envelopes fall into two major categories (Figure 1.5.1): Gram positive and Gram negative.

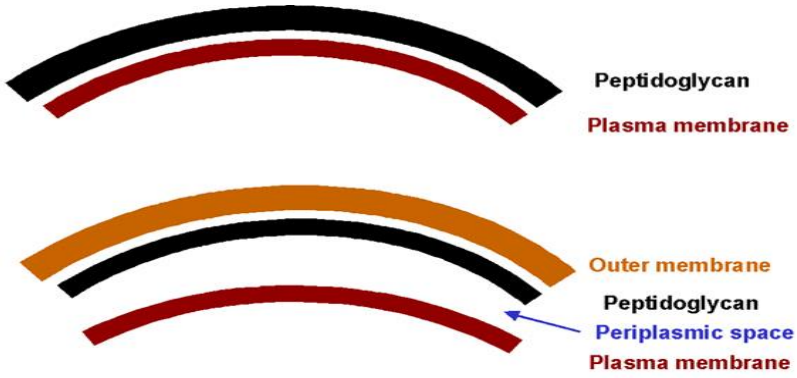

FIGURE 1.5.1--1.5.6 DIAGNOSTIC MICROBIOLOGY 12TH EDITION.

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This is based on Gram staining characteristics that reflect major structural differences between the two groups. Other types of cell wall are found in a few bacterial species (neither Gram positive nor Gram negative).

The peptidoglycan is a single bag-shaped, highly cross-linked macromolecule that surrounds the bacterial cell membrane and provides rigidity. It is huge (billions in molecular weight; compare proteins which are thousands in molecular weight). Peptidoglycan consists of a glycan (polysaccharide) backbone consisting of N-acetyl muramic acid and N-acetyl glucosamine with peptide side chains containing D- and L- amino acids and in some instances diaminopimelic acid. The side chains are cross-linked by peptide bridges. These peptide bridges vary in structure among bacterial species. Muramic acid, D-amino acids and diaminopimelic acid are not synthesized by mammals. PG is found in all eubacteria except *Chlamydia* and *Mycoplasma*.

3.2 Gram Positive Cell Envelope (Figure 2.1.2)

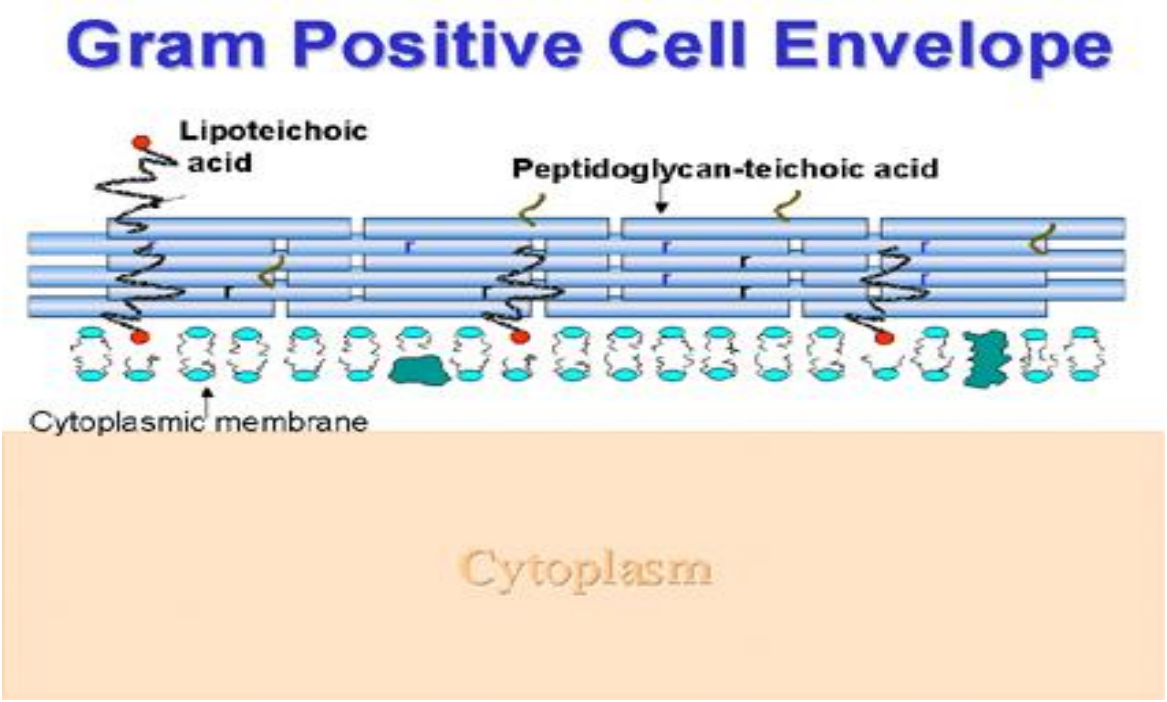

FIGURE 1.5.GRAM POSITIVE CELL ENVELOP

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Covalently bound to the thick peptidoglycan are teichoic acid (their backbones are usually phosphorus- containing polymers of ribitol or glycerol) or teichuronic acid (glucuronic acid- containing polysaccharides). These negatively charged molecules are believed to be involved in concentrating metal ions from the surroundings. Teichoic acids can also direct autolytic enzymes to sites of peptidoglycan digestion (autolysis), one of the steps in cell wall biosynthesis. In some instances, neutral polysaccharides are present. Lipoteichoic acid, in many bacteria, is generally associated with the cell membrane. In other instances, it occurs in the fimbriae on the outside of the cell. When expressed on the cell exterior it can be involved in adhesion to epithelial cells allowing colonization of the throat (e.g. by the group A streptococcus).

3.3 THE GRAM NEGATIVE CELL ENVELOPE (FIGURE 1.5.3)

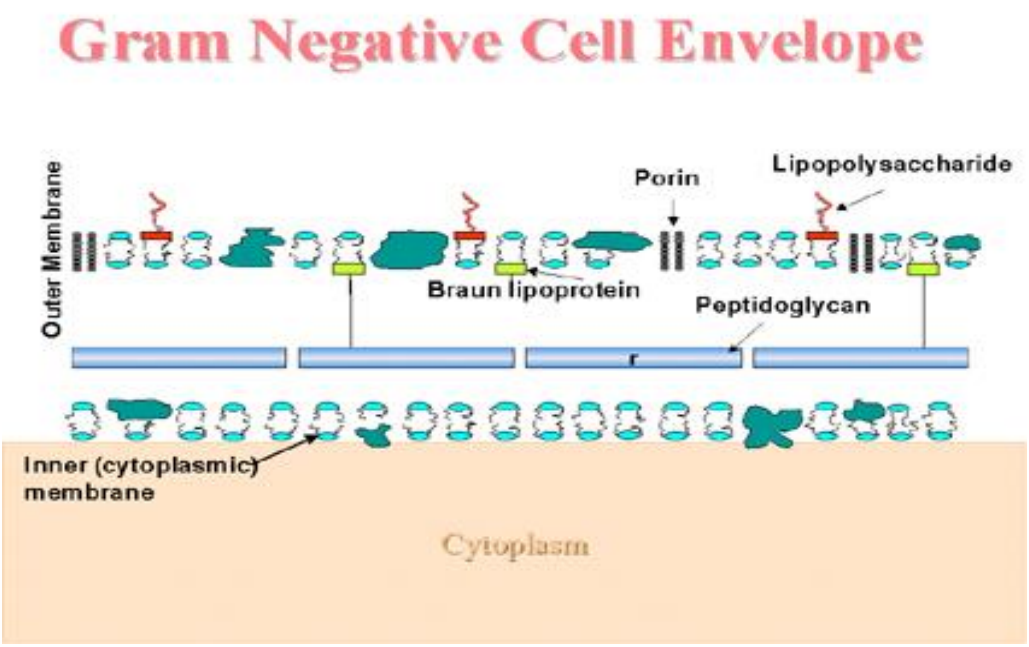

FIGURE 1.5.3; GRAM NEGATIVE CELL ENVELOP

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Covalently linked to the thin peptidoglycan is the Braun lipoprotein which binds the outer membrane to the cell wall. Like other membranes, the outer membrane contains proteins and phospholipids. Unlike other membranes, it contains additional molecules (lipopolysaccharide). The lipopolysaccharide is important to the bacterial cell since it helps to provide a permeability barrier to hydrophobic substances. The lipopolysaccharide consists of three regions: an outer O antigen, a middle core and an inner lipid A region. The core contains several sugars (heptoses and ketodeoxyoctonic acid), not found elsewhere in nature**, and lipid A contains β hydroxyfatty** acids (uncommon in nature). The molecule displays endotoxin activity. Porins in the outer membrane help form channels to allow passage of small hydrophilic nutrients (such as sugars) through the outer membrane.

Acid fast and related bacteria (*mycobacteria, nocardia and corynebacteria*)

The cell envelopes of these organisms are considerably more complex than other bacteria. Mycolic acid (long, branch chained fatty acids) covalently bound via a polysaccharide to

peptidoglycan. Other mycolic acid-containing compounds and other complex lipids form a thick waxy membranous layer outside the peptidoglycan layer.

3.4 SYNTHESIS OF CELL ENVELOPE MACROMOLECULES

Peptidoglycan (Figures 1.5.4 and 1.5.5):

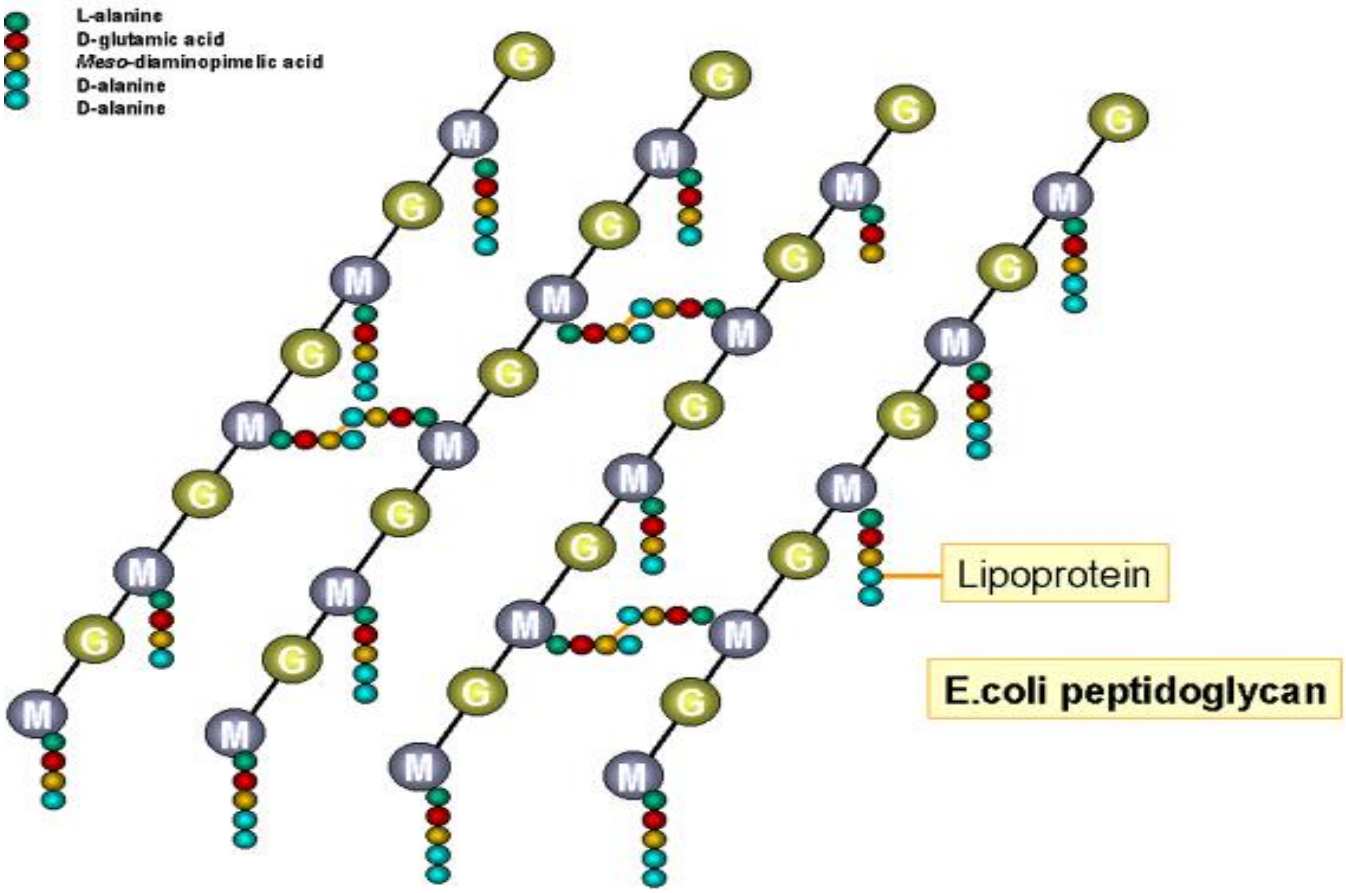

FIGURE 2.1.4 E.COLI PEPTIDOGLYCAN

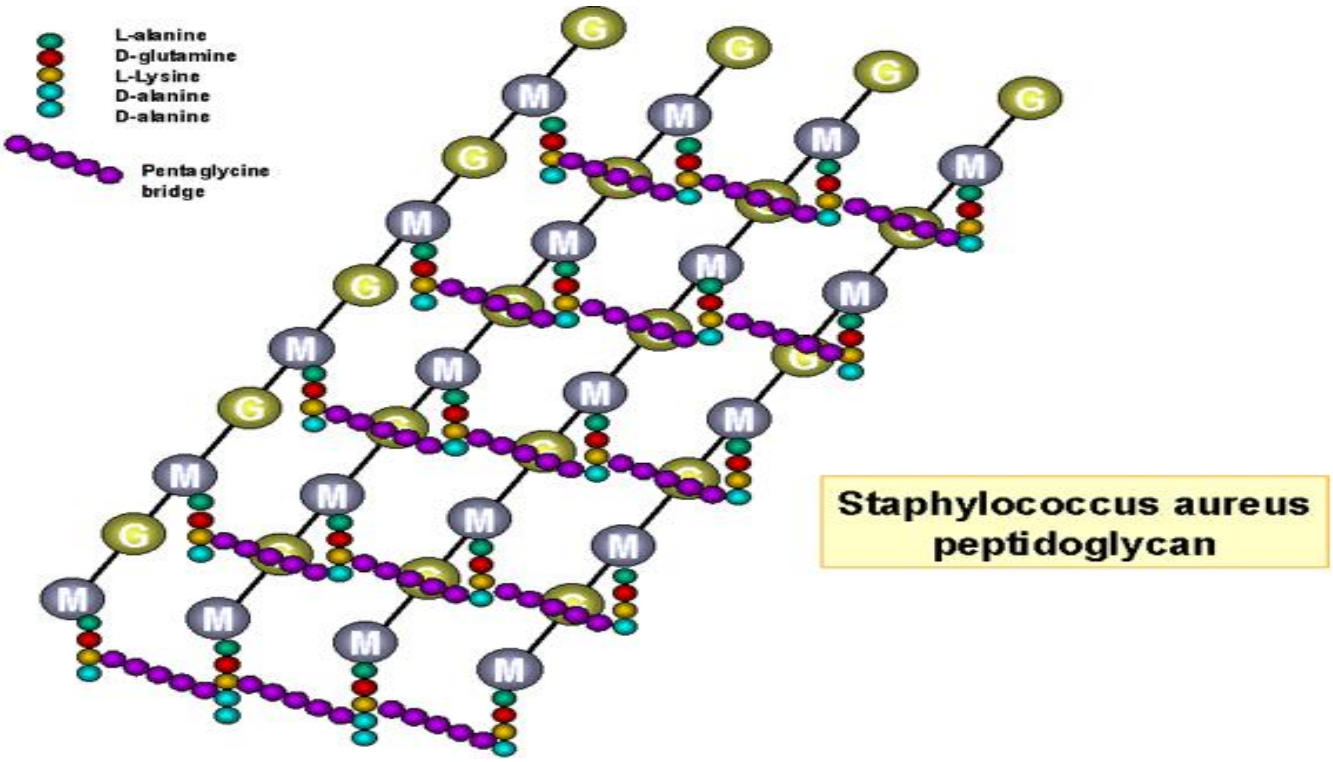

FIGURE 1.5.5 ; STAPHYLOCOCCUS AUREUS PEPTIDOGLYCAN

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The precursor subunit (muramyl pentapeptide attached to uridine diphosphate, UDP) is synthesized in the cytoplasm and passed to the cell membrane. The subunit is moved enzymatically from the nucleotide to a lipid carrier (undecaprenol/bactoprenol) and built into a completed subunit (disaccharide pentapeptide with attached bridge peptide). The completed subunits are then exported to the cell wall. After release of the monomer the undecaprenol is recirculated in the cell membrane and used again. The glycan backbones of the existing cell wall are enzymatically broken (by autolysins) to allow insertion of the newly synthesized subunit. If these enzymes are overactive, the cell wall becomes degraded and the high osmotic pressure of the cell bursts the cytoplasmic membrane killing the cell ("autolysis"). Cross-linking of the peptide side-chain of the inserted subunit to the existing chain then occurs enzymatically (penicillin binding proteins). Completed subunits of teichoic and teichuronic acids are also synthesized in the cell membrane (on lipid carriers) before transport and insertion into the existing cell wall.

Lipopolysaccharide

Lipid A is assembled in the cell membrane and the core sugars attached sequentially. O-antigen subunits are independently synthesized (on a lipid carrier as in peptidoglycan synthesis). The fully synthesized O-antigen is then attached to the lipid A-core (generating lipopolysaccharide) in the cell membrane before passage/insertion into the outer membrane (Figure 1.5.6).

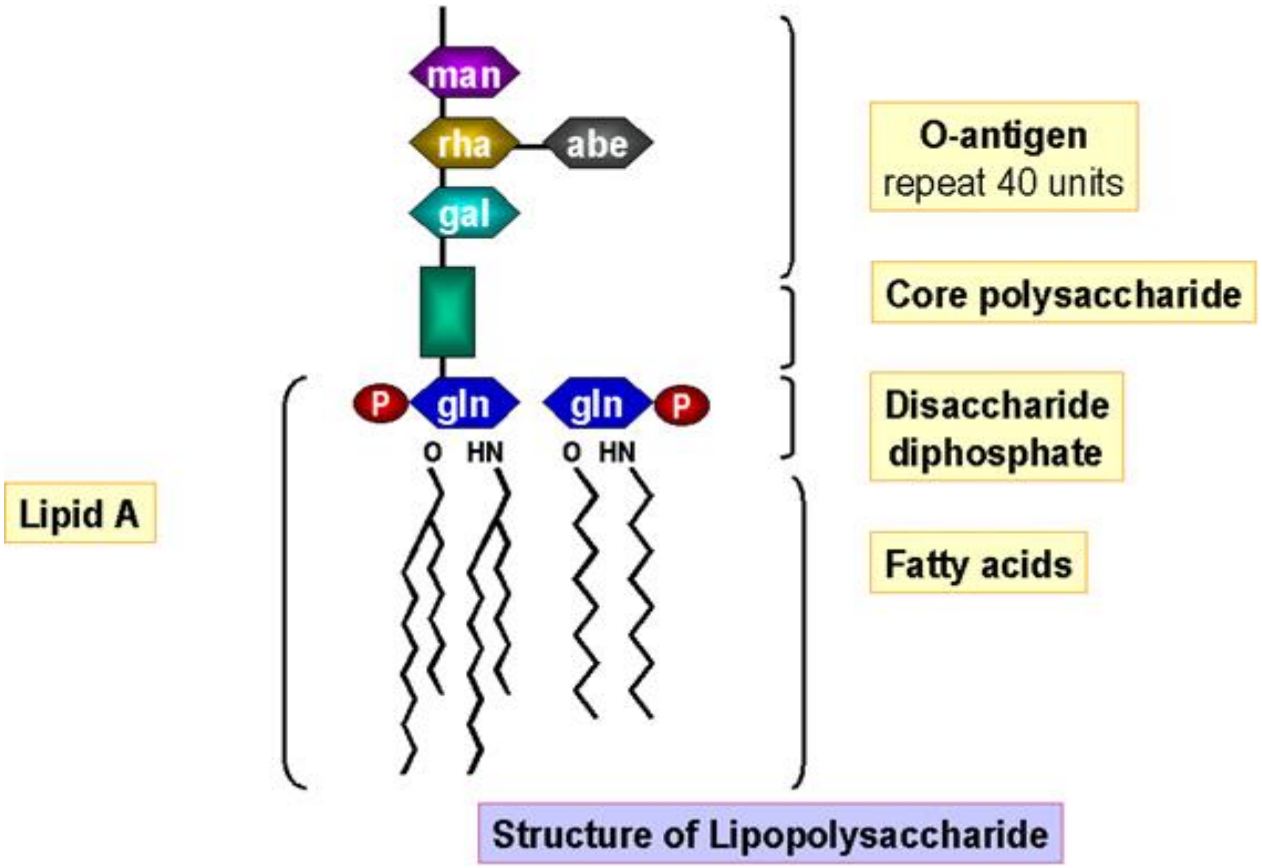

FIGURE 1.5.1--1.5.6 COURTESY; STRUCTURE OF LIPOPOLYSACCHARIDE

DIAGNOSTIC MICROBIOLOGY 12TH EDITION

Endospores: These modified Gram positive bacterial cells have an unusual cell envelope that contains a cell membrane and an outer membrane. The peptidoglycan layer is less cross-linked than in most bacterial cells and contains a dehydrated form

of muramic acid. The spore peptidoglycan is referred to as a cortex and is found between the two membranes. A coat consisting of highly cross-linked keratin is found around the outside of the cell. The bacterial spore is highly resistant to chemical agents because of this coat. Normally in bacterial replication, as cells divide, a septum forms dividing the mother cell into two roughly equally sized daughters. When sporulation occurs, cell division is unequal and the larger so-called "mother cell" envelops the daughter cell. The cell membrane of the daughter cell constitutes the inner membrane of the spore and the cell membrane of the mother forms the outer membrane (Figure 1.5.7 and 1.5.8).

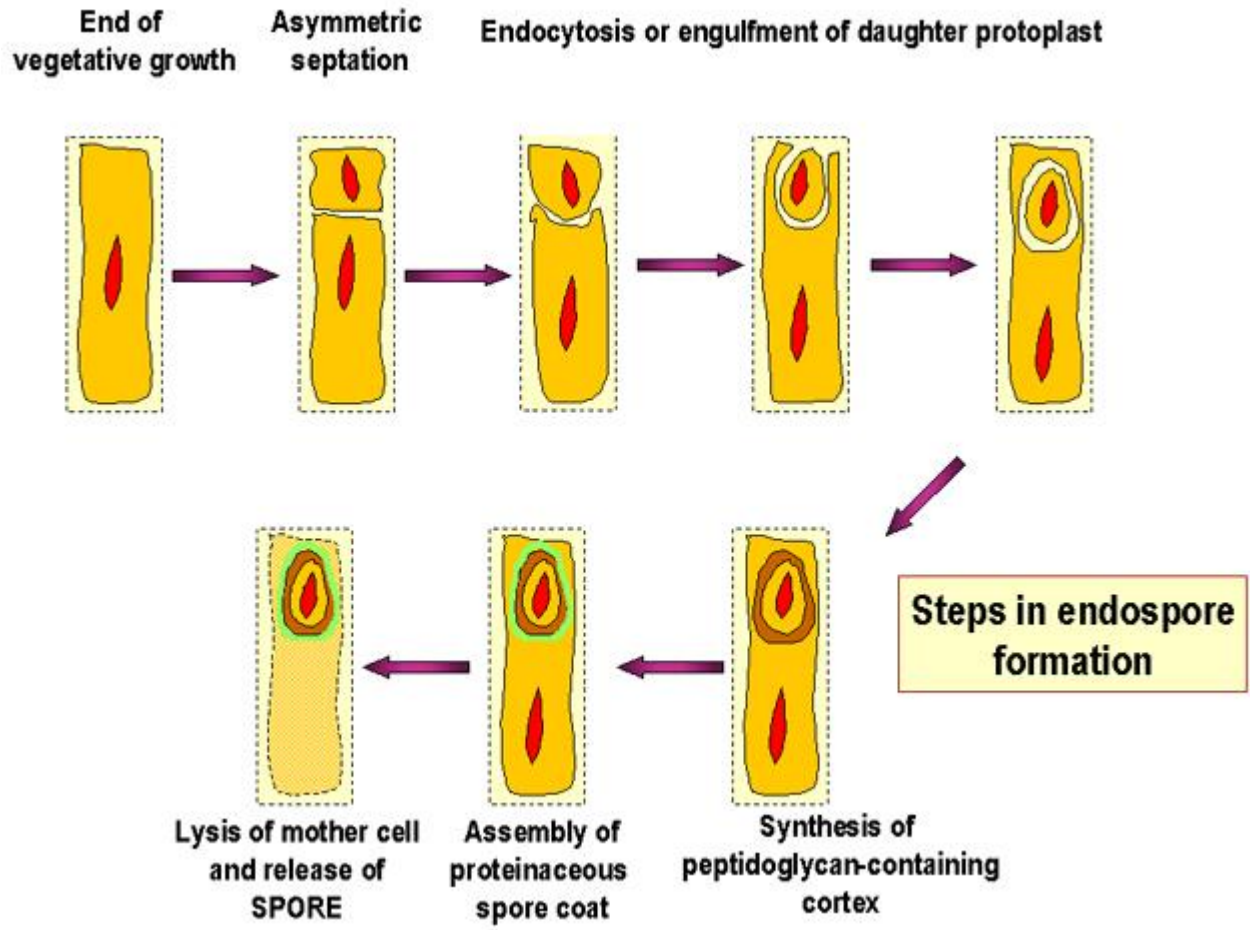

FIGURE 1.5.7

COURTESY; DIAGNOSTIC MICROBIOLOGY 12TH EDITION

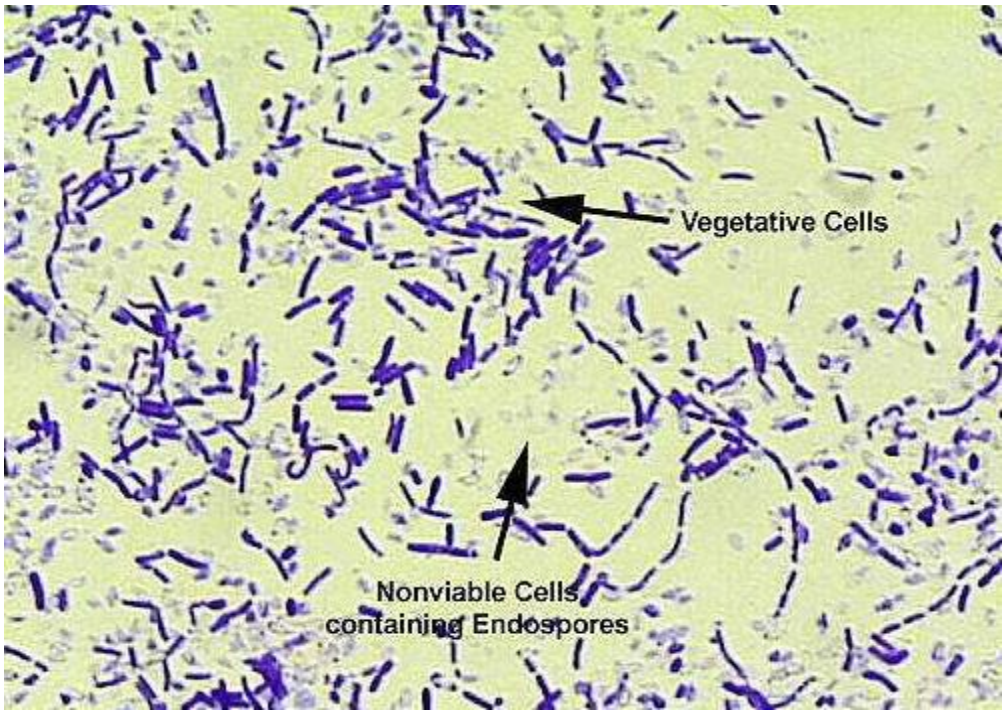

FIGURE 1.5.8A

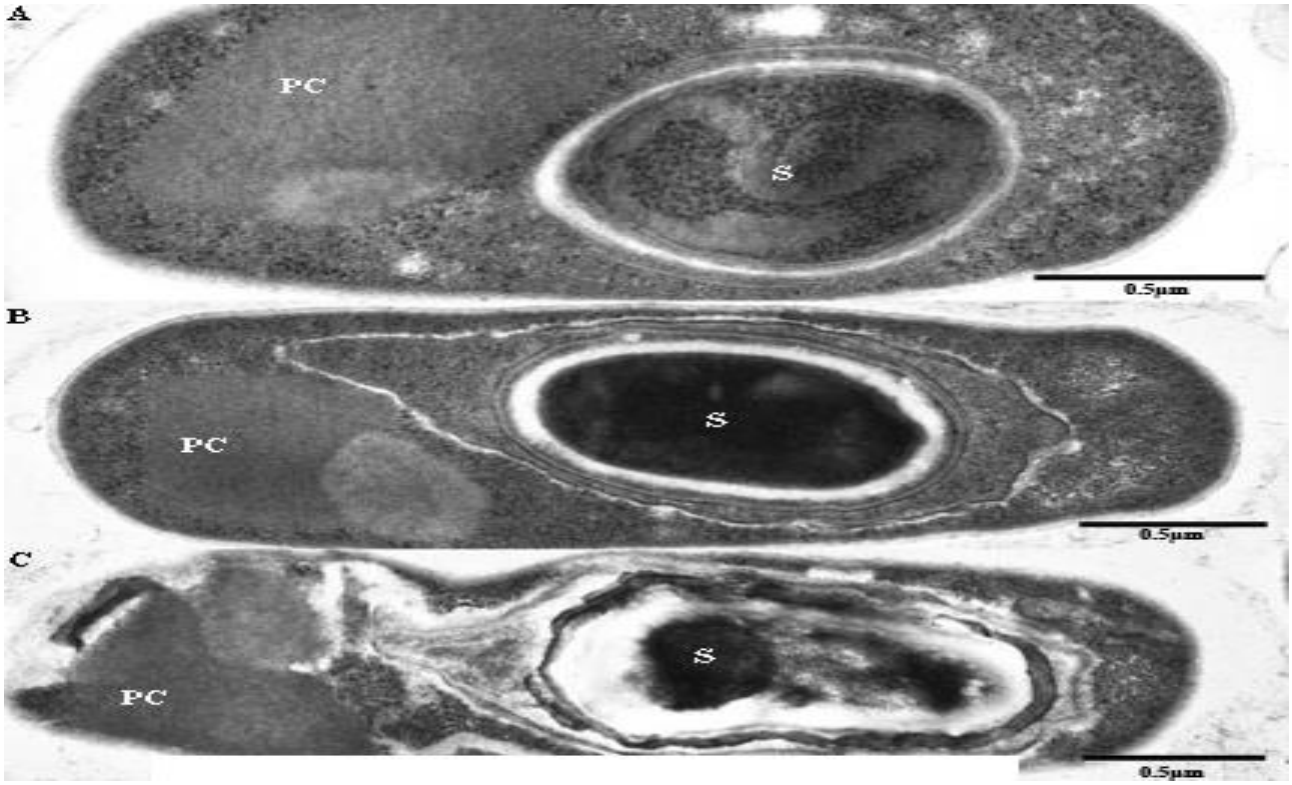

FIGURE 1.5.8B

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4.0 Conclusion

In this section, we have learnt about the structure of the Gram negative, Gram positive and acid fast cell envelopes of bacteria. Most of the principles of Gram reaction, antibiotics activity, bacteria susceptibility to attacks, and other vital events reside in the composition and function of unique cell envelope macromolecules and their biosynthesis. In addition, endospores, which are unusual in many ways (including cell envelope structure), had been explained.

5.0 Summary

Viewed in this perspective, the following are of prime importance in bacteria metabolism;

- the bacterial Cell Envelope.
- Gram Positive Cell Envelope.
- Gram negative cell envelope.
- Acid fast and related bacteria (mycobacteria, nocardia and
- Corynebacteria).
- Synthesis of cell envelope macromolecules

6.0 Tutor-Marked Assignment

Describe the cell envelope of Gram positive and Gram Negative bacteria.
What are the key differences between these and Acid fast bacteria Cell envelope?
How are cell envelope macromolecules synthesized?

7.0 References/Further Reading

Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby.
Murray P.R. *et al*, editor: *Medical Microbiology,* ed.5 St. Louis 2005,
Textbook of Microbiology by R. Vasanthakumari
Todar's Online textbook of Bacteriology by Kenneth Todar, PhD 2014 edition

UNIT 6: EFFECTS OF ANTIBIOTICS ON BACTERIA CELL ENVELOPE;

CONTENTS

1.0 INTRODUCTION: EFFECT OF ANTIBIOTICS ON BACTERIA CELL ENVELOPE;

Sterilization as we saw earlier refers to killing (or removal) of **ALL** bacteria in a non-selective fashion. For example, autoclaving involves heating liquids (e.g. media) or solids to 121^{o}C under steam pressure. The materials must be heat resistant. Ethylene oxide is sometimes used in hospitals for equipment that cannot be heated. Membrane filters have pores that trap bacteria, but allow drugs and small chemicals to pass through; thus pre-sterilized filters can be used to sterilize delicate solutions. UV light is used for decreasing bacterial levels on surfaces such as in operating rooms; however, it is not totally effective. Ionizing radiation is more efficient and can be used for sterilizing instruments and food.

Disinfectants (e.g. phenol-based) can be useful in killing many bacteria on certain instruments, but cannot be used for internal consumption or on skin. Antiseptics (e.g. iodine or 70% alcohol) are used topically (e.g. on skin surfaces) to reduce bacterial load.

ANTIBIOTICS

In contrast, antibiotics are agents that are "selectively" toxic for bacteria (either killing them [bactericidal] or inhibiting their growth [bacteriostatic]) without harm to the patient. They can thus be ingested. By definition, these compounds must act on structures found in bacteria, but not in the host. Antibiotics work most efficiently in conjunction with an active immune system to kill infecting bacteria in the host. After isolation of pure colonies, the susceptibility of bacterial isolates can be tested to a variety of antibiotics. The minimal inhibitory concentration (MIC) refers to the lowest concentration of an antibiotic that stops visible growth. More simply, the zone of inhibition around a disk impregnated with antibiotic (Kirby-Bauer) is another measure of antibiotic activity.

INHIBITORS OF CELL WALL SYNTHESIS

One major class of antibiotics inhibits the synthesis of peptidoglycan (figure 1.6.1).

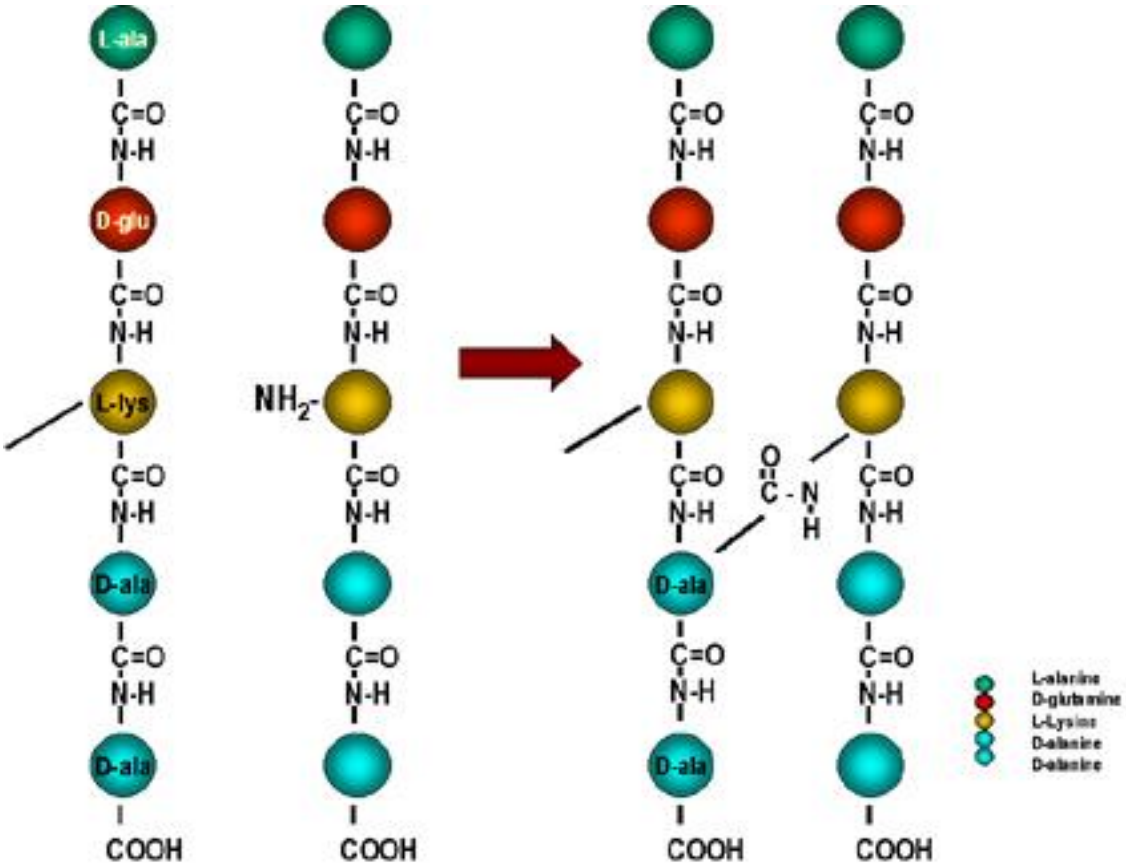

Figure 1.6.1 **INHIBITORS OF CELL WALL SYNTHESIS**

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Once cell wall synthesis (involving penicillin binding proteins) is inhibited, enzymatic autolysis of the cell wall can occur. Without the restraining influence of the cell wall the high osmotic pressure inside the cell bursts the inner and/or outer membranes of bacteria. Thus, these antibiotics are generally bactericidal. Several mechanisms are involved in inhibition of peptidoglycan synthesis:

(1) The terminal two amino acids of a peptide side chain of peptidoglycan are unusual amino acids (D-alanine as opposed to its isomer L-alanine). The antibiotic cycloserine is an analog of D-alanine and interferes with enzymatic conversion of L-alanine to D-alanine in the cytoplasm. Thus, subsequent synthesis of peptidoglycan cannot occur.

(2) The peptidoglycan subunit (containing one side-chain and an attached peptide to be used in cross-bridge formation) is passed across the cytoplasmic membrane attached to undecaprenol diphosphate. After the nascent peptidoglycan monomer leaves the carrier on reaching the cell wall, the undecaprenol diphosphate is dephosphorylated to its monophosphate form. Bacitracin inhibits the dephosphorylation

reaction and in the absence of monophosphorylated carrier peptidoglycan subunit synthesis stops.

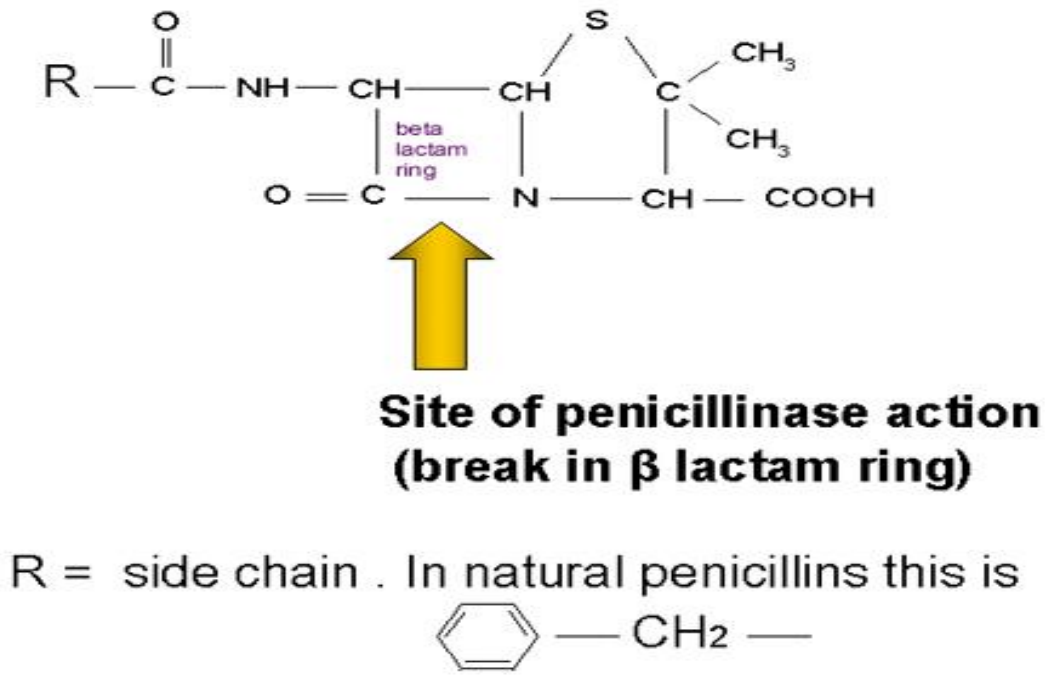
(3) The final step in peptidoglycan synthesis involves linking the sugar portion of the peptidoglycan subunit to the glycan backbone of the existing cell wall polymer. Cross-linking of the peptide portion of the subunit to a peptide in the cell wall then occurs. During this process D-alanine is enzymatically excised from the end of a pre-existing peptide side chain allowing it to be cross-linked (by a new peptide bond) to the recently synthesized peptidoglycan subunit. Vancomycin binds to D-Alanine-D-alanine thus sterically inhibits transpeptidation (cross-linking).

(4) The beta lactam antibiotics include penicillins (e.g. ampicillin), Cephalosporins and monobactams. They bind to and inhibit enzymes (penicillin binding proteins) involved in the transpeptidation (cross-linking) of peptidoglycan. These antibiotics have in common the four membered lactam ring. Attached to the lactam, penicillins have an additional five membered ring and Cephalosporins a six membered ring. Monobactams consist of the lactam ring alone and display antibiotic activity

3.1 THE MODE OF ACTION OF BETA-LACTAM ANTIBIOTICS;

PENICILLIN

Penicillin is made by the mould *Penicillium chrysogenum*. During fermentation the mould forms 6-aminopenicillanic acid which has a thiazolidine ring and a beta-lactam ring fused together (figure 1.6.2).



R = side chain . In natural penicillins this is

FIGURE 1.6.2 PENICILLIN

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This, however, is acid labile and subject to degradation by bacterial enzymes. More stable derivatives are made biochemically so that in addition to increased stability, they are better absorbed from the gastro-intestinal tract and have a wider spectrum of bactericidal effects.

Various chemical side chains have been synthetically linked to the ring structures producing a host of antibiotics with different properties in the host. Many penicillins display little activity against Gram negative bacteria, since they do not penetrate the outer membrane. Cephalosporins and other newer penicillins are active against Gram negative bacteria, since they can penetrate the outer membrane. Other chemically modified penicillins have lower elimination rates from the patient; decreasing the frequency of administration of these drugs.
Penicillins can be destroyed by beta lactamase (penicillinase) produced by resistant bacterial strains (figure 1.6.3).

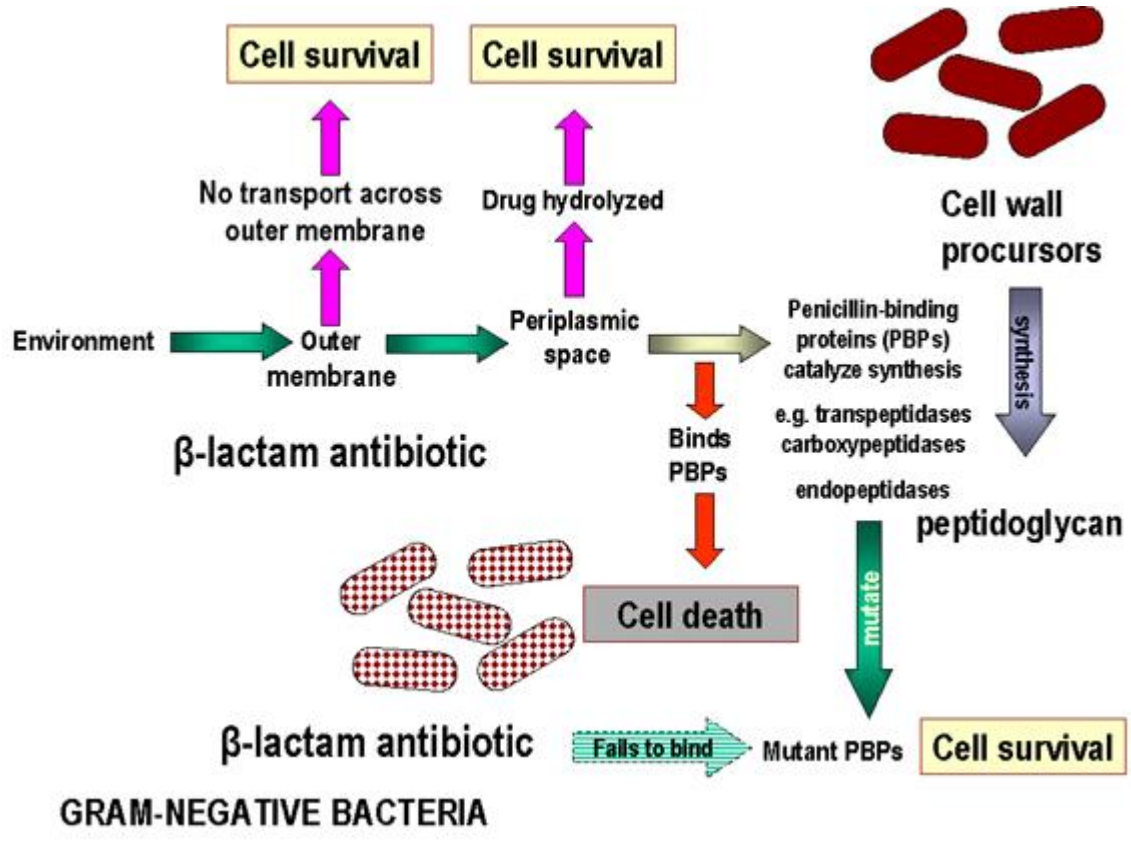

FIGURE 1.6.3 GRAM NEGATIVE BACTERIA

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Clavulaninic acid also has a beta lactam component which binds strongly to beta lactamases inhibiting their activity. It is used in conjunction with certain penicillins allowing their use against otherwise resistant bacteria. Another form of resistance involves a change in the structure of penicillin binding proteins such that the antibiotic does not bind efficiently (figure 1.6.4).

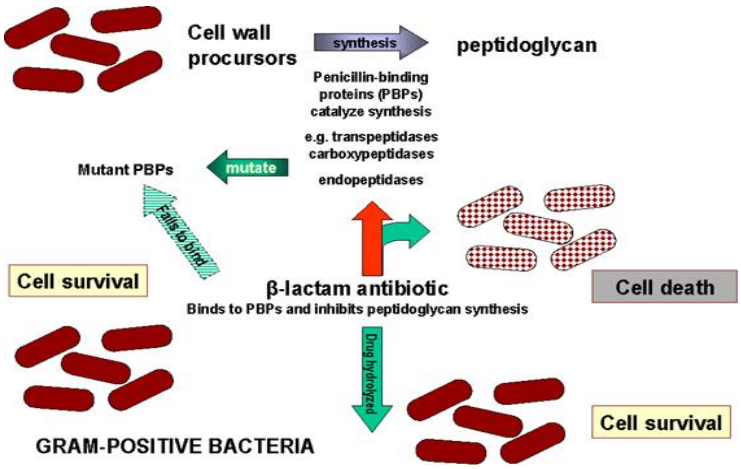

FIGURE 1.6.4 GRAM POSITIVE BACTERTIA

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In the case of Gram negative bacteria, penicillins pass across the outer membrane

using porins. Resistance may develop from mutation leading to modified porins.

POLYMYXIN B

Polymyxin B (figure 1.6.5) binds to the lipid A portion of lipopolysaccharide and also to phospholipids.

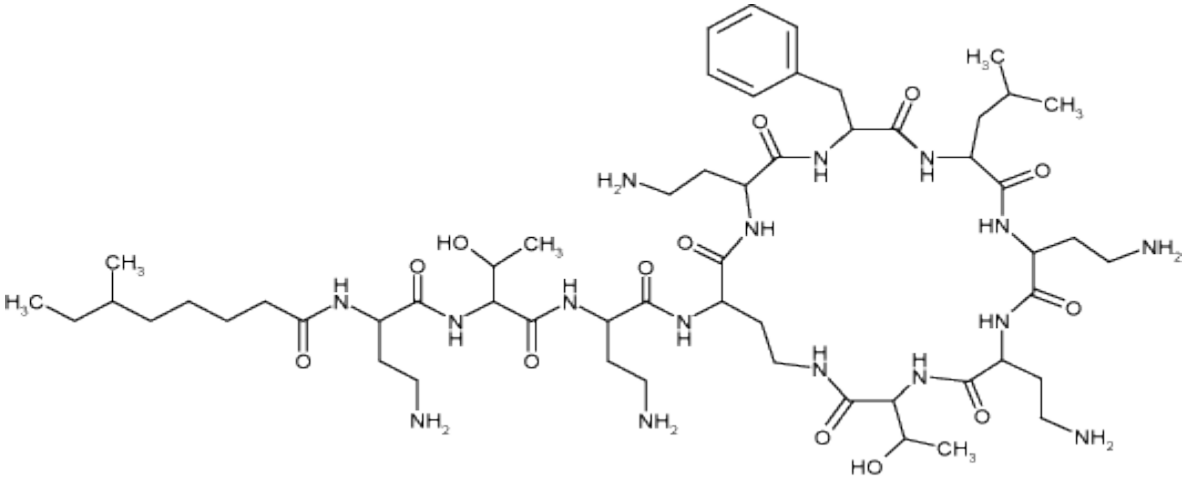

FIGURE 1.6.5 POLYMYXIN B

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However, it binds preferentially to lipid A. This disrupts the outer membrane of Gram negative bacteria. Since the cell membrane is not exposed in Gram positive bacteria polymyxin has little
activity against them. This drug is toxic to human cells, since it can also lyze eukaryotic membranes; this explains its limited clinical use.

VANCOMYCIN

Vancomycin is a drug of last resort against Gram-positive bacteria. It is a glycopeptide (figure 1.6.5A) made by an Acinobacter species.

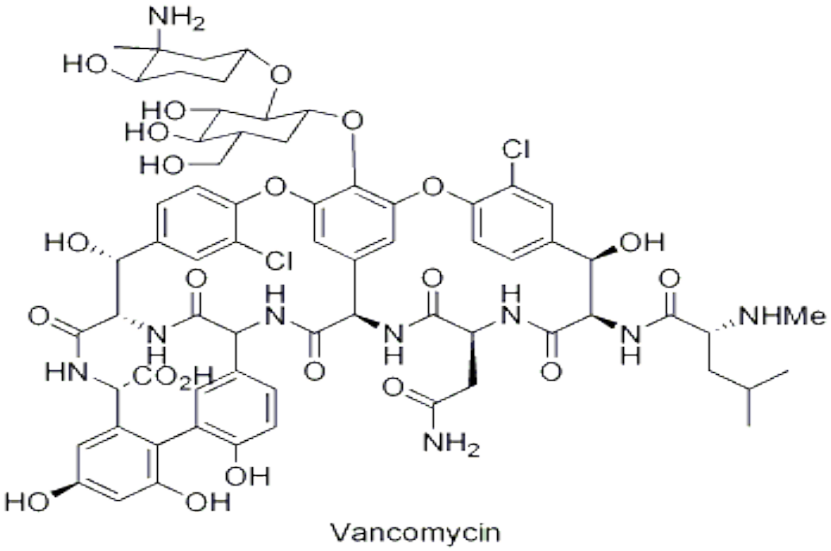

FIGURE 1.6.5A:VANCOMYCIN

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Vancomycin-resistance has arisen making this antibiotic less useful. It is very hydrophilic and forms hydrogen binds with terminal D-alanyl-D-alanine moieties of the NAM/NAG-subunits and stops polymerization of the subunits to form long chains. It also prevents polymer cross-linking.

Vancomycin use is often replaced by Daptomycin.

DAPTOMYCIN

Daptomycin (Cubicin - figure 1.6.6) is a natural lipopeptide that is used to treat multi-resistant Gram-positive bacterial infections.

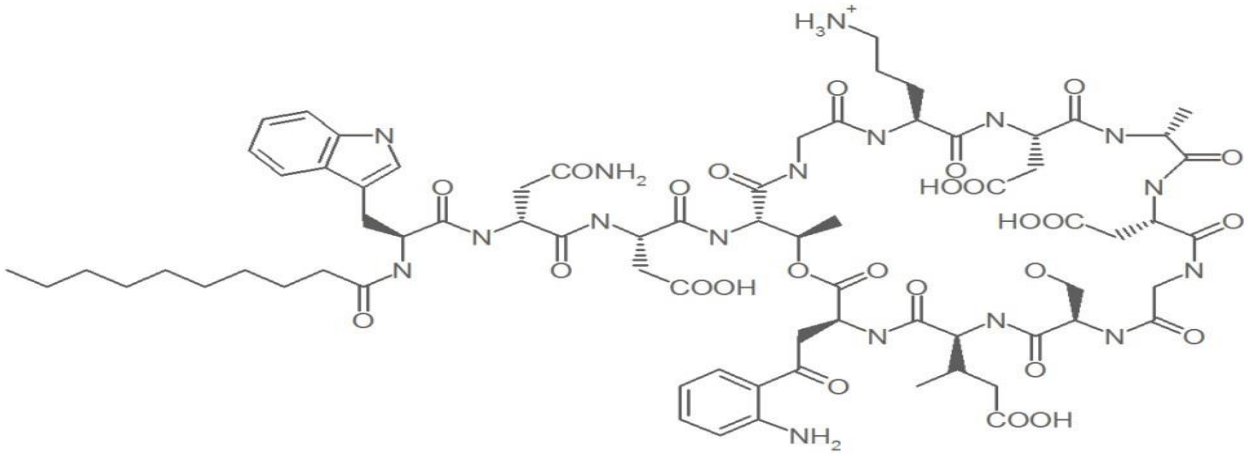

FIGURE 1.6.6:DAPTOMYCIN

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It is a natural product made by the soil fungus Streptomyces roseosporus. The lipid portion of the molecule binds to the cell membrane resulting in depolarization (loss of membrane potential). It can be used to treat:

- Enterococci (including glycopeptide-resistant Enterococci (GRE))
- Staphylococci (including methicillin-resistant Staphylococcus aureus)
- Streptococci
- Corynebacteria.

It is used in the United States against Gram-positive skin infections, Staphylococcus aureus bacteraemia and right-sided Staphylococcus aureus endocarditis. Daptomycin cannot be used to treat pneumonia because it binds to pulmonary surfactant. Daptomycin may cause life-threatening eosinophilic pneumonia in people over 60 years.

BACITRACIN

Bacitracin is a cyclic polypeptide produced by Bacillus subtilis var Tracy. It is used topically against Gram-positive bacterial eye and skin infections but is not used systemically. Bacitracin inhibits dephosphorylation of C55-isoprenyl pyrophosphate which transports peptidoglycan components bacterial cell walls outside the inner membrane

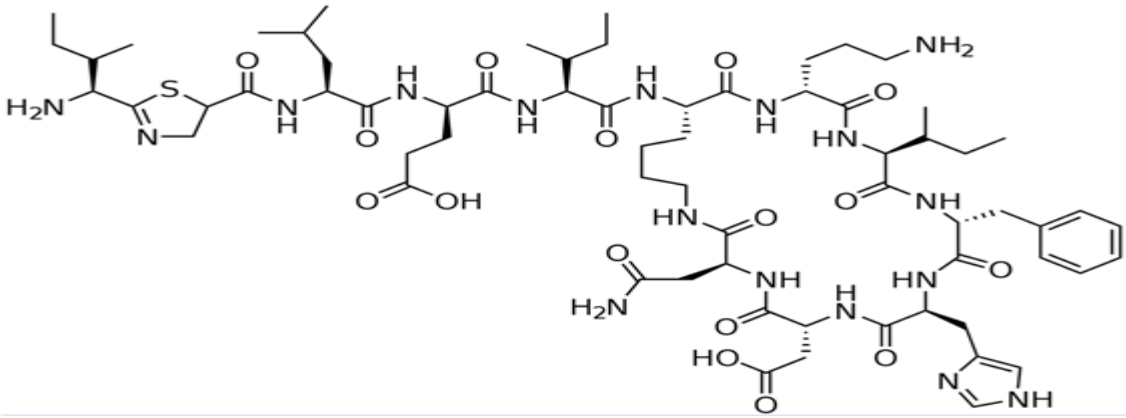
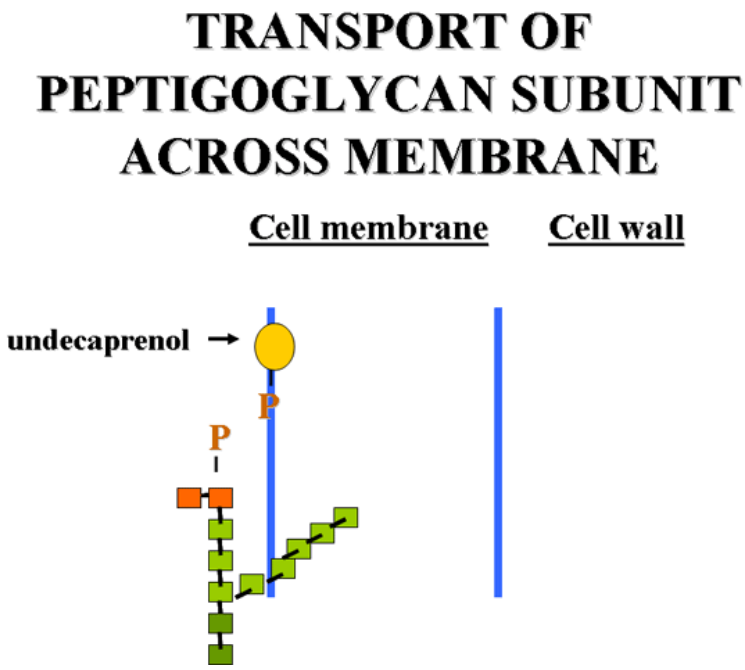


FIGURE 1.6.7

COURTESY OF PROFESSOR ALBERT LENINGER PRINCIPLES OF BIOCHEMISTRY

Transport of a peptidoglycan subunit across the cell membrane to the cell wall



Undecaprenol phosphate and the peptidoglycan subunit are phosphorylated

Figure 1.6.8A : Transport of peptidoglycan subunit across membrane

Dr Alvin Fox Emeritus Professor University of South Carolina School of Medicine

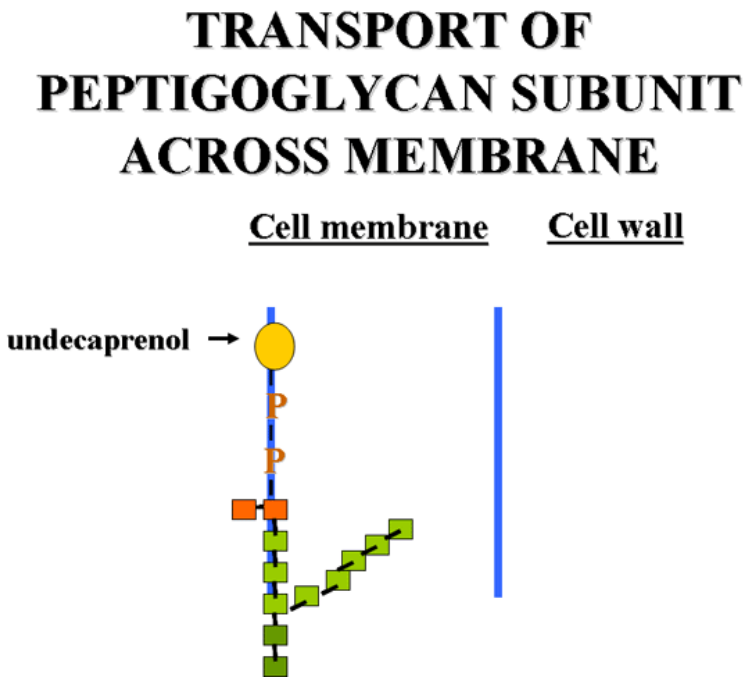
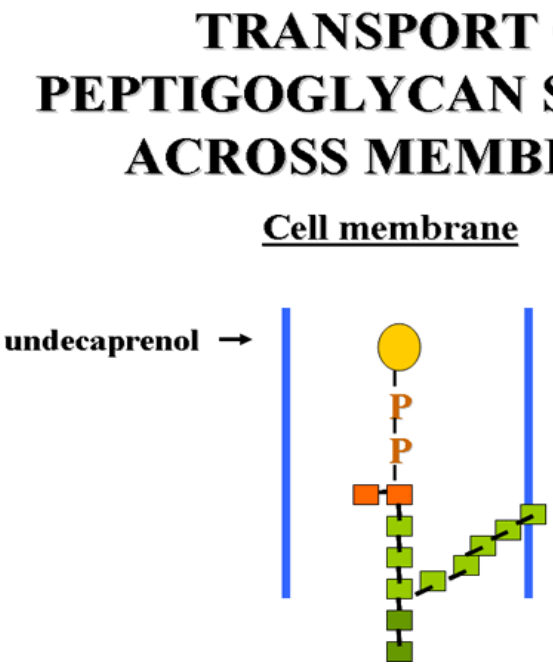


Figure 2.2.8B : Transport of peptidoglycan subunit across membrane

Dr Alvin Fox Emeritus Professor University of South Carolina School of Medicine

A pyrophosphate bond is formed between the undecaprenol and the subunit



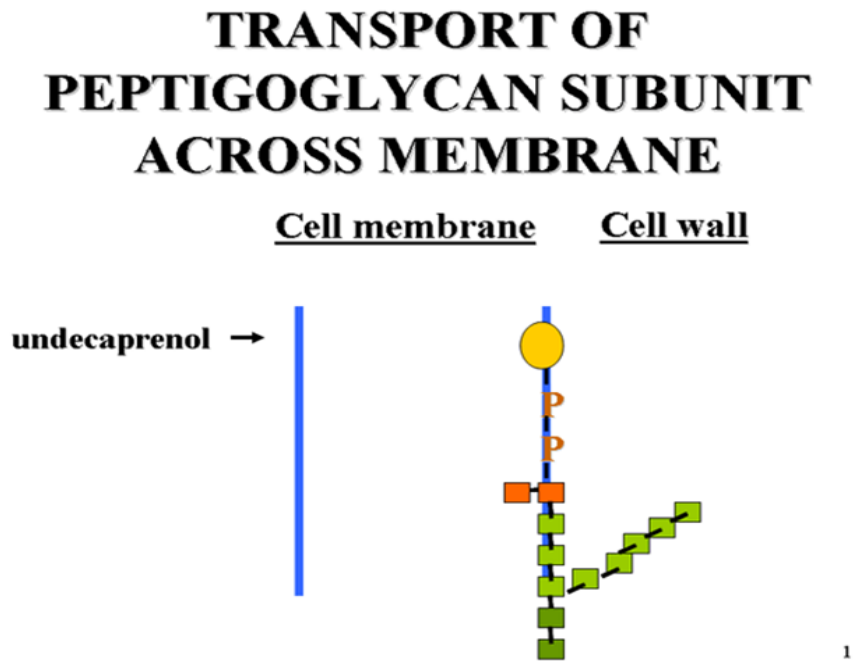


Figure 1.6.8C :Transport of Peptidoglycan subunit across membrane

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The subunit is transferred from the membrane to the cell wall

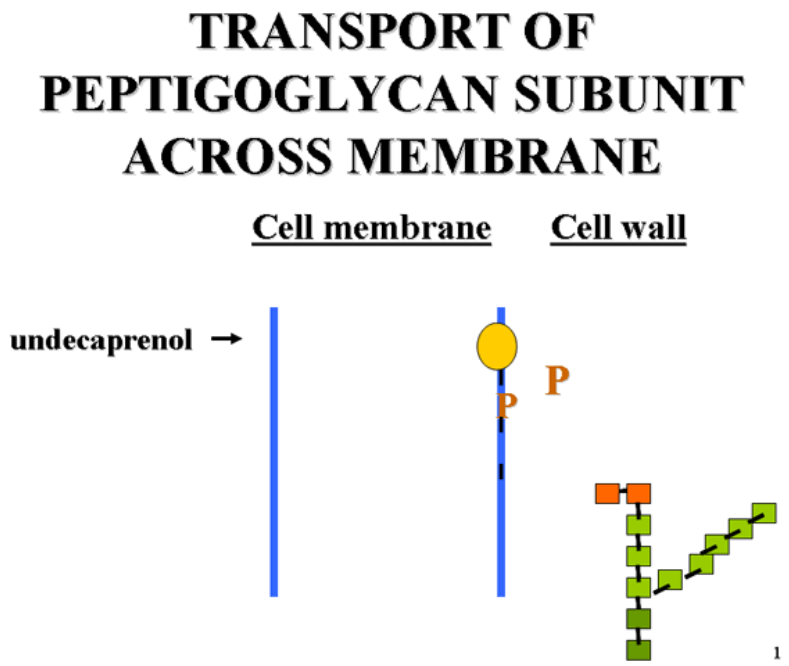

Figure 1.6.8D

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The subunit is released and the pyrophosphate cleaved in the absence of bacitracin

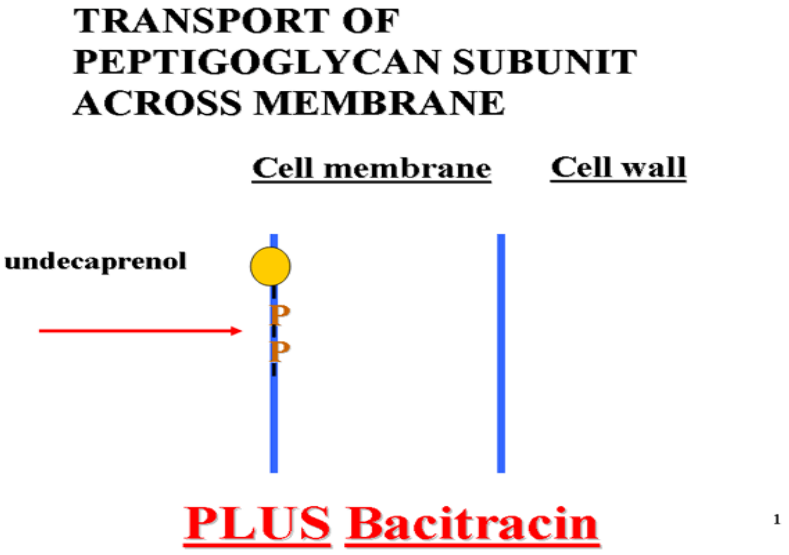


Figure 1.6.8E Courtesy Baileys And Scott

In the presence of bacitracin, the pyrophosphate is not cleaved

3.2 ANTIBIOTICS - PROTEIN SYNTHESIS, NUCLEIC ACID SYNTHESIS AND METABOLISM

Major Principles and Definitions

Selectivity

Clinically effective antimicrobial agents all exhibit selective toxicity toward the bacterium rather than the host. It is this characteristic that distinguishes antibiotics from disinfectants. The basis for selectivity will vary depending on the particular antibiotic. When selectivity is high the antibiotics are normally not toxic. However, even highly selective antibiotics can have side effects.

Therapeutic Index

The therapeutic index is defined as the ratio of the dose toxic to the host to the effective therapeutic dose. The higher the therapeutic index the better the antibiotic.

Categories of Antibiotics

Antibiotics are categorized as bactericidal if they kill the susceptible bacteria or bacteriostatic if they reversibly inhibit the growth of bacteria. In general the use of bactericidal antibiotics is preferred but many factors may dictate the use of a bacteriostatic antibiotic. When a bacteriostatic antibiotic is used the duration of therapy must be sufficient to allow cellular and humoral defence mechanisms to eradicate the bacteria. If possible, bactericidal antibiotics should be used to treat infections of the endocardium or the meninges. Host defences are relatively ineffective at these sites and the dangers imposed by such infections

For an antibiotic to be effective the MIC or MBC must be able to be achieved at the site of the infection. The pharmacological absorption and distribution of the antibiotic will influence the dose, route and frequency of administration of the antibiotic in order to achieve an effective dose at the site of infection.

In clinical laboratories, a more common test for antibiotic susceptibility is a disk

diffusion test.

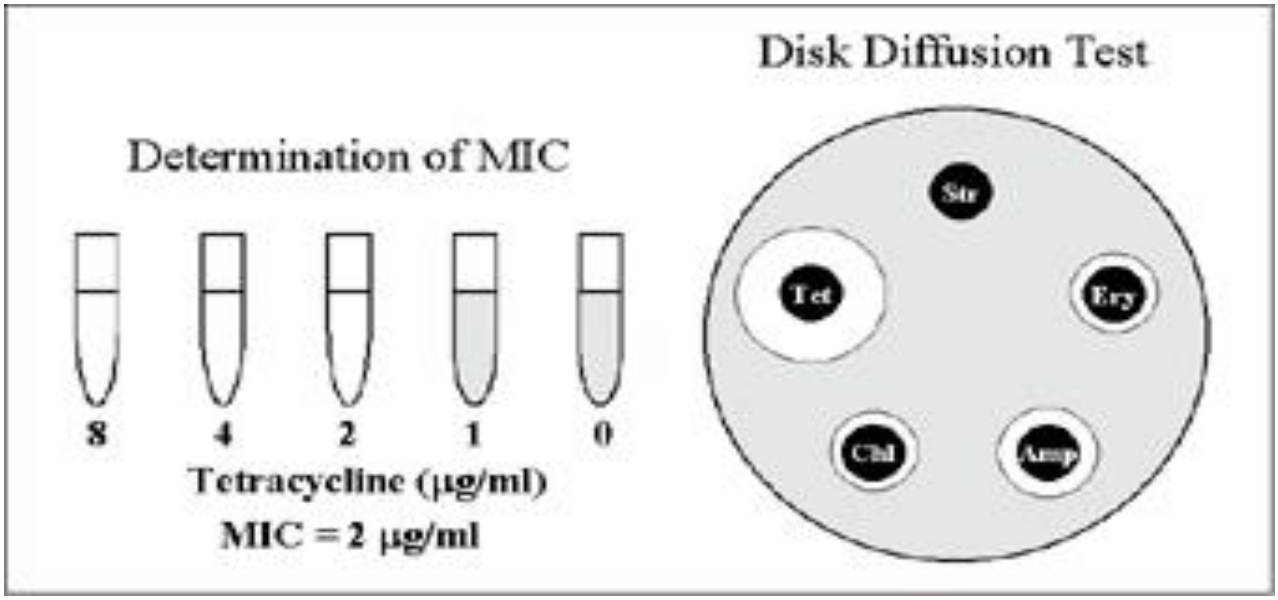


FIGURE 1.6.9: **Disk Diffusion Test**

DR ALVIN FOX EMERITUS PROFESSOR UNIVERSITY OF SOUTH CAROLINA SCHOOL OF MEDICINE

In this test the bacterial isolate is inoculated uniformly onto the surface of an agar plate. A filter disk impregnated with a standard amount of an antibiotic is applied to the surface of the plate and the antibiotic is allowed to diffuse into the adjacent medium. The result is a gradient of antibiotic surrounding the disk. Following incubation, a bacterial lawn appears on the plate. Zones of inhibition of bacterial growth may be present around the antibiotic disk. The size of the zone of inhibition is dependent on the diffusion rate of the antibiotic, the degree of sensitivity of the microorganism, and the growth rate of the bacterium. The zone of inhibition in the disk diffusion test is inversely related to the MIC.

The test is performed under standardized conditions and standard zones of inhibition have been established for each antibiotic. If the zone of inhibition is equal to or greater than the standard, the organism is considered to be sensitive to the antibiotic. If the zone of inhibition is less than the standard, the organism is considered to be resistant. Figure 1.6.9 also illustrates how the disk diffusion test is done and Figure 1.6.10 illustrates some of the standard zones of inhibition for several antibiotics.

Combination Therapy

Combination therapy with two or more antibiotics is used in special cases:

- To prevent the emergence of resistant strains
- To treat emergency cases during the period when an etiological diagnosis is still in progress
- To take advantage of antibiotic synergism.

Antibiotic synergism occurs when the effects of a combination of antibiotics is greater than the sum of the effects of the individual antibiotics. Antibiotic antagonism occurs when one antibiotic, usually the one with the least effect, interferes with the effects of another antibiotic.

3.3 Antibiotics and Chemotherapeutic agents

The term antibiotic strictly refers to substances that are of biological origin whereas the term chemotherapeutic agent refers to a synthetic chemical. The distinction between these terms has been blurred because many of our newer "antibiotics" are actually chemically modified biological products or even chemically synthesized biological products. The generic terms to refer to either antibiotics or chemotherapeutic agents are antimicrobic or antimicrobial agent. However, the term antibiotic is often used to refer to all types of antimicrobial agents.

Figure 1.6.10
Zone diameter interpretive standards and approximate MIC correlates used to define the interpretive categories

Antimicrobial agent (amount per disk) and organism	Zone diameter (nearest whole millimeter) for each interpretive category)				Approximate MIC correlates (micro gm/ml) for:	
	R	I	MS	S	R	S
Ampicillin (10 micro gm)						
Enterobacteriaceae	≤11	12-13		≥14	≥32	≤8

Staphylococcus spp.	≤28			≥29	beta-Lactamase	≤0.25
Haemophilus spp.	≤19			≥20	≥4	≤2
Enterococci	≤16		≥17		≥16	
Other streptococci	≤21		22-29	≥30	≥4	≤0.12
Chloramphenicol (30 micro gm)	≤12	13-17		≥18	≥25	≤12.5
Erythromycin (15 mcro gm)	≤13	14-17		≥18	≥8	≤2
Nalidixic acid (30 micro gm)	≤13	14-18		≥19	≥32	≤12
Streptomycin (10 micro gm)	≤11	12-14		≥15		
Tetracycline (30 micro gm)	≤14	15-18		≥19	≥16	≤4
Trimethprim 5 micro gm)	≤10	11-15		≥16	≥16	≤4

PROTEIN SYNTHESIS AND SITE OF ACTION OF ANTIMICROBIALS

THAT INHIBIT PROTEIN SYNTHEIS

Initiation of Protein Synthesis

Figure 1.6.11 illustrates the initiation of protein synthesis and the site of action of antimicrobials that inhibit this process.

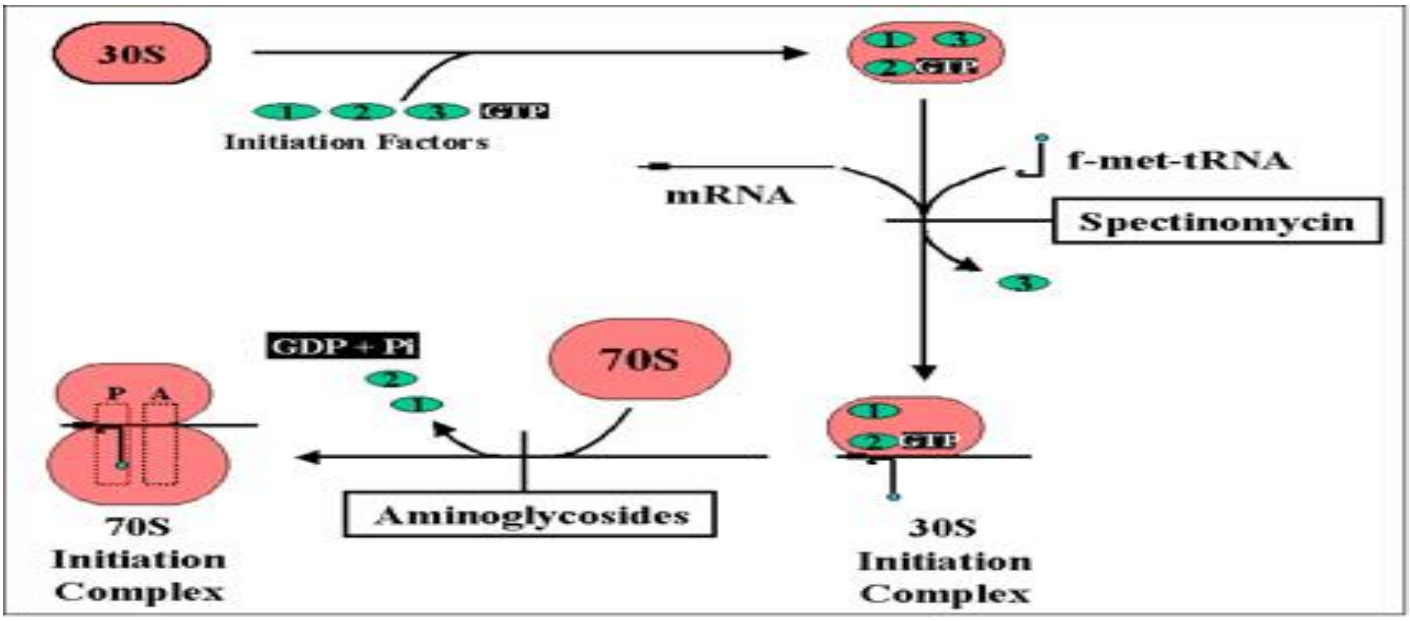


FIGURE 1.6.11

Elongation

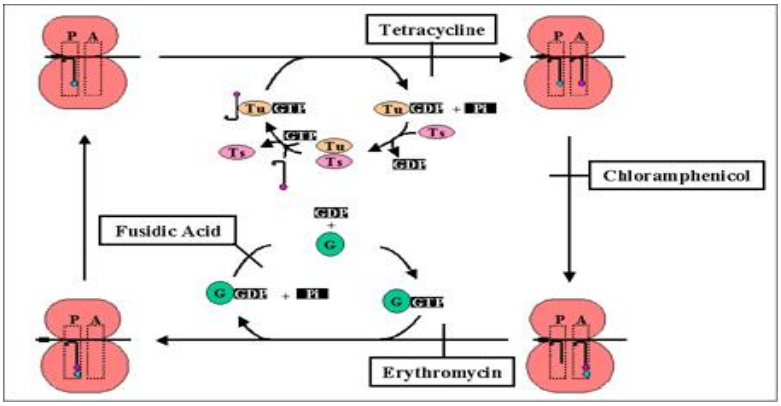


FIGURE 1.6.12 ILLUSTRATES THE PROCESS OF ELONGATION AND THE SITE OF ACTION OF ANTIMICROBIALS THAT INHIBIT THIS PROCESS

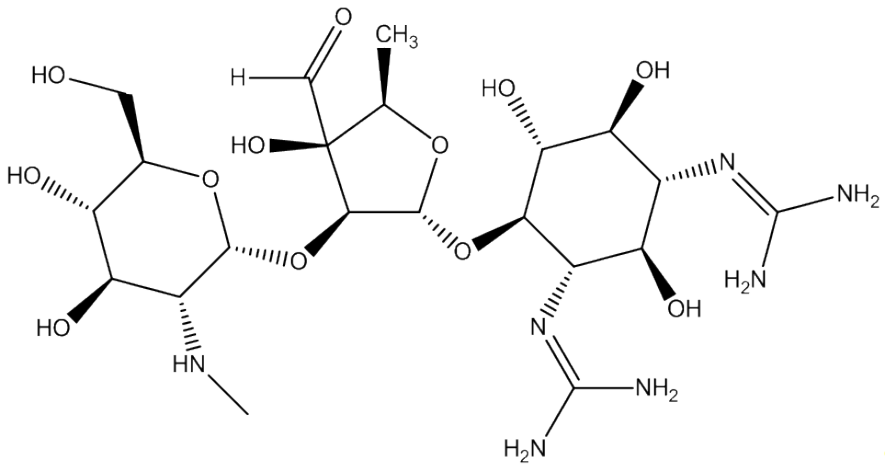
Courtesy: **Dr Alvin Fox** Emeritus Professor University of South Carolina School of Medicine

Inhibitors of Protein Synthesis

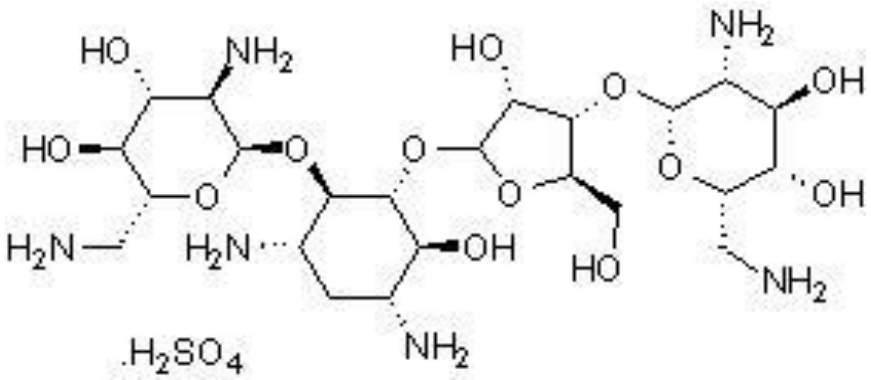
The selectivity of these agents is a result of differences in the prokaryotic 70S ribosome and the 80S eukaryotic ribosome. Since mitochondrial ribosomes are similar to prokaryotic ribosomes; these antimetabolites can have some toxicity. They are mostly bacteriostatic.

Antimicrobials that Bind to the 30S Ribosomal Subunit

Aminoglycosides (bactericidal)
Streptomycin, kanamycin, gentamicin, tobramycin, amikacin, netilmicin and neomycin (topical)



STREPTOMYCIN



NEOMYCIN

a. **Mode of action**

The aminoglycosides irreversibly bind to the 30S ribosome and freeze the 30S initiation complex (30S-mRNA-tRNA), so that no further initiation can occur. The aminoglycosides also slow down protein synthesis that has already initiated and induce misreading of the mRNA.

b. **Spectrum of Activity**

Aminoglycosides are active against many gram-negative and some gram-positive bacteria. They are not useful for anaerobic bacteria, since oxygen is required for uptake of the antibiotic, or for intracellular bacteria.

c. **Resistance**

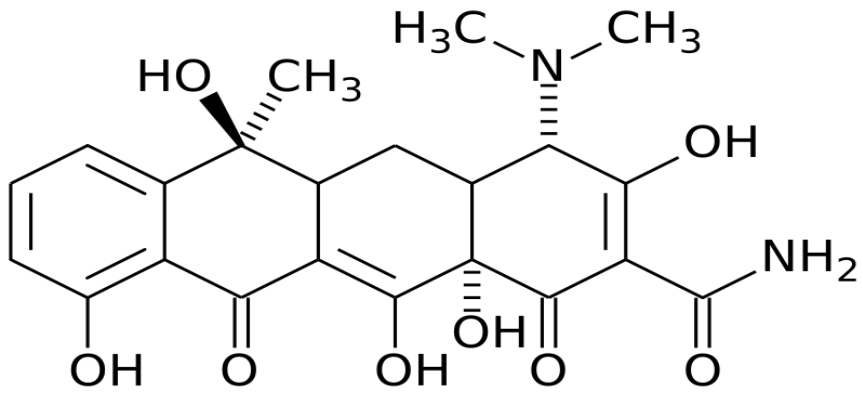
Resistance to these antibiotics is common

d. **Synergy**

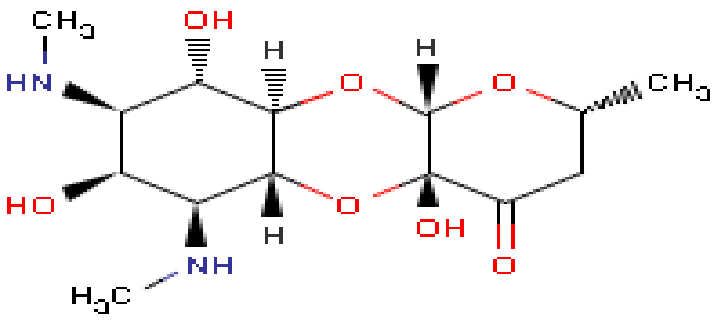
The aminoglycosides synergize with β-lactam antibiotics such as the Penicillins. The β-lactams inhibit cell wall synthesis and thereby increase the permeability of the bacterium to the aminoglycosides.

Tetracyclines (bacteriostatic)

Tetracycline, minocycline and doxycycline



TETRACYCLINE



SPECTINOLYCIN

a. **Mode of action**

The tetracyclines reversibly bind to the 30S ribosome and inhibit binding of aminoacyl-t-RNA to the acceptor site on the 70S ribosome.

b. **Spectrum of activity -**

These are broad spectrum antibiotics and are useful against intracellular bacteria

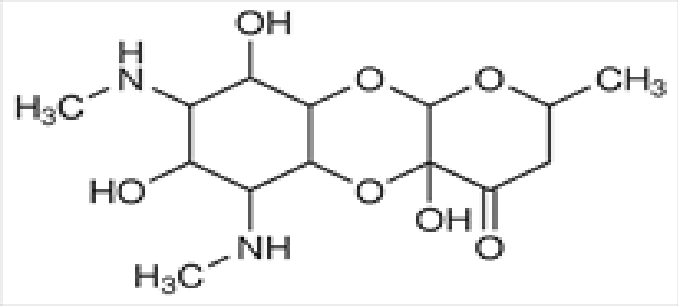
c. **Resistance**

Resistance to these antibiotics is common

d. **Adverse effects**

Destruction of normal intestinal flora often occurs, resulting in increased secondary infections. There can also be staining and impairment of the structure of bone and teeth

Spectinomycin (bacteriostatic)



SPECTINOMYCIN

a. **Mode of action**

Spectinomycin reversibly interferes with mRNA interaction with the 30S ribosome. It is structurally similar to aminoglycosides but does not cause misreading of mRNA

b. **Spectrum of activity -**

Spectinomycin is used in the treatment of penicillin-resistant Neisseria gonorrhoea

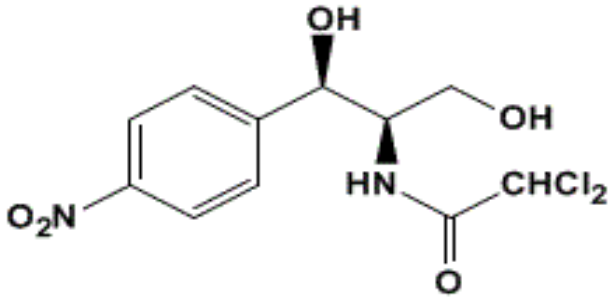
c. **Resistance**

This is rare in Neisseria gonorrhoea

Antimicrobials that Bind to the 50S Ribosomal Subunit

Chloramphenicol, lincomycin, clindamycin (bacteriostatic)

Chloramphenicol



a. Mode of action

These antimicrobials bind to the 50S ribosome and inhibit peptidyl transferase activity.

b. Spectrum of activity

•Chloramphenicol - Broad range

•Lincomycin and clindamycin - Restricted range

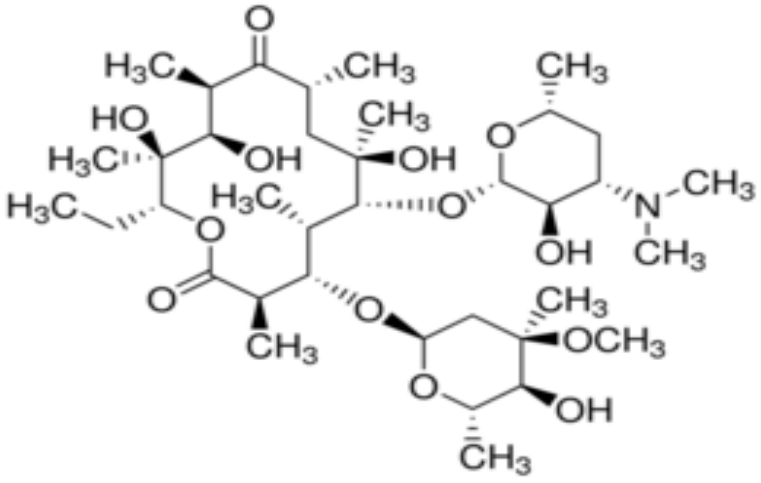
c. Resistance

Resistance to these antibiotics is common

d. Adverse effects

Chloramphenicol is toxic (bone marrow suppression) but it is used in the treatment of bacterial meningitis.

Macrolides (bacteriostatic) - Erythromycin (also azithromycin, clarithromycin)



ERYTHROMYCIN

a. Mode of action

The macrolides inhibit translocation of the peptidyl tRNA from the A to the P site on the ribosome by binding to the 50S ribosomal 23S RNA.

b. Spectrum of activity

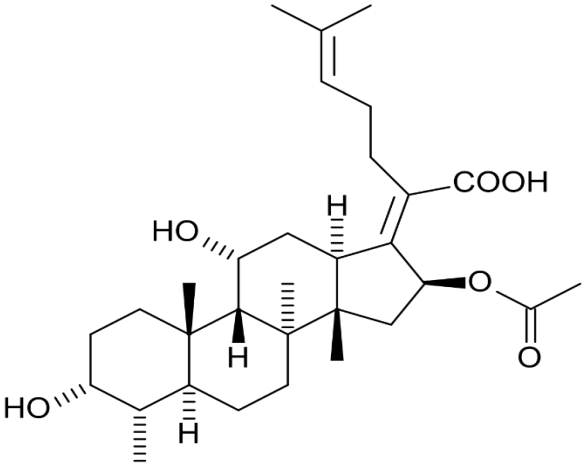
Gram-positive bacteria, *Mycoplasma, Legionella*

c. Resistance

Resistance to these antibiotics is common. Most gram-negative antibiotics are resistant to macrolides.

ANTIMICROBIALS THAT INTERFERE WITH ELONGATION FACTORS

Fusidic acid (bacteriostatic)



FUSIDIC ACID

a. **Mode of action**

Fusidic acid binds to elongation factor G (EF-G) and inhibits release of EF-G from the EF-G/GDP complex.

b. **Spectrum of activity**

Fusidic acid is only effective against gram-positive bacteria such as *Streptococcus*, *Staphylococcus aureus* and *Corynebacterium minutissimum*.

INHIBITORS OF NUCLEIC ACID SYNTHESIS AND FUNCTION

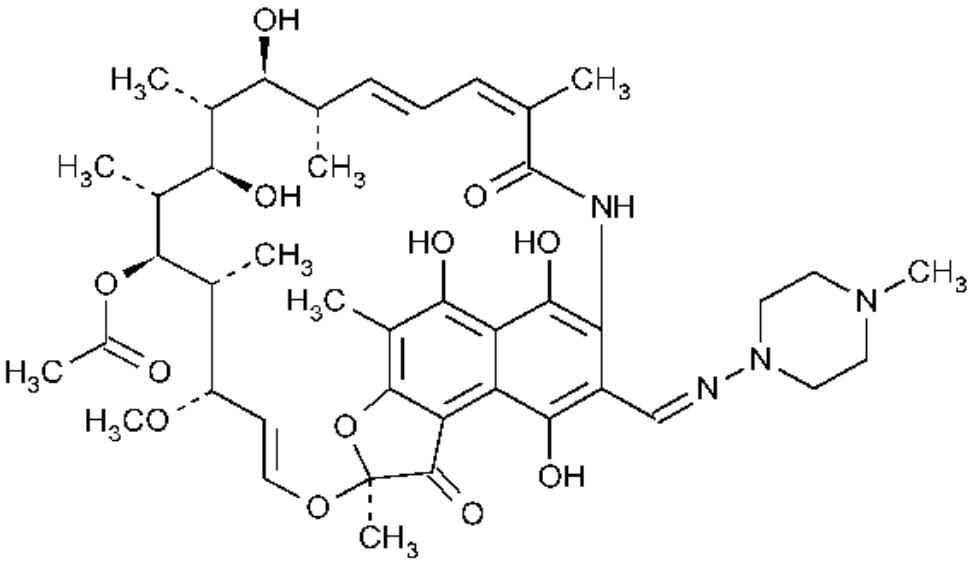
The selectivity of these agents is a result of differences in prokaryotic and eukaryotic enzymes affected by the antimicrobial agent.

INHIBITORS OF RNA SYNTHESIS AND FUNCTION

Rifampin, rifamycin, rifampicin (bactericidal)

a. Mode of action

These antimicrobials bind to DNA-dependent RNA polymerase and inhibit initiation of RNA synthesis.



RIFAMPIN

b. Spectrum of activity

They are wide spectrum antibiotics but are used most commonly in the treatment of tuberculosis

c. Resistance

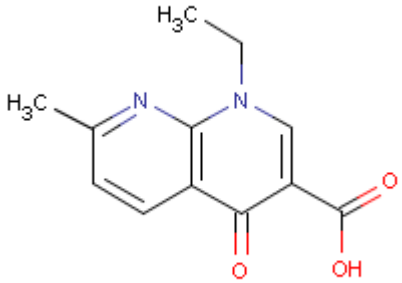
Resistance to these antibiotics is common.

d. Combination therapy

Since resistance is common, Rifampin is usually used in combination therapy

INHIBITORS OF DNA SYNTHESIS AND FUNCTION

Quinolones - nalidixic acid, ciprofloxacin, oxolinic acid (bactericidal)



NALIDIXIC ACID

a. Mode of action

These antimicrobials bind to the A subunit of DNA gyrase (topoisomerase) and prevent supercoiling of DNA, thereby inhibiting DNA synthesis.

b. Spectrum of activity -

These antibiotics are active against Gram-positive cocci and are used in urinary tract infections

c. Resistance

This is common for nalidixic acid and is developing for ciprofloxacin

ANTIMETABOLITE ANTIMICROBIALS

Inhibitors of folic acid synthesis.

The selectivity of these antimicrobials is a consequence of the fact that bacteria cannot use pre-formed folic acid and must synthesize their own folic acid. In contrast, mammalian cells use folic acid obtained from food. Figure 2.2.13 summarizes the pathway of folic acid metabolism and indicates the sites at which antimetabolites act.

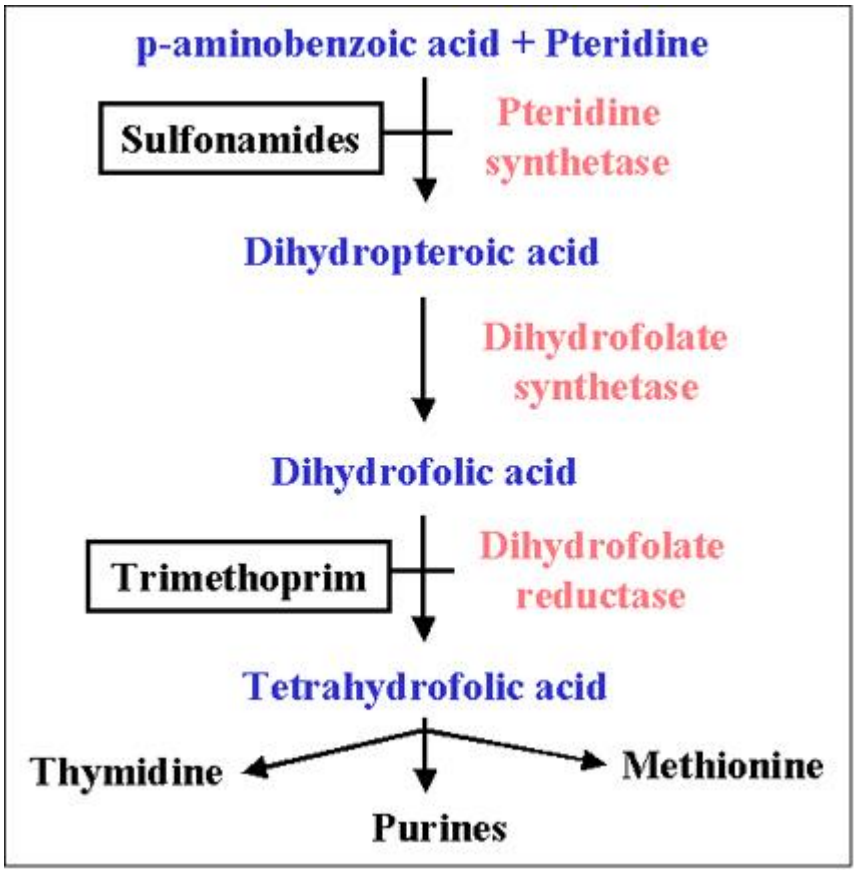
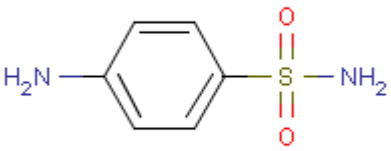


FIGURE 2.2.13

Sulfonamides, sulfones (bacteriostatic)



SULFANILAMIDE

a. Mode of action

These antimicrobials are analogues of para-aminobenzoic acid and competitively inhibit formation of dihydropteric acid.

b. Spectrum of activity

They have a broad range activity against gram-positive and gram-negative bacteria and are used primarily in urinary tract infections and in Nocardia infections.

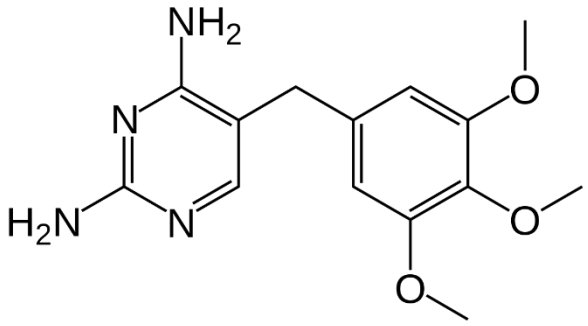
c. Resistance

Resistance to these antibiotics is common

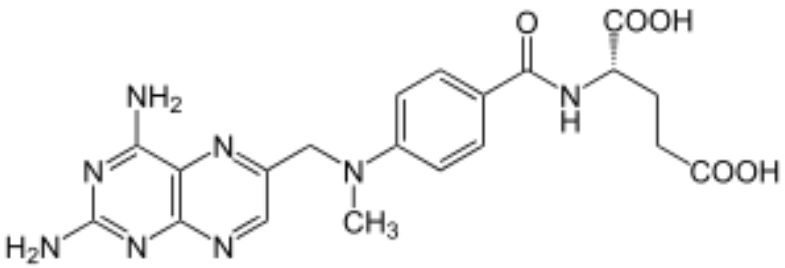
d. Combination therapy

The sulphonamides are used in combination with trimethoprim. This combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

Trimethoprim, methotrexate, pyrimethamine (bacteriostatic)



TRIMETHOPRIM



METHOTREXATE

a. Mode of action

These antimicrobials bind to dihydrofolate reductase and inhibit formation of tetrahydrofolic acid.

b. Spectrum of activity

They have a broad range activity against gram-positive and gram-negative bacteria and are used primarily in urinary tract infections and in Nocardia infections.

c. Resistance

Resistance to these antibiotics is common

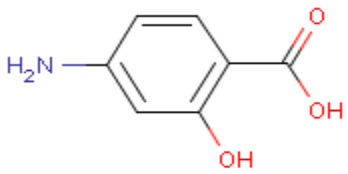
d. Combination therapy

These antimicrobials are used in combination with the sulphonamides. This combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

Anti-Mycobacterial agents

Anti-mycobacterial agents are generally used in combination with other antimicrobials since treatment is prolonged and resistance develops readily to individual agents.

Para-amino salicylic acid (PSA) (bacteriostatic)



AMINO SALICYLIC ACID

a. Mode of action

This is similar to sulphonamides

b. Spectrum of activity

PSA is specific for Mycobacterium tuberculosis

Dapsone (bacteriostatic)

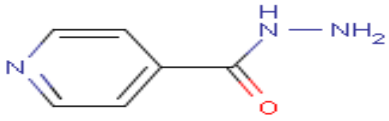
DAPSONE

a. *Mode of action*

Similar to sulphonamides

b. *Spectrum of activity*
Dapsone is used in treatment of leprosy

Isoniazid (INH) (bacteriostatic)



ISONIAZID

a. Mode of action
Isoniazid inhibits synthesis of mycolic acids.
b. *Spectrum of activity* -
INH is used in treatment of tuberculosis
c. *Resistance*
Resistance has developed

Antimicrobial Drug Resistance

Principles and Definitions: Clinical resistance to an antimicrobial agent occurs when the MIC of the drug for a particular strain of bacteria exceeds that which is capable of being achieved with safety in vivo. Resistance to an antimicrobial can arise:

- By mutation in the gene that determines sensitivity/resistance to the agent
- By acquisition of extrachromosomal DNA (plasmid) carrying a resistance gene.

Resistance that appears after introduction of an antimicrobial agent into the environment usually results from a selective process, i.e. the antibiotic selects for survival of those strains possessing a resistance gene. Resistance can develop in a single step or it can result from the accumulation of multiple mutations.

Cross Resistance

Cross resistance implies that a single mechanism confers resistance to multiple antimicrobial agents while multiple resistance implies that multiple mechanisms are involved. Cross resistance is commonly seen with closely related antimicrobial agents while multiple resistance is seen with unrelated antimicrobial agents.

Mechanisms of Resistance

Altered permeability of the antimicrobial agent
Altered permeability may be due to the inability of the antimicrobial agent to enter the bacterial cell or alternatively to the active export of the agent from the cell.

Inactivation of the antimicrobial agent
Resistance is often the result of the production of an enzyme that is capable of inactivating the antimicrobial agent.

Altered target site
Resistance can arise due to alteration of the target site for the antimicrobial agent.

Replacement of a sensitive pathway
Resistance can result from the acquisition of a new enzyme to replace the sensitive one.

4.0: CONCLUSION

Several methods are normally applied to completely eradicate (bactericidal, 'kill') bacteria or partially inhibit them (bacteriostatic). *In vitro*, within the environment of the laboratory, sterilization could be applied to achieve this. *In vivo*, within the living cell, some antibiotic could be used to achieve this complete killing (for the purposes of infectious diseases agent control in infected individuals or to render a particular environment free of these pathogens) , and as such are referred to as being bactericidal. This is done selectively during infection of a human or animal host. In other words, the antibiotics only kill the bacteria without much effect on the host cell. Disinfectants differ from antibiotics in their lack of selectivity and the impossibility of *in-vivo* application after isolation of pure colonies, the

susceptibility of bacterial isolates can be tested to a variety of antibiotics. The minimal inhibitory concentration (MIC) refers to the lowest concentration of an antibiotic that stops visible growth. Several mechanisms as earlier seen had been proposed for antibiotics activities on bacteria cell metabolism.

5.0 SUMMARY

In this unit we have so far discussed the following, they must be committed into memory and be well understood because of their useful clinical applications and relevance in public health microbiology.

- The mode of action of beta-lactam antibiotics;
- Antibiotics - Protein Synthesis, Nucleic Acid Synthesis and Metabolism
- Antibiotics and Chemotherapeutic agents
- Antibiotic susceptibility testing
- The mode of action of antibacterial chemotherapeutic agents
- The mechanism by which bacteria express resistance to antibiotic

6.0 TUTOR-MARKED ASSIGNMENT

Ques. 1 Define the following

(I) Antibiotics

(II) Disinfectants

(III) Sterilization

Ques. 2 What are the modes of action of beta-lactam antibiotics?

Ques. 3 Write briefly on Antibiotic susceptibility testing.

7.0 References/Further Reading

Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby.

Murray P.R. *et al*, editor: *Medical Microbiology,* ed.5 St. Louis 2005,

Antibiotics: Containing the Beta-Lactam Structure: By Arnold L. Demain, Nadine A. SolomonMosby.

Textbook of Microbiology by R. Vasanthakumari

Todar's online textbook of Bacteriology by Kenneth Todar, PhD

University of Sothern Carolina: Online Microbiology Teaching Aids and Resources, 2013.

UNIT 7: Bacteriophage/ Exchange of Genetic Information / Genetic Regulatory Mechanisms in Bacteria.

CONTENT

1.0 INTRODUCTION

Bacteriophage (phage) are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery (i.e., viruses that infect bacteria.).

There are many similarities between bacteriophages and animal cell viruses. Thus, bacteriophage can be viewed as model systems for animal cell viruses. In addition, knowledge of the life cycle of bacteriophage is necessary to understand one of the mechanisms by which bacterial genes can be transferred from one bacterium to another.

At one time it was thought that the use of bacteriophage might be an effective way to treat bacterial infections, but it soon became apparent that phage is quickly removed from the body and thus, were of little clinical value. However, bacteriophage is used in the diagnostic laboratory for the identification of pathogenic bacteria (phage typing). Although phage typing is not used in the routine clinical laboratory, it is used in reference laboratories for epidemiological purposes. Recently, new interest has developed in the possible use of bacteriophage for treatment of bacterial infections and in prophylaxis. Whether bacteriophage will be used in clinical medicine remains to be determined. In bacterial populations mutations are constantly arising due to errors made during replication. If there is any selective advantage for a particular mutation (e.g. antibiotic resistance), the mutant will quickly become the major component of the population due to the rapid growth rate of bacteria. In addition, since bacteria are haploid organisms, even mutations that might normally be recessive will be expressed. Thus, mutations in bacterial populations can pose a problem in the treatment of bacterial infections. Not

only are mutations a problem, bacteria have mechanisms by which genes can be transferred to other bacteria. Thus, a mutation arising in one cell can be passed on to other cells. Gene transfer in bacteria is unidirectional from a donor cell to a recipient cell and the donor usually gives only a small part of its DNA to the recipient. Thus, complete zygotes are not formed; rather, partial zygotes (merozygotes) are formed. Bacterial genes are usually transferred to members of the same species but occasionally transfer to other species can also occur. Figure 1.7.1 illustrates gene transfers that have been shown to occur between different species of bacteria

2.0 OBJECTIVE

At the end of this Unit, you should be able to:

- Describe the Composition and Structure of Bacteriophage
- Know how Host Cells Are Infected
- Fully Describe Phage Multiplication Cycle
- Describe Lysogenic or Temperate Phage
- Know the Events Leading to Termination of Lysogeny
- Get conversant with Gene Transfer Mechanisms in Bacteria

Such as:

- Transformation
- Transduction
- Conjugation
- Describe Transposable Genetic elements
- Know Types of Transposable Genetic Elements
- Mode of action of resistance genes

  Know what Plasmids are

- Fully describe Regulation of Gene Expression in Bacteria
- Fully know the regulation of Enzyme Activity in Bacteria

3.0 CONTENTS

3.1 COMPOSITION AND STRUCTURE OF BACTERIOPHAGE

Composition

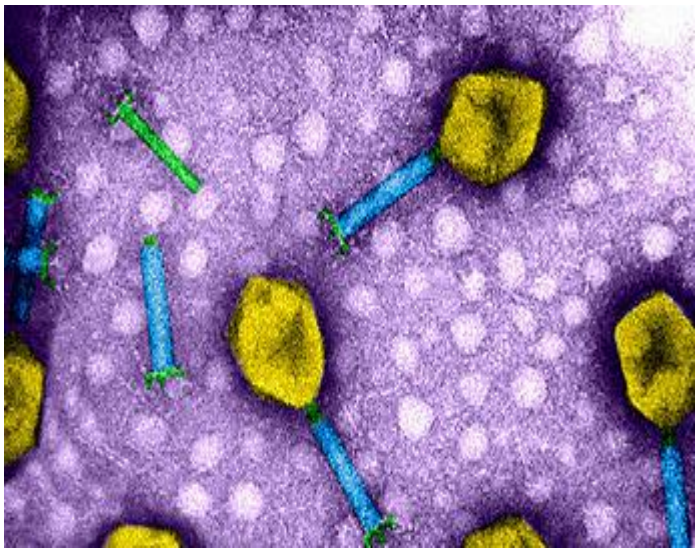
Although different bacteriophages may contain different materials they all contain nucleic acid and protein.

Depending upon the phage, the nucleic acid can be either DNA or RNA but not both and it can exist in various forms. The nucleic acids of phages often contain unusual or modified bases. These modified bases protect phage nucleic acid from nucleases that break down host nucleic acids during phage infection. The size of the nucleic acid varies depending upon the phage. The simplest phages only have enough nucleic acid to code for 3-5 average size gene products while the more complex phages may code for over 100 gene products.

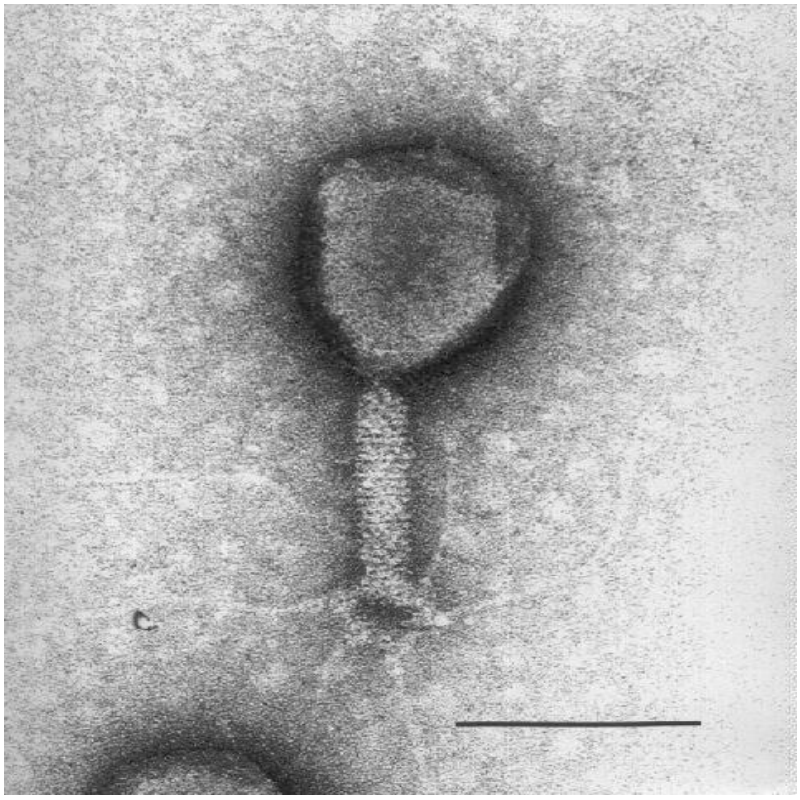
The number of different kinds of protein and the amount of each kind of protein in the phage particle will vary depending upon the phage. The simplest phage has many copies of only one or two different proteins while more complex phages may have many different kinds. The proteins function in infection and to protect the nucleic acid from nucleases in the environment.

Structure

Bacteriophage comes in many different sizes and shapes. The basic structural features of bacteriophages are illustrated in Figure 1.7.1, which depicts the phage called T4.



T4 BACTERIOPHAGE (TEM x390,000)



T4 BACTERIOPHAGE NEGATIVE STAIN ELECTRON MICROGRAPH

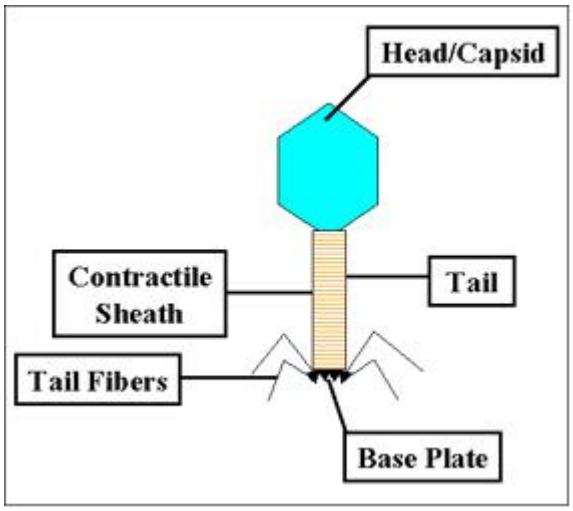


FIGURE 1.7.1

Courtesy: Dr. Avin Fox Emeritus Professor of Microbiology University of South Carolina.

Size

T4 is among the largest phages; it is approximately 200 nm long and 80-100 nm wide. Other phages are smaller. Most phages range in size from 24-200 nm in length.

Head or Capsid

All phages contain a head structure which can vary in size and shape. Some are icosahedral (20 sides) others are filamentous. The head or capsid is composed of many copies of one or more different proteins. Inside the head is found the nucleic acid. The head acts as the protective covering for the nucleic acid.

Tail

Many but not all phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. The size of the tail can vary and some phages do not even have a tail structure. In the more complex phages like T4 the tail is surrounded by a contractile sheath which contracts during infection of the bacterium. At the end of the tail the more complex phages like T4 have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers. In

these instances, other structures are involved in binding of the phage particle to the bacterium.

3.2 INFECTION OF HOST CELLS

Adsorption

The first step in the infection process is the adsorption of the phage to the bacterial cell. This step is mediated by the tail fibers or by some analogous structure on those phages that lack tail fibers and it is reversible. The tail fibers attach to specific receptors on the bacterial cell and the host specificity of the phage (i.e. the bacteria that it is able to infect) is usually determined by the type of tail fibers that a phage has. The nature of the bacterial receptor varies for different bacteria. Examples include proteins on the outer surface of the bacterium, LPS, pili, and lipoprotein. These receptors are on the bacteria for other purposes and phage have evolved to use these receptors for infection.

Irreversible attachment

Irreversible attachment is the attachment of the phage to the bacterium via the tail fibers is a weak one and is reversible. Irreversible binding of phage to a bacterium is mediated by one or more of the components
of the base plate. Phages lacking base plates have other ways of becoming tightly bound to the bacterial cell

Sheath Contraction

The irreversible binding of the phage to the bacterium results in the contraction of the sheath (for those phages which have a sheath) and the hollow tail fiber is pushed through the bacterial envelope (Figure 1.7.2).

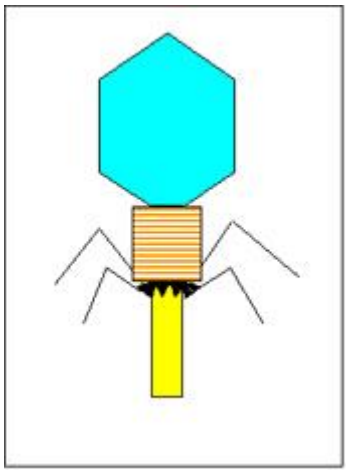


FIGURE 1.7.2 COURTESY: DR. AVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

Phages that don't have contractile sheaths use other mechanisms to get the phage particle through the bacterial envelope. Some phages have enzymes that digest various components of the bacterial envelope.

Nucleic Acid Injection

When the phage has gotten through the bacterial envelope the nucleic acid from the head passes through the hollow tail and enters the bacterial cell. Usually, the only phage component that actually enters the cell is the nucleic acid. The remainder of the phage remains on the outside of the bacterium. There are some exceptions to this rule. This is different from animal cell viruses in which most of the virus particle usually gets into the cell. This difference is probably due to the inability of bacteria to engulf materials.

3.3 PHAGE MULTIPLICATION CYCLE

Lytic or Virulent Phages

Definition

Lytic or virulent phages are phages which can only multiply on bacteria and kill the cell by lysis at the end of the life cycle.

Life cycle

The life cycle of a lytic phage is illustrated in Figure 1.7.3 .

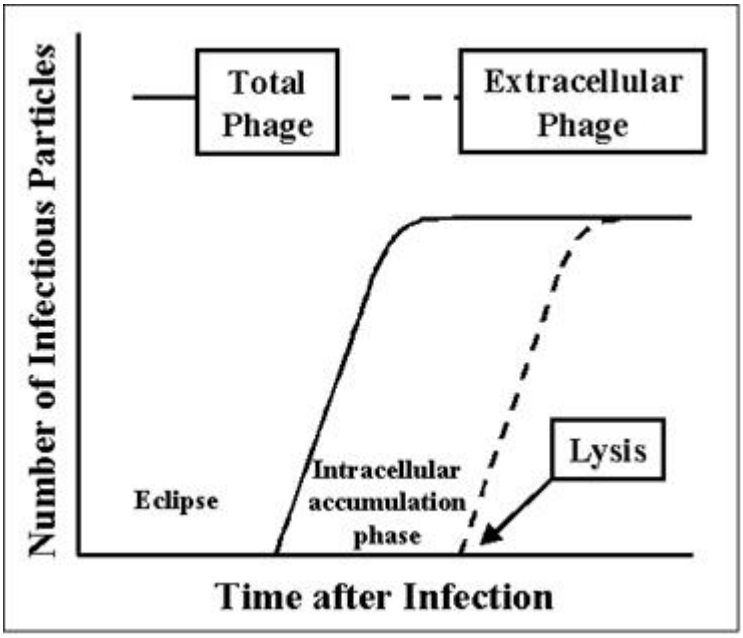


FIGURE 1.7.3 COURTESY: DR. AVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

Eclipse period

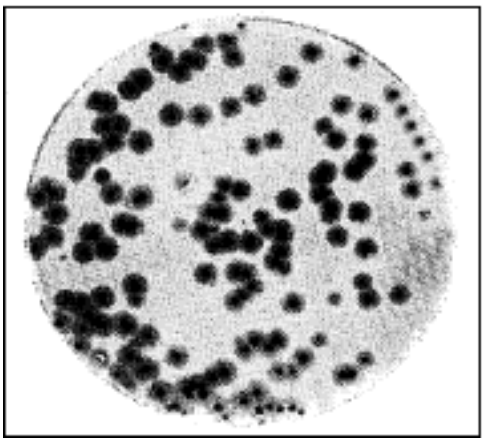
During the eclipse phase, no infectious phage particles can be found either inside or outside the bacterial cell. The phage nucleic acid takes over the host biosynthetic machinery and phage specified m-RNA's and proteins are made. There is an orderly expression of phage directed macromolecular synthesis, just as one sees in animal virus infections. Early m-RNA's code for early proteins which are needed for phage DNA synthesis and for shutting off host DNA, RNA and protein biosynthesis. In some cases, the early proteins actually degrade the host chromosome. After phage DNA is made late m-RNA's and late proteins are made. The late proteins are the structural proteins that comprise the phage as well as the proteins needed for lysis of the bacterial cell.

Intracellular Accumulation Phase

In this phase the nucleic acid and structural proteins that have been made are assembled and infectious phage particles accumulate within the cell.

Lysis and Release Phase

After a while the bacteria begin to lyse due to the accumulation of the phage lysis protein and intracellular phage are released into the medium. The number of particles released per infected bacteria may be as high as 1000.



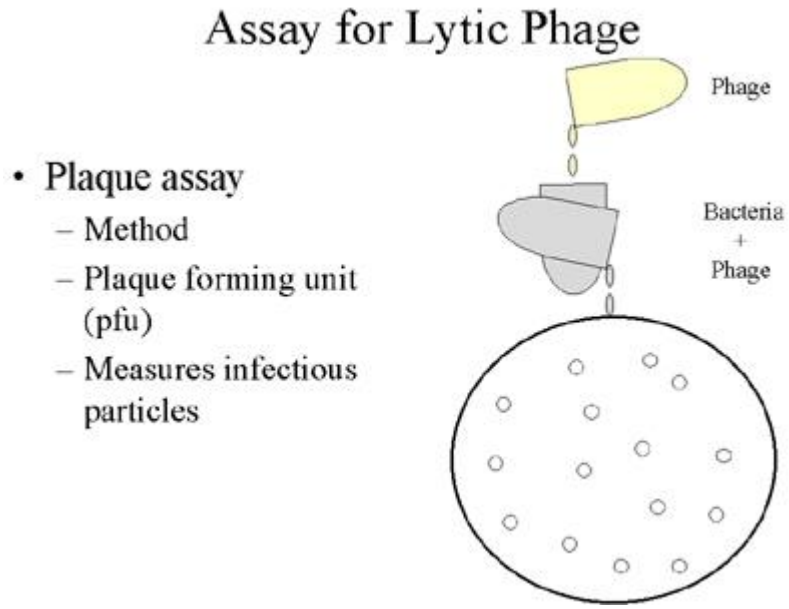


FIGURE 1.7.4 *ASSAY FOR LYTIC PHAGE*

Plaque assay

Lytic phage is enumerated by a plaque assay. A plaque is a clear area which results from the lysis of bacteria (Figure 1.7.4). Each plaque arises from a single infectious phage. The infectious particle that gives rise to a plaque is called a pfu (plaque forming unit).

3.4 LYSOGENIC OR TEMPERATE PHAGE

Definition

Lysogenic or temperate phages are those that can either multiply via the lytic cycle or enter a quiescent state in the cell. In this quiescent state most of the phage genes are not transcribed; the phage genome exists in a repressed state. The phage DNA in this repressed state is called a prophage because it is not a phage but it has the potential to produce phage. In most cases the phage DNA actually integrates into the host chromosome and is replicated along with the host chromosome and passed on to the daughter cells. The cell harbouring a prophage is not adversely affected by the presence of the prophage and the lysogenic state may persist indefinitely. The cell harbouring a prophage is termed a Lysogeny.

3.5 EVENTS LEADING TO LYSOGENY

The Prototype Phage: Lambda

Circularization of the phage chromosome

Lambda DNA is a double stranded linear molecule with small single stranded regions at the 5' ends. These single stranded ends are complementary (cohesive ends) so that they can base pair and produce a circular molecule. In the cell the free ends of the circle can be ligated to form a covalently closed circle as illustrated in Figure 1.7.5.

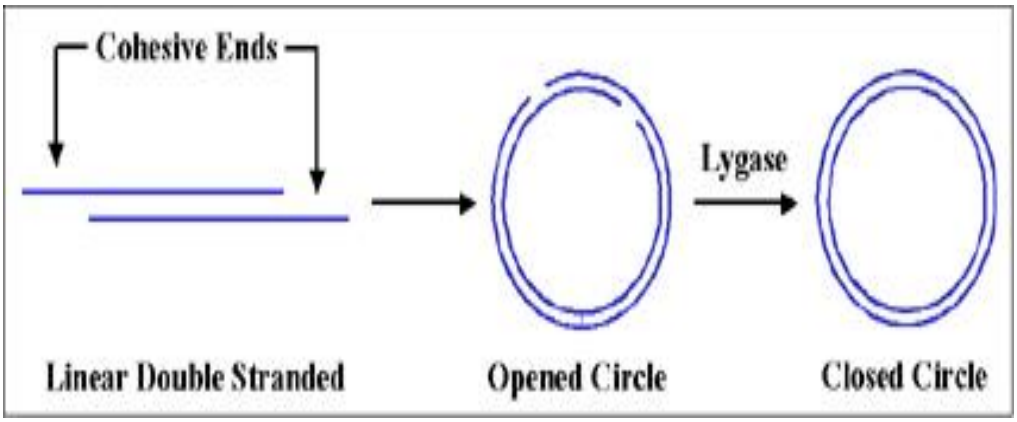


FIGURE 1.7.5: CIRCULARIZATION OF PHAGE CHROMOSOME: COHESIVE ENDS

Courtesy: Dr. Avin Fox Emeritus Professor of Microbiology University of South Carolina

Site-specific recombination

A recombination event, catalysed by a phage coded enzyme, occurs between a particular site on the circularized phage DNA and a particular site on the host chromosome. The result is the integration of the phage DNA into the host chromosome

as illustrated in Figure 1.7.6.

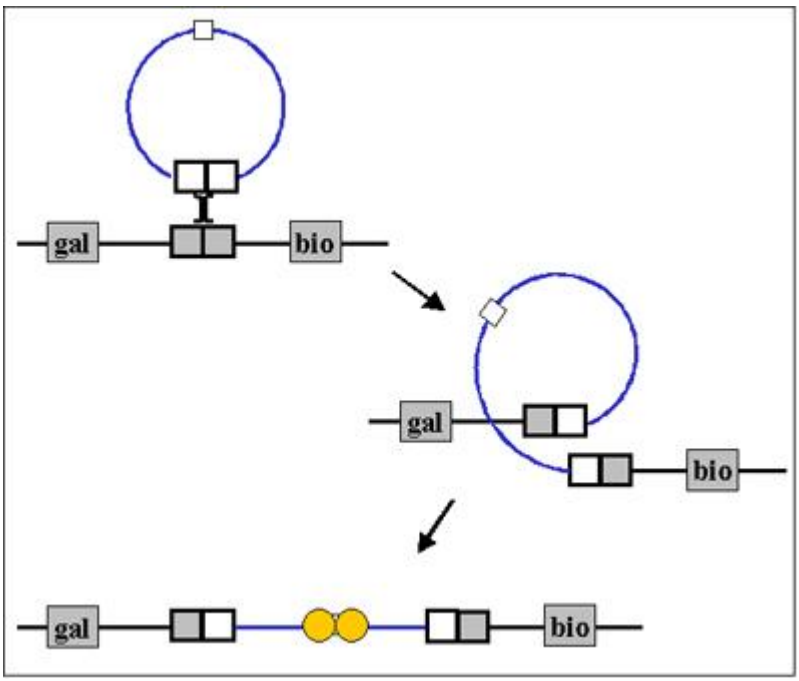


FIGURE 1.7.6: SITE-SPECIFIC RECOMBINATION COURTESY: DR. AVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF S

Repression of the phage genome

A phage coded protein, called a repressor, is made which binds to a particular site on the phage DNA, called the operator, and shuts off transcription of most phage genes EXCEPT the repressor gene. The result is a stable repressed phage genome which is integrated into the host chromosome. Each temperate phage will only repress its own DNA and not that from other phage, so that repression is very specific (immunity to superinfection with the same phage).

3.6 EVENTS LEADING TO TERMINATION OF LYSOGENY

Anytime a lysogenic bacterium is exposed to adverse conditions, the lysogenic state can be terminated. This process is called induction. Conditions which favour the termination of the lysogenic state include: desiccation, exposure to UV or ionizing radiation, exposure to mutagenic chemicals, etc. Adverse conditions lead to the production of proteases (rec A protein) which destroy the repressor protein. This in turn leads to the expression of the phage genes, reversal of the integration process and lytic multiplication.

Lytic vs Lysogenic Cycle

The decision for lambda to enter the lytic or lysogenic cycle when it first enters a cell is determined by the concentration of the repressor and another phage protein called *cro* in the cell. The cro protein turns off the synthesis of the repressor and thus prevents the establishment of lysogeny. Environmental conditions that favour the production of cro will lead to the lytic cycle while those that favour the production of the repressor will favour lysogeny.

SIGNIFICANCE OF LYSOGENY

Model for animal virus transformation

Lysogeny is a model system for virus transformation of animal cells

Lysogenic conversion

When a cell becomes lysogenized, occasionally extra genes carried by the phage get expressed in the cell. These genes can change the properties of the bacterial cell. This process is called lysogenic or phage conversion. This can be of significance clinically. e.g. Lysogenic phages have been shown to carry genes that can modify the Salmonella O antigen, which is one of the major antigens to which the immune response is directed. Toxin production by *Corynebacterium diphtheria* is mediated by a gene carried by a phage. Only those strain that have been converted by lysogeny are pathogenic.

3.7 GENE TRANSFER MECHANISMS IN BACTERIA

3.61 Transformation

Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a donor cell. Certain bacteria (e.g. *Bacillus, Haemophilus, Neisseria, and Pneumococcus)* can take up DNA from the environment and the DNA that is taken up can be incorporated into the recipient's chromosome.

Factors affecting transformation

a. DNA size state

Double stranded DNA of at least 5 X 10^5 Daltons works best. Thus, transformation is sensitive

to nucleases in the environment.

b. Competence of the recipient

Some bacteria are able to take up DNA naturally. However, these bacteria only take up DNA a particular time in their growth cycle when they produce a specific protein called a competence factor. At this stage the bacteria are said to be competent. Other bacteria are not able to take up DNA naturally. However, in these bacteria competence can be induced in vitro by treatment with chemicals (e.g. $CaCl_2$).

Steps in transformation

a. Uptake of DNA

Uptake of DNA by Gram+ and Gram- bacteria differs. In Gram + bacteria the DNA is taken up as a single stranded molecule and the complementary strand is made in the recipient. In contrast, Gram- bacteria take up double stranded DNA.

b. Legitimate/Homologous/General Recombination

After the donor DNA is taken up, a reciprocal recombination event occurs between the chromosome and the donor DNA. This recombination requires homology between the donor DNA and the chromosome and results in the substitution of DNA between the recipient and the donor as illustrated in Figure 1.7.7.

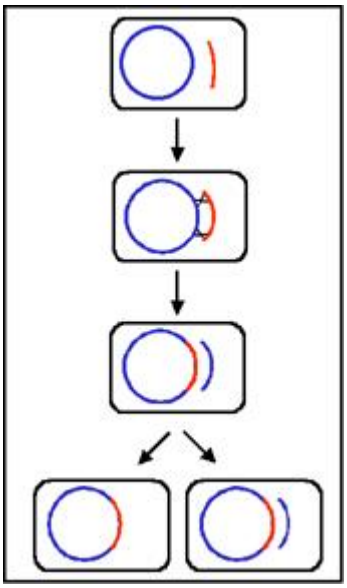


FIGURE 1.7.7: GENERAL RECOMBINATION. DONOR DNA IS RED AND RECIPIENT DNA IN BLUE.

Courtesy: Albert Lehninger Text book on Biochemistry

Recombination requires the bacterial recombination genes (rec A, B and C) and homology between the DNA's involved. This type of recombination is called legitimate or homologous or general recombination. Because of the requirement for homology between the donor and host DNA, only DNA from closely related bacteria would be expected to successfully

transform, although in rare instances gene transfer between distantly related bacteria has been shown to occur.

Significance

Transformation occurs in nature and it can lead to increased virulence. In addition, transformation is widely used in recombinant DNA technology.

3.62 Transduction

Transduction is the transfer of genetic information from a donor to a recipient by way of a bacteriophage. The phage coat protects the DNA in the environment so that transduction, unlike transformation, is not affected by nucleases in the environment. Not all phages can mediate transduction. In most cases gene transfer is between members of the same bacterial species. However, if a particular phage has a wide host range then transfer between species can occur. The ability of a phage to mediated transduction is related to the life cycle of the phage.

Types of Transduction

a. **Generalized Transduction -** Generalized transduction is transduction in which potentially any bacterial gene from the donor can be transferred to the recipient. The mechanism of generalized transduction is illustrated in Figure 1.7.8.

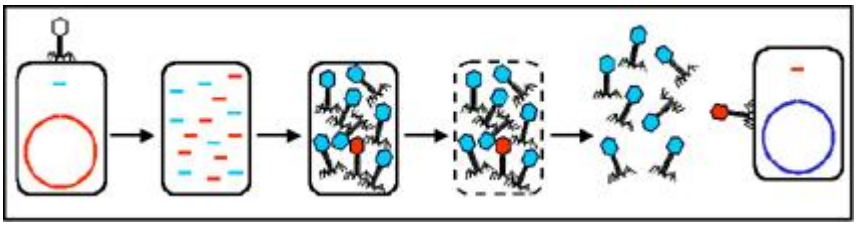


FIGURE 1.7.8: THE MECHANISM OF GENERALIZED TRANSDUCTION

Courtesy: Dr. Avin Fox Emeritus Professor of Microbiology University of South Carolina

Phages that mediate generalized transduction generally breakdown host DNA into smaller pieces and package their DNA into the phage particle by a "head-full" mechanism. Occasionally one of the pieces of host DNA is randomly packaged into a phage coat. Thus, any donor gene can be potentially transferred but only enough DNA as can fit into a phage head can be transferred. If a recipient cell is infected by a phage that contains donor DNA, donor DNA enters the recipient. In the recipient a generalized recombination event can occur which substitutes the donor DNA and recipient DNA (See Figure 1.7.7).

Specialized transduction - Specialized transduction is transduction in which only certain

donor genes can be transferred to the recipient. Different phages may transfer different genes but an individual phage can only transfer certain genes. Specialized transduction is mediated by lysogenic or temperate phage and the genes that get transferred will depend on where the prophage has inserted in the chromosome. The mechanism of specialized transduction is illustrated in Figure 1.7.9.

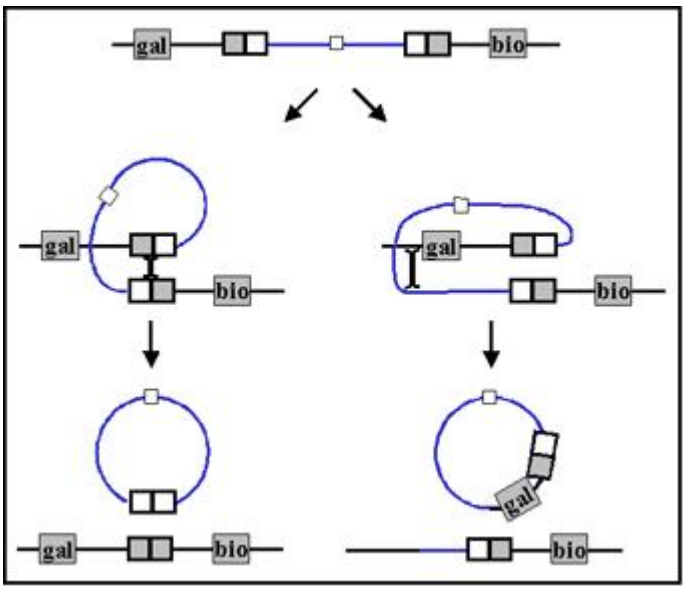


FIGURE 1.7.9: THE MECHANISM OF SPECIALIZED TRANSDUCTION.

Courtesy: Dr. Avin Fox Emeritus Professor of Microbiology University of South Carolina

During excision of the prophage, occasionally an error occurs where some of the host DNA is excised with the phage DNA. Only host DNA on either side of where the prophage has inserted can be transferred (i.e. specialized transduction). After replication and release of phage and infection of a recipient, lysogenization of recipient can occur resulting in the stable transfer of donor genes. The recipient will now have two copies of the gene(s) that were transferred. Legitimate recombination between the donor and recipient genes is also possible.

Significance

Lysogenic (phage) conversion occurs in nature and is the source of virulent strains of bacteria

3.63 Conjugation

Transfer of DNA from a donor to a recipient by direct physical contact between the cells. In bacteria there are two mating types a donor (male) and a recipient (female) and the direction of transfer of genetic material is one way; DNA is transferred from a donor to a

recipient.

Mating types in bacteria

a. Donor

The ability of a bacterium to be a donor is a consequence of the presence in the cell of an extra piece of DNA called the F factor or fertility factor or sex factor. The F factor is a circular piece of DNA that can replicate autonomously in the cell; it is an independent replicon. Extrachromosomal pieces of DNA that can replicate autonomously are given the general name of plasmids. The F factor has genes on it that are needed for its replication and for its ability to transfer DNA to a recipient. One of the things the F factor codes for is the ability to produce a sex pilus (F pilus) on the surface of the bacterium. This pilus is important in the conjugation process. The F factor is not the only plasmid that can mediate conjugation but it is generally used as the model.

b. Recipient

The ability to act as a recipient is a consequence of the lack of the F factor.

PHYSIOLOGICAL STATES OF THE F FACTOR

a. Autonomous (F+)

In this state the F factor carries only those genes necessary for its replication and for DNA transfer. There are no chromosomal genes associated with the F factor in F+ strains.
In crosses of the type F+ X F- the F- becomes F+ while F+ remains F+. Thus, the F factor is infectious. In addition, there is only low level transfer of chromosomal genes.

b. Integrated (Hfr)

In this state the F factor has integrated into the bacterial chromosome via a recombination event as illustrated in the Figure 1.7.10A

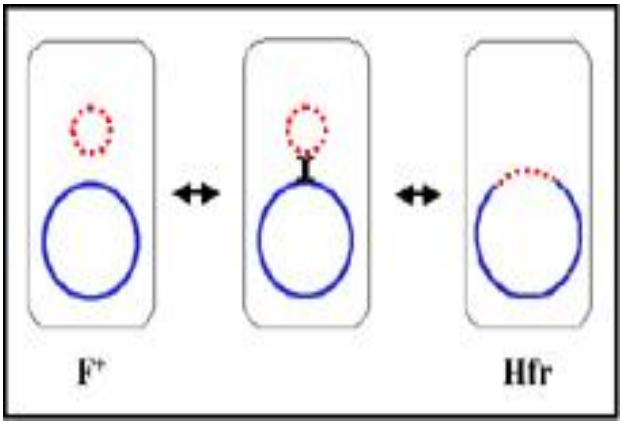


FIGURE 1.7.10A

Courtesy: Dr. Avin Fox Emeritus Professor of Microbiology University of South Carolina

In crosses of the type Hfr X F- the F- rarely becomes Hfr and Hfr remains Hfr.
In addition, there is a high frequency of transfer of donor chromosomal genes.

c. Autonomous with chromosomal genes (F')

In this state the F factor is autonomous but it now carries some chromosomal genes. F' factors are produced by excision of the F factor from an Hfr, as illustrated in Figure 1.7.10B. Occasionally, when the F factor is excising from the Hfr chromosome, donor genes on either side of the F factor can be excised with the F factor generating an F'. F' factors are named depending on the chromosomal genes that they carry.

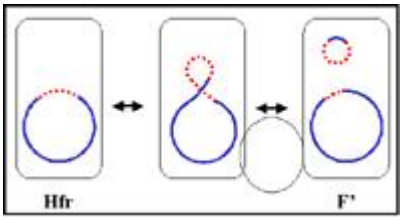


FIGURE 1.7.10B

COURTESY: DR. AVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

In crosses of the type F' X F- the F- becomes F' while F' remains F'. In addition, there is high frequency of transfer of those chromosomal genes on the F' and low Frequency transfer of other donor chromosomal genes.

Mechanism of conjugation

a. F+ X F- crosses (Figure 1.7.11)

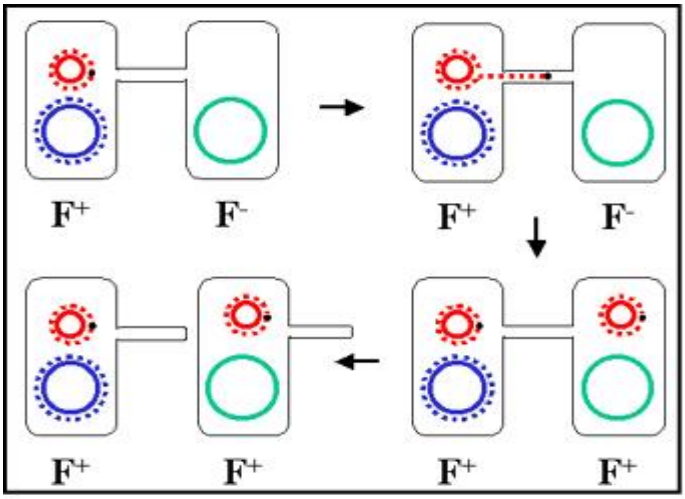


FIGURE 1.7.11

Courtesy: Dr. Avin Fox Emeritus Professor of Microbiology University of South Carolina

i) Pair formation

The tip of the sex pilus comes in contact with the recipient and a conjugation bridge is formed between the two cells. It is through this bridge that the DNA will pass from the donor to the recipient. Thus, the DNA is protected from environmental nucleases. The mating pairs can be separated by shear forces and conjugation can be interrupted. Consequently, the mating pairs remain associated for only a short time.

ii) DNA transfer

The plasmid DNA is nicked at a specific site called the origin of transfer and is replicated by a rolling circle mechanism. A single strand of DNA passes through the conjugation bridge and enters the recipient where the second strand is replicated.

iii) This process explains the characteristics of F+ X F- crosses. The recipient becomes F+, the donor remains F+ and there is low frequency of transfer of donor chromosomal genes. Indeed, as depicted in Figure 1.7.12 there is no transfer of donor chromosomal genes. In practice however, there is a low level of transfer of donor chromosomal genes in such crosses.

b. Hfr X F- crosses (Figure 1.7.12)

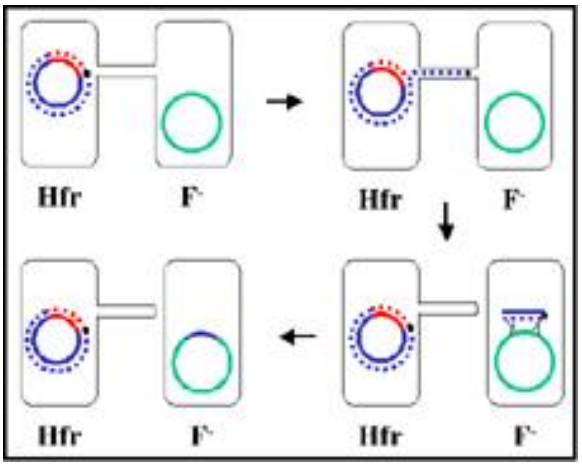


FIGURE 1.7.12: MECHANISM OF HFR X F- CROSSES

Courtesy: Dr. Avin Fox Emeritus Professor of Microbiology University of South Carolina

i) Pair Formation

ii) DNA transfer

The DNA is nicked at the origin of transfer and is replicated by a rolling circle mechanism. But the DNA that is transferred first is the chromosome. Depending upon where in the chromosome the F factor has integrated and in what orientation, different chromosomal genes will be transferred at different times. However, the relative order and distances of the genes will always remain the same. Only when the entire chromosome is transferred will the F factor be transferred. Since shearing forces separate the mating pairs it is rare that the entire chromosome will be transferred. Thus, the recipient does not receive the F factor in a Hfr X F- cross.

iii) Legitimate recombination

Recombination between the transferred DNA and the chromosome results in the exchange of genetic material between the donor and recipient.

iv) This mechanism explains the characteristics of Hfr X F- crosses. The recipient remains F-, the donor remains Hfr and there is a high frequency of transfer of donor chromosomal genes.

c. F' X F- crosses (Figure 1.7.13)

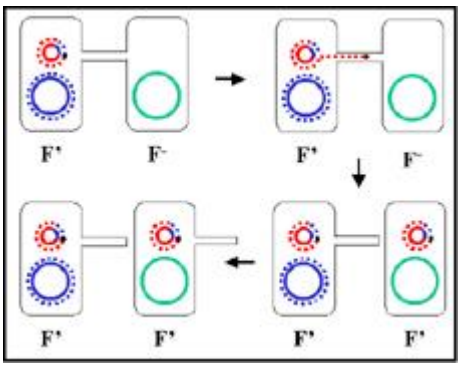


FIGURE 1.7.13: THE MECHANISM OF F" X F- CROSSES

Courtesy: Dr. Avin Fox Emeritus Professor of Microbiology University of South Carolina

i) Pair formation

ii) DNA transfer
This process is similar to F+ X F- crosses. However, since the F' has some chromosomal genes on it these will also be transferred.
iii) Homologous recombination is not necessary although it may occur.

iv) This mechanism explains the characteristics of F' X F- crosses. The F- becomes F', the F' remains F' and the is high frequency transfer of donor genes on the F' but low frequency transfers of other donor chromosomal genes.

Significance

Among the Gram negative bacteria this is the major way that bacterial genes are transferred. Transfer can occur between different species of bacteria. Transfer of multiple antibiotic resistance by conjugation has become a major problem in the treatment of certain bacterial diseases. Since the recipient cell becomes a donor after transfer of a plasmid it is easy to see why an antibiotic resistance gene carried on a plasmid can quickly convert a sensitive population of cells to a resistant one.

Gram positive bacteria also have plasmids that carry multiple antibiotic resistance genes, in some cases these plasmids are transferred by conjugation while in others they are transferred by transduction. The mechanism of conjugation in Gram + bacteria is different than that for Gram -. In Gram + bacteria the donor makes an adhesive material which causes aggregation with the recipient and the DNA is transferred.

3.8 TRANSPOSABLE GENETIC ELEMENTS

Transposable genetic elements are segments of DNA that have the capacity to move from one location to another (i.e. jumping genes).

Properties of Transposable Genetic Elements

Random movement

Transposable genetic elements can move from any DNA molecule to any DNA other molecule or even to another location on the same molecule. The movement is not totally random; there are preferred sites in a DNA molecule at which the transposable genetic element will insert.

Not capable of self-replication

The transposable genetic elements do not exist autonomously (exception - some transposable phages) and thus, to be replicated they must be a part of some other replicon.

Transposition mediated by site-specific recombination

Transposition requires little or no homology between the current location and the new site. The transposition event is mediated by a transposase coded for by the transposable genetic element. Recombination that does not require homology between the recombining molecules is called site-specific or illegitimate or non-homologous recombination.

Transposition can be accompanied by duplication

In many instances transposition of the transposable genetic element results in removal of the element from the original site and insertion at a new site. However, in some cases the transposition event is accompanied by the duplication of the transposable genetic element. One copy remains at the original site and the other is transposed to the new site.

3.9: TYPES OF TRANSPOSABLE GENETIC ELEMENTS

Insertion sequences (IS)

Insertion sequences are transposable genetic elements that carry no known genes except those that are required for transposition.

a. Nomenclature

Insertion sequences are given the designation IS followed by a number. e.g. IS1

b. Structure (Figure 1.7.14)

Insertion sequences are small stretches of DNA that have at their ends repeated sequences, which are involved in transposition.

FIGURE 1.7.14: STRUCTURE OF TRANSPOSABLE GENETIC ELEMENTS

In between the terminal repeated sequences there are genes involved in transposition and sequ that can control the expression of the genes but no other nonessential genes are present.

c. Importance

i) Mutation

The introduction of an insertion sequence into a bacterial gene will result in the inactivation of the gene.

ii) Plasmid insertion into chromosomes

The sites at which plasmids insert into the bacterial chromosome are at or near insertion sequence in the chromosome.

iii) Phase Variation

The flagella antigens are one of the main antigens to which the immune response is directed in our attempt to fight off a bacterial infection. In Salmonella there are two genes which code for two antigenically different flagella antigens. The expression of these genes is regulated by an insertion sequences. In one orientation one of the genes is active while in the other orientation the other flagella gene is active. Thus, Salmonella can change their flagella in response to the immune systems' attack. Phase variation is not unique to Salmonella flagella antigens. It is also seen with other bacterial surface antigens. Also the mechanism of phase variation may differ in different species of bacteria (e.g. Neisseria; transformation).

Transposons (Tn)

Transposons are transposable genetic elements that carry one or more other genes in addition to those which are essential for transposition.

a. Nomenclature

Transposons are given the designation Tn followed by a number.

b. Structure

The structure of a transposon is similar to that of an insertion sequence. The extra genes are located between the terminal repeated sequences. In some instances, (composite transposons) the terminal repeated sequences are actually insertion sequences. (See Figure 1.7.15).

FIGURE 1.7.15: TRANSPOSON STRUCTURE

c. Importance

Many antibiotic resistance genes are located on transposons. Since transposons can jump from one DNA molecule to another, these antibiotic resistance transposons are a major factor in the development of plasmids which can confer multiple drug resistance on a bacterium harbouring such a plasmid. These multiple drug resistance plasmids have become a major medical problem because the indiscriminate use of antibiotics have provided a selective advantage for bacteria harbouring these plasmids.

4.0: PLASMIDS

Definition

Plasmids are Extrachromosomal genetic elements capable of autonomous replication.

An episome is a plasmid that can integrate into the bacterial chromosome.

Classification of Plasmids

Transfer properties

a. Conjugative plasmids

Conjugative plasmids are those that mediated conjugation. These plasmids are usually large and have all the genes necessary for autonomous replication and for transfer of DNA to a recipient (e.g. genes for sex pilus).

b. Non-conjugative plasmids

Non-conjugative plasmids are those that cannot mediate conjugation. They are usually smaller than conjugative plasmids and they lack one or more of the genes needed for transfer of DNA. A non-conjugative plasmid can be transferred by conjugation if the cell also harbours a conjugative plasmid.

Phenotypic effects

a. Fertility plasmid (F factor)

b. Bacteriocinogenic plasmids

These plasmids have genes which code for substances that kill other bacteria. These substances are called bacteriocins or colicins.

c. Resistance plasmids 7 factors)

These plasmids carry antibiotic resistance genes.

i) Origin - The origin of the R factors is not known. It is likely that they evolved for other purposes and the advent of the antibiotic age provided a selective advantage for their wide-spread dissemination.

ii) Structure - R plasmids are conjugative plasmids in which the genes for replication and transfer are located on one part of the R factor and the resistance genes are located on another part as illustrated in Figure 1.7.16.

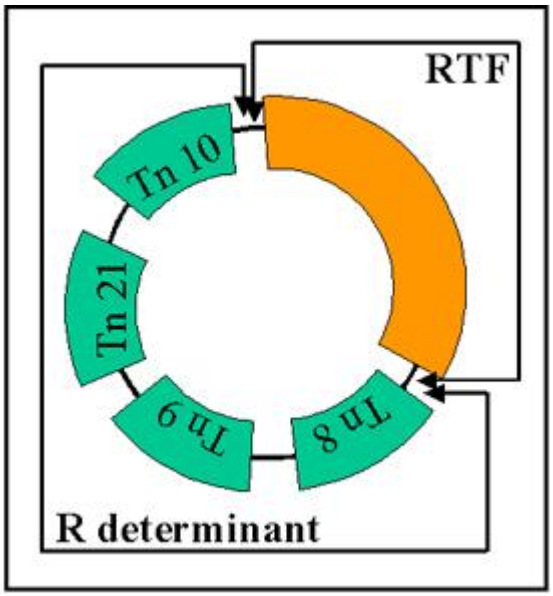


FIGURE 1.7.16: R PLASMID STRUCTURE: COURTESY: DR. AVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CA

RTF (Resistance Transfer Factor)
Carries the transfer genes.

R determinant
Carries the resistance genes. The resistance genes are often parts of transposons.

Mode of action of resistance genes
a) Modification (detoxification) of antibiotic - e.g. β-lactamase

b) Alteration of target site - e.g. Streptomycin resistance

c) Alteration of uptake - Tetracycline resistance

d) Replacement of sensitive pathway - e.g. new folic acid pathway for resistance to sulfa drugs

4.1 REGULATION OF GENE EXPRESSION

Bacteria do not make all the proteins that they are capable of making all of the time. Rather, they can adapt to their environment and make only those gene products that are essential for them to survive in a particular environment. For example, bacteria do not synthesize the enzymes needed to make tryptophan when there is an abundant supply of tryptophan in the environment. However, when tryptophan is absent from the environment the enzymes are made. Similarly, just because a bacterium has a gene for resistance to an antibiotic does not mean that that gene will be expressed. The resistance gene may only be expressed when the antibiotic is present in the environment.

Bacteria usually control gene expression by regulating the level of mRNA transcription. In bacteria, genes with related function are generally located adjacent to each other and they are regulated co-ordinately (i.e. when one is expressed, they all are expressed). Co-ordinate regulation of clustered genes is accomplished by regulating the production of a polycistronic mRNA (i.e. a large mRNA containing the information for several genes). Thus, bacteria are able to "sense" their environment and express the appropriate set of genes needed for that environment by regulating transcription of those genes.

KEY WORDS

Coordinate gene expression

Polycistronic m-RNA

Promoter

Operon

Inducible operon

Inducer

Structural gene

Regulatory gene

Repressor

Operator

Negative control

Catabolite répression

CAP protein

Positive control

Repressible operon

Co-repressor

Apo-repressor

Attenuation

Leader region

Feedback inhibition

Epigenetic modification

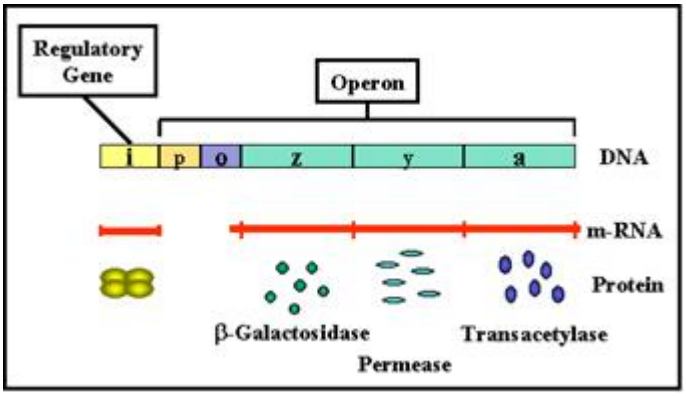


Figure 1.7.17: The lactose operon

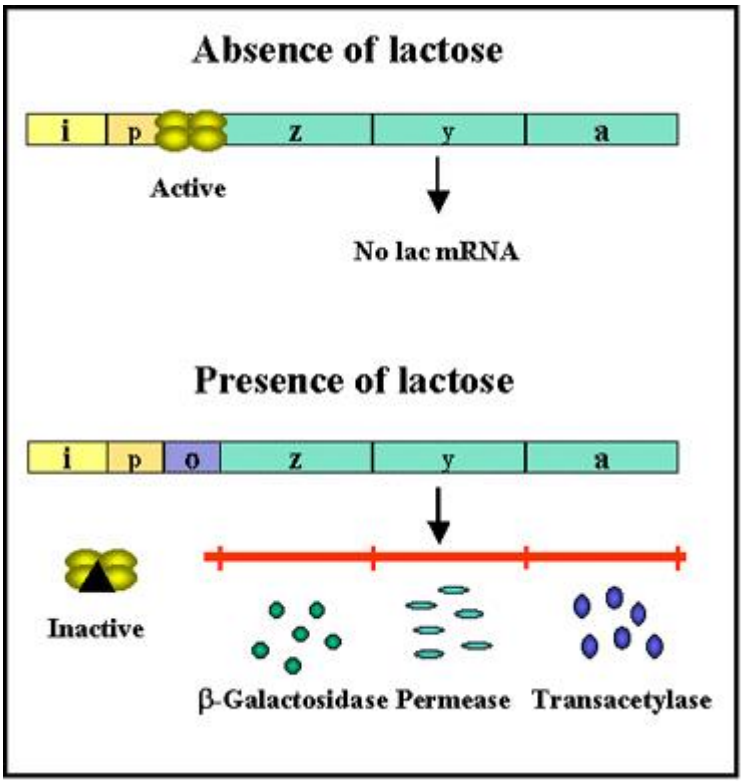


Figure 1.7.18: Transcription of lac genes in the presence and absence of glucose

Inducible genes - The operon model

Courtesy: Albert Lehninger Biochemistry Text Book

Definition

An inducible gene is a gene that is expressed in the presence of a substance (an inducer) in the environment. This substance can control the expression of one or

more genes (structural genes) involved in the metabolism of that substance. For example, lactose induces the expression of the lac genes that are involved in lactose metabolism. A certain antibiotic may induce the expression of a gene that leads to resistance to that antibiotic.
Induction is common in metabolic pathways that result in the catabolism of a substance and the inducer is normally the substrate for the pathway.

Lactose Operon

Structural genes

The lactose operon (figure 1.7.17) contains three structural genes that code for enzymes involved in lactose metabolism.

- The lac z gene codes for β-galactosidase, an enzyme that breaks down lactose into glucose and galactose
- The lac y gene codes for a permease, which is involved in uptake of lactose
- The lac a gene codes for a galactose transacetylase.

These genes are transcribed from a common promoter into a polycistronic mRNA, which is translated to yield the three enzymes.

Regulatory gene

The expression of the structural genes is not only influenced by the presence or absence of the inducer, it is also controlled by a specific regulatory gene. The regulatory gene may be next to or far from the genes that are being regulated.
The regulatory gene codes for a specific protein product called a REPRESSOR.

Operator

The repressor acts by binding to a specific region of the DNA called the operator which is adjacent to the structural genes being regulated. The structural genes together with the operator region and the promoter is called an OPERON.
However, the binding of the repressor to the operator is prevented by the inducer and the inducer can also remove repressor that has already bound to the operator. Thus, in the presence of the inducer the repressor is inactive and does not bind to the operator, resulting in transcription of the structural genes.
In contrast, in the absence of inducer the repressor is active and binds to the operator, resulting in inhibition of transcription of the structural genes. This kind

of control is referred to a NEGATIVE CONTROL since the function of the regulatory gene product (repressor) is to turn off transcription of the structural genes.

Inducer

Transcription of the lac genes is influenced by the presence or absence of an inducer (lactose or other β-galactosides) (Figure 1.7.18).

e.g. + inducer expression

- inducer no expression

CHIME

Image: rotatable molecular structure of the lac repressor bound to the DNA of the lac operon. Requires Netscape and a Chime plug-in.

Figure 1.7.19: Catabolite repression

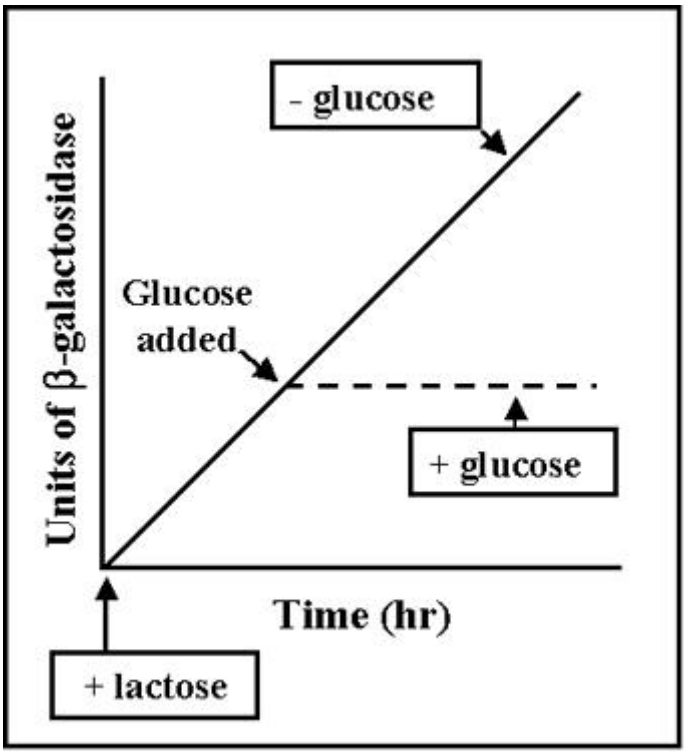


FIGURE 1.7.19

Figure 1.7.20: Effect of glucose on expression of proteins encoded by the lac operon

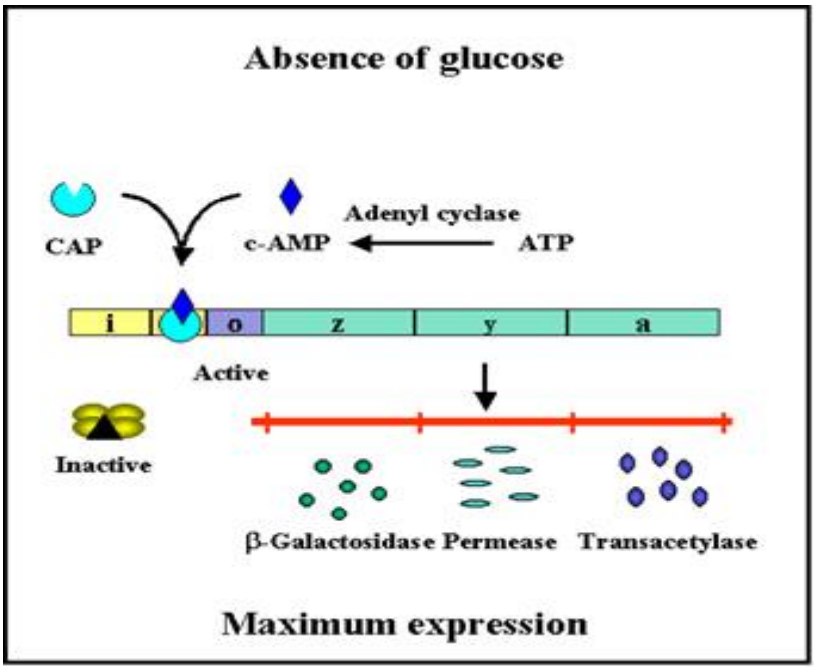
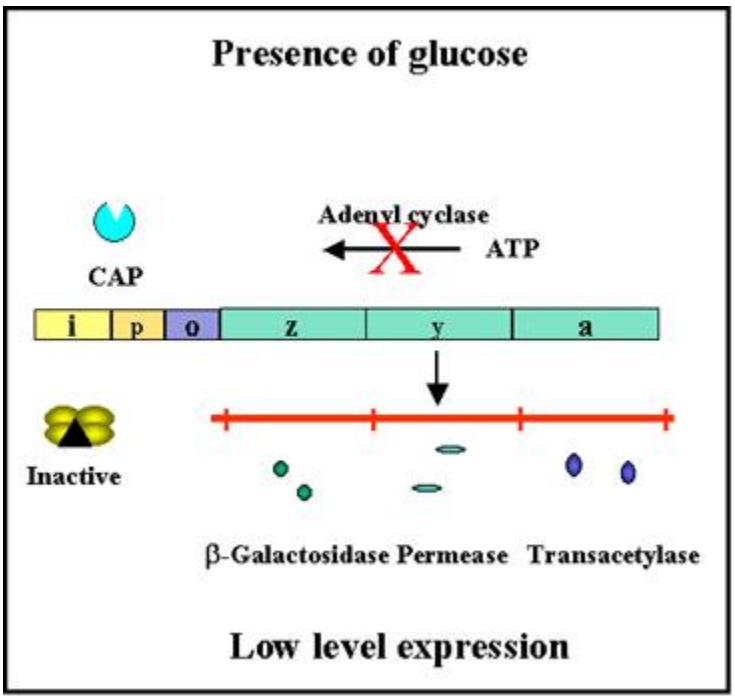


Figure 1.7.21: Effect of glucose on expression of proteins encoded by the lac operon



Courtesy: Albert Lehninger Biochemistry Text book

Catabolite repression (Glucose Effect)

Many inducible operons are not only controlled by their respective inducers and regulatory genes, but they are also controlled by the level of glucose in the environment. The ability of glucose to control the expression of a number of different inducible operons is called CATABOLITE REPRESSION. This is illustrated in Figure 1.7.19.

Catabolite repression is generally seen in those operons which are involved in the degradation of compounds used as a source of energy. Since glucose is the preferred energy source in bacteria, the ability of glucose to regulate the expression of other operons ensures that bacteria will utilize glucose before any other carbon source as a source of energy.

Mechanism

There is an inverse relationship between glucose levels and cyclic AMP (cAMP) levels in bacteria. When glucose levels are high cAMP levels are low and when glucose levels are low cAMP levels are high. This relationship exists because the transport of glucose into the cell inhibits the enzyme adenyl cyclase which produces cAMP. In the bacterial cell cAMP binds to a cAMP binding protein called CAP or CRP. The cAMP-CAP complex, but not free CAP protein, binds to a site in the promoters of catabolite repression-sensitive operons. The binding of the complex results in a more efficient promoter and thus more initiations of transcriptions from that promoter as illustrated in Figures 1.7.20 and 1.7.21. Since the role of the

CAP-cAMP complex is to turn on transcription this type of control is said to be POSITIVE CONTROL. The consequences of this type of control is that to achieve maximal expression of a catabolite repression sensitive operon glucose must be absent from the environment and the inducer of the operon must be present. If both are present, the operon will not be maximally expressed until glucose is metabolized. Obviously, no expression of the operon will occur unless the inducer is present.

Figure 1.7.22 The tryptophan operon

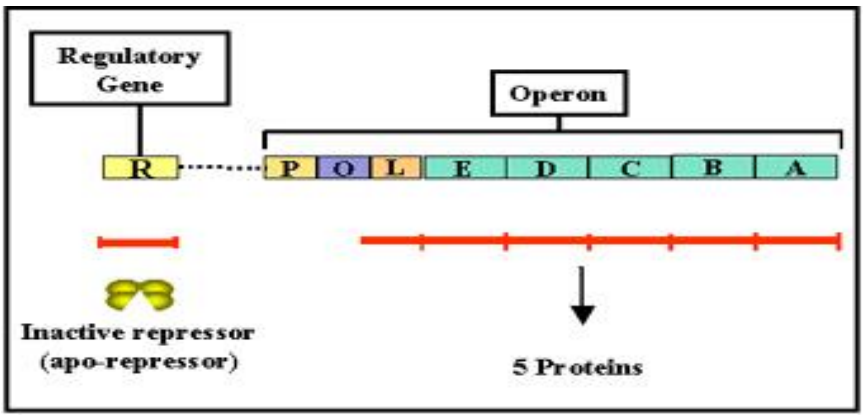
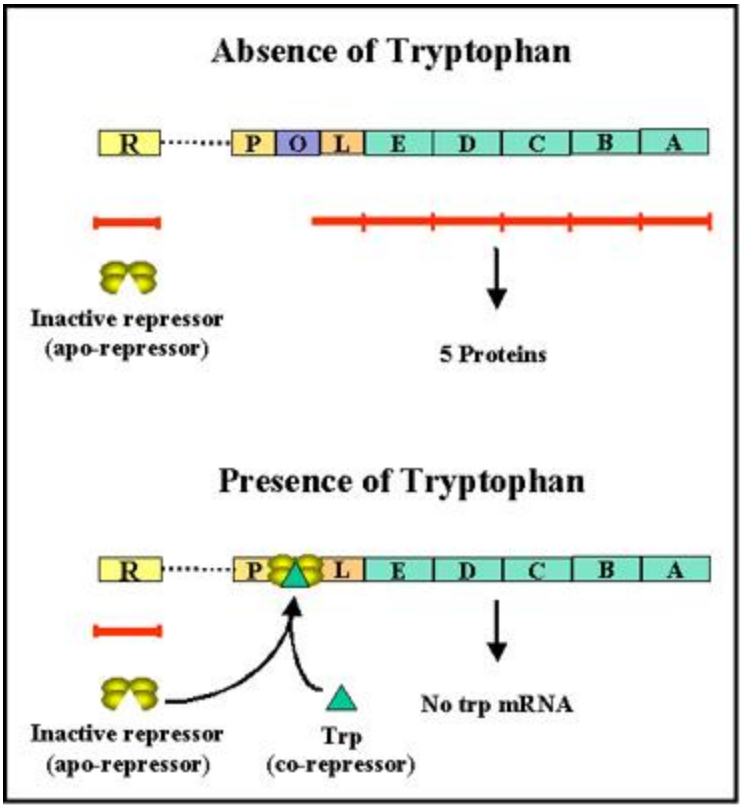


Figure 1.7.23 The effect of tryptophan on expression from the tryp. operon



Courtesy: Albert Lehninger Biochemistry Text Book

Repressible genes - The operon model

Definition

Repressible genes are those in which the presence of a substance (a co-repressor) in the environment turns off the expression of those genes (structural genes) involved in the metabolism of that substance.
e.g., Tryptophan represses the expression of the trp genes.
Repression is common in metabolic pathways that result in the biosynthesis of a substance and the co-repressor is normally the end product of the pathway being regulated.

Operon

Structural genes

The tryptophan operon (figure 1.7.22) contains five structural genes that code for enzymes involved in the synthesis of tryptophan. These genes are transcribed from a common promoter into a Polycistronic mRNA, which is translated to yield the five enzymes.

Regulatory gene

The expression of the structural genes is not only influenced by the presence or absence of the co-repressor, it is also controlled by a specific regulatory gene. The regulatory gene may be next to or far from the genes that are being regulated. The regulatory gene codes for a specific protein product called an REPRESSOR (sometimes called an apo-repressor). When the repressor is synthesized it is inactive. However, it can be activated by complexing with the co-repressor (i.e. tryptophan).

Operator

The active repressor/co-repressor complex acts by binding to a specific region of the DNA called the operator which is adjacent to the structural genes being regulated. The structural genes together with the operator region and the promoter is called an OPERON. Thus, in the presence of the co-repressor the repressor is active and binds to the operator, resulting in repression of transcription of the structural genes. In contrast, in the absence of co-repressor the repressor is inactive and does not bind to the operator, resulting in transcription of the structural genes. This kind of control is referred to as NEGATIVE CONTROL since the function of the regulatory gene product (repressor) is to turn off transcription of the structural genes.

Co-repressor

Transcription of the tryptophan genes is influenced by the presence or absence of a co-repressor (tryptophan) (Figure 1.7.23).

e.g. + co-repressor no expression

- co-repressor expression

Figure 1.7.24 Mechanism of attenuation

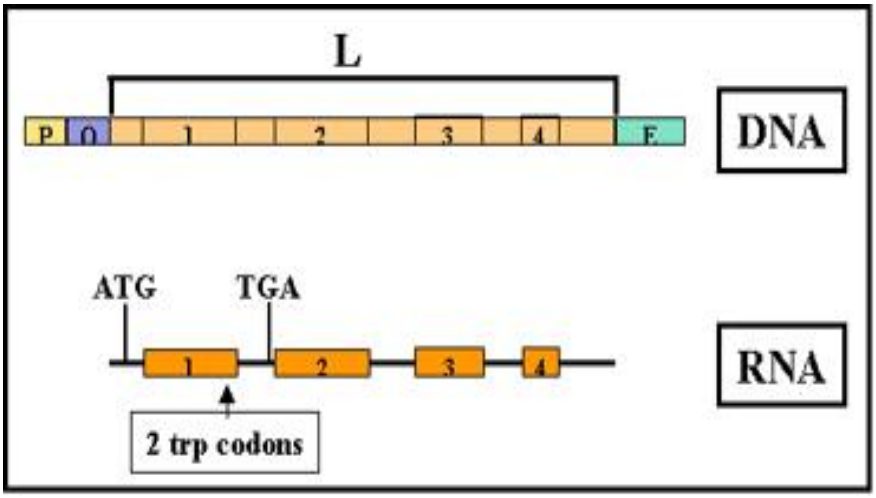
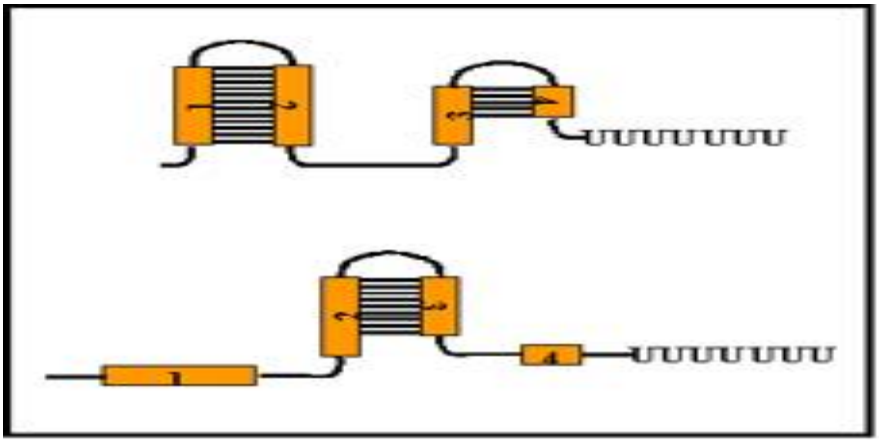


Figure 1.7.25 Formation of stem-loops



Attenuation

In many repressible operons, transcription that initiates at the promoter can terminate prematurely in a leader region that precedes the first structural gene. (i.e. the polymerase terminates transcription before it gets to the first gene in the operon). This phenomenon is called ATTENUATION; the premature termination of Transcription. Although attenuation is seen in a number of operons, the mechanism is best understood in those repressible operons involved in amino acid biosynthesis. In these instances, attenuation is regulated by the availability of the cognate aminoacylated t-RNA.

Mechanism (See Figure 1.7.24)

When transcription is initiated at the promoter, it actually starts before the first structural gene and a leader transcript is made. This leader region contains

a start and a stop signal for protein synthesis. Since bacteria do not have a nuclear membrane, transcription and translation can occur simultaneously. Thus, a short peptide can be made while the RNA polymerase is transcribing the leader region. The test peptide contains several tryptophan residues in the middle of the peptide. Thus, if there is a sufficient amount of tryptophanyl-t-RNA to translate that test peptide, the entire peptide will be made and the ribosome will reach the stop signal. If, on the other hand, there is not enough tryptophanyl-t-RNA to translate the peptide, the ribosome will be arrested at the two tryptophan codons before it gets to the stop signal.

The sequence in the leader m-RNA contains four regions, which have complementary sequences (Figure 1.7.25). Thus, several different secondary stem and loop structures can be formed. Region 1 can only form base pairs with region 2; region 2 can form base pairs with either region 1 or 3; region 3 can form base pairs with region 2 or 4; and region 4 can only form base pairs with region 3. Thus three possible stem/loop structures can be formed in the RNA.

region 1: region 2

region 2: region 3

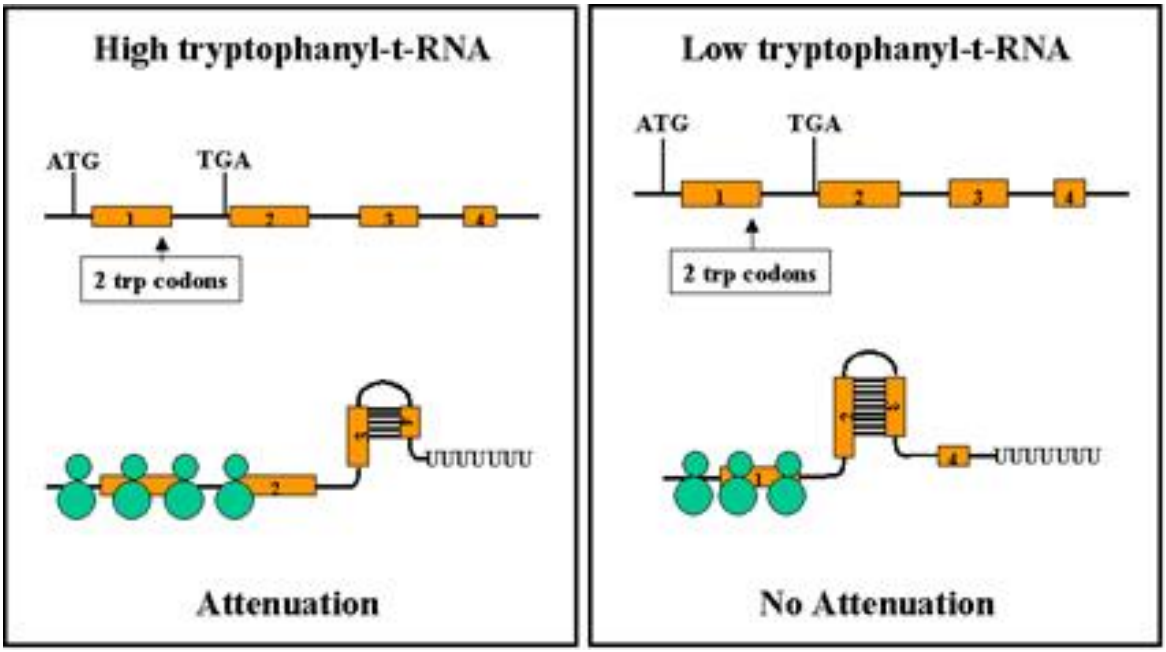
region 3: region 4

One of the possible structures (region 3 base pairing with region 4) generates a signal for RNA polymerase to terminate transcription (i.e. to attenuate transcription). However, the formation of one stem and loop structure can preclude the formation of others. If region 2 forms base pairs with region 1 it is not available to base pair with region 3. Similarly if region 3 forms base pairs with region 2 it is not available to base pair with region 4.

The ability of the ribosomes to translate the test peptide will affect the formation of the various stem and loop structures Figure 1.7.26. If the ribosome reaches the stop signal for translation it will be covering up region 2 and thus region 2 will not be available for forming base pairs with other regions. This allows the generation of the transcription termination signal because region 3 will be available to pair with region 4. Thus, when there is enough tryptophanyl-t-RNA to translate the test peptide attenuation will occur and the structural genes will not be transcribed. In contrast, when there is an insufficient amount of tryptophanyl-t-RNA to translate the test peptide no attenuation will occur. This is because the ribosome will stop at the two tryptophan codons in region 1, thereby allowing region 2 to base pair with region 3 and preventing the formation of the attenuation signal

(i.e. region 3 base paired with region 4). Thus, the structural genes will be transcribed.

Figure 1.7.26 Mechanism of attenuation



Courtesy: Albert Lehninger Biochemistry Text Book

4.2 REGULATION OF ENZYME ACTIVITY

Bacteria also have ways of regulating the activities of their enzymes.

Feedback Inhibition

The activity of bacterial enzymes is often subject to feedback inhibition. Usually it is the end product of a pathway that is the inhibitor and the first enzyme in the pathway is the step that is regulated.

Epigenetic Modification

The activities of bacterial enzymes can also be regulated by covalent modifications of enzymes. Such modifications are called EPIGENETIC MODIFICATIONS.
e.g. Adenylation of glutamine synthetase

Phosphorylation of glycogen synthetase

5.0 Conclusion

Bacteriophage (phage) are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery (i.e., viruses that infect bacteria.). In Bacteria both the Gene expression and enzyme activity are well regulated as we have seen in the above note.

6.0 Summary

- In this unit we have learnt about the composition and structure of Bacteriophage in terms of size, head or capsid and the Tail.

- How Infection of Host Cells occurs through adsorption, irreversible attachment with the aid of some special structures as described over leaf**.**

- Phage Multiplication Cycle such as the virulent or lytic phages and the Lysogenic or Temperate Phage.

- Events Leading to Lysogeny and that Leading to Termination of Lysogeny.

- Gene Transfer Mechanisms in Bacteria such as- Transformation Transduction and Conjugation in details we have seen.

- Transposable Genetic elements and types of these transposable Genetic Elements.
- Extrachromosomal Plasmids which are self-replicating as fully explained above.

- Regulation of Gene Expression and Regulation of Enzyme Activity in Bacteria.

8.0 Tutor-Marked Assignment

A) What is a Bacteriophage**?**

b) Explain briefly the following;

c) Temperate phage

d) Transduction

e) Conjugation

f) What are the events Leading to Lysogeny and those Leading to its Termination?

g) What is an Operon?

8.0 References/Further Reading

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UNIT 8: THE GENERAL ASPECTS OF BACTERIAL PATHOGENESIS;

1.0 Introduction
2.0 Objectives
3.0 Contents
3.1 Exotoxins
3.2 Endotoxins,
3.4 Transmission
3.5 Adhesion,
3.6 Immunopathology

4.0 Conclusion

5.0 Summary

6.0 Tutor-Marked Assignment

7.0 References/Further Reading

1.0 INTRODUCTION: Pathogenesis is a multi-factorial process which depends on the immune status of the host, the nature of the species or strain (virulence factors) and the number of organisms in the initial exposure.

A limited number of bacterial species are responsible for the majority of infectious diseases in healthy individuals. Due to the success of vaccination, antibiotics, and effective public health measures, until recently, epidemics were felt to be a thing of the past. Due to the development of antibiotic resistant organisms, this situation is changing rapidly.

All humans are infected with bacteria (the normal flora) living on their external surfaces (including the skin, gut and lungs). We are constantly also exposed to bacteria (including air, water, soil and food). Normally due to our host defences most of these bacteria are harmless. In compromised patients, whose defences are weakened, these bacteria often cause opportunistic infectious diseases when entering the bloodstream (after surgery, catheterization or other treatment modalities). When initiated in the hospital, these infectious diseases are referred to as nosocomial. Some common bacteria found in the normal flora include Staphylococcus aureus, S. epidermidis and Propionibacterium acnes (found on the skin) and Bacteroides and Enterobacteriaceae found in the intestine (the latter in much smaller numbers).

Koch's postulate

1. The organism must always be found in humans with the infectious disease but not found in healthy ones.

2. The organism must be isolated from humans with the infectious disease and grown in pure culture.

3. The organism isolated in pure culture must initiate disease when re-inoculated into susceptible animals.

4. The organism should be re-isolated from the experimentally infected animals.

Postulates 3. and 4. are extremely important in definite proof of the role of agent in human disease. However, this depends on the ability to develop animal models that resemble the human disease. In many cases such models do not exist.

8.0 OBJECTIVES

At the end of this unit you should be able to

- Explain the term exotoxins in bacteria pathogenesis
- Explain the term Endotoxins in bacteria pathogenesis,
- Do similar explanation in Transmission pathogenesis in bacteria
- Describe Adhesion in bacteria pathogenesis,
- Finally describe the process of Immunopathology

3.0 CONTENT

3.1 Transmission

Specific bacterial species (or strains within a species) initiate infection after being transmitted by different routes to specific sites in the human body. For example, bacteria are transmitted in airborne droplets to the respiratory tract, by ingestion of food or water or by sexual contact.

3.2 Adhesion

Bacterial infections are usually initiated by adherence of the microbe to a specific epithelial surface of the host. Otherwise the organism is removed e.g. by peristalsis and defecation (from the gut) ciliary action, coughing and sneezing (from the respiratory tract) or urination (from the urogenital tract).

Adhesion is not non-specific "stickiness". Specific interactions between external constituents on the bacterial cell (adhesins) and on the host cell (receptors) occur i.e. an adhesin-receptor interaction.

S. pyogenes has surface fimbriae which contain two major components the M protein and lipoteichoic acid. The protein fibronectin binds to epithelial cells and fatty acid moieties of lipoteichoic acid in turn interact with fibronectin.

Strains of E. coli with different surface characteristics cause distinct diseases. Among the most thoroughly studied pili are those of uropathogenic E. coli. Certain adhesins present on the tips of fimbriae of E. coli facilitate binding to epithelial cells.

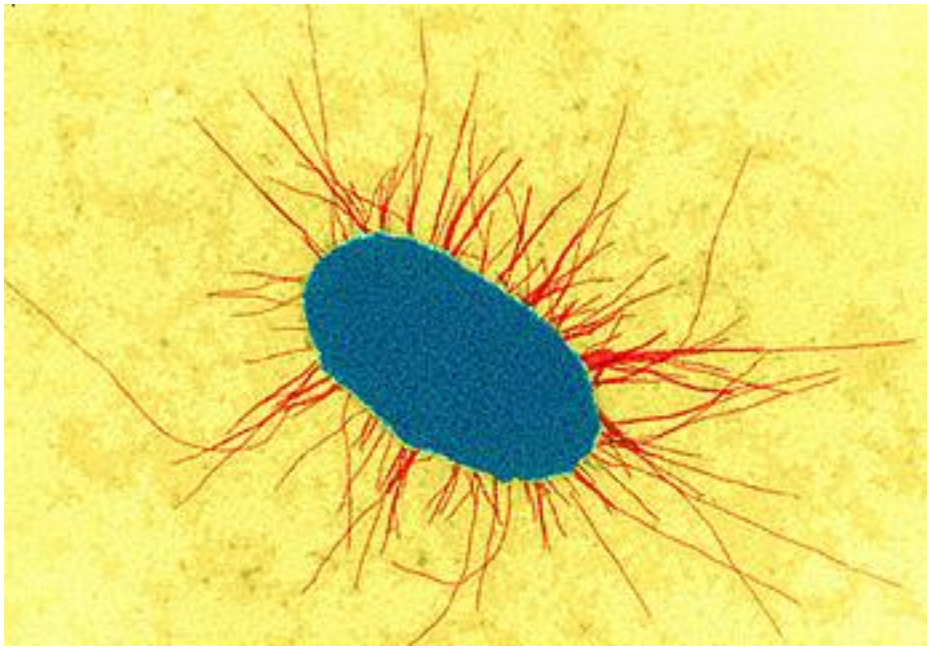

FIGURE 1.8.1

Courtesy: **Bailey and Scott's**: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby,USA

Type 1 fimbriae bind to mannose containing receptors. Whilst P fimbriae allow binding to galactose containing glycolipids (e.g. cerebrosides) and glycoproteins present on epithelial cells. They are referred to as "P" fimbriae since they were originally shown to bind to P blood group antigens on human erythrocytes.

3.3 Penetration and spread

Some bacterial pathogens reside on epithelial surfaces e.g. *Vibrio cholerae*. Other species are able to penetrate these barriers but remain locally. Others pass into the bloodstream or from there onto other systemic sites. This often occurs in the intestine, urinary tract and respiratory tract, and much less commonly through the skin. For example, Shigella penetrates by activating epithelial cells of the intestine to become endocytic; the Shigella do not usually spread into the bloodstream. In other cases, bacteria (e.g. Salmonella typhi) pass through epithelial cells into the bloodstream. Thus, invasion can refer to the ability of an organism to enter a cell, although in some instances it can mean further passage into the systemic vasculature. *Borrelia burgdorferi* is transmitted into the bloodstream through the skin by a tick bite. Certain degradative exotoxins secreted by some bacteria (e.g. hyaluronidase or collagenase) can loosen the connective tissue matrix increasing the ease of passage of bacteria through these sites.

3.4 Survival in the host

Many bacterial pathogens are able to resist the cytotoxic action of plasma and other body fluids involving antibody and complement (classical pathway) or complement alone (alternate pathway) or lysozyme. Killing of extracellular pathogens largely occurs within phagocytes after opsonization (by antibody and/or complement) and phagocytosis. Circumvention of phagocytosis by extracellular pathogens is thus a major survival mechanism. Capsules (many pathogens), protein A (S. aureus) and M protein (S. pyogenes) function in this regard.

Protein A is a surface constituent of S. aureus as well as a secreted product and binds to the Fc portion of immunoglobulins. Bacteria, on binding antibody, activate the classical complement cascade which results in the attachment of fragments of C3. Phagocytosis occurs after binding of the opsonized bacteria to receptors for the Fc portion of IgG or C3 regions. Protein A is anti-complementary (since, on binding to IgG, the complement cascade is activated, depleting complement levels). Thus in the presence of protein A, interaction of bacteria (via bound complement) with C3 receptors will be inhibited. Free protein A binds to the Fc portion of IgG, thus phagocytosis via Fc receptors may not occur because of steric hindrance.

Peptidoglycan, like lipopolysaccharide, can activate the alternate complement cascade. In S. pyogenes peptidoglycan is sufficiently exposed that it is able to bind complement. The M protein of group A streptococcus is the anti-phagocytic component of the fimbriae. M protein binds fibrinogen from plasma which blocks complement binding to the underlying peptidoglycan layer. Thus streptococci in non-immune serum are not phagocytosed.

Intracellular pathogens (both obligate and facultative) must be able to avoid being killed within phagolysozomes. This can occur from by-passing or lysing these vesicles and then residing free in the cytoplasm. Alternatively, they can survive in phagosomes (fusion of phagosomes with lysosomes may be inhibited or the organism may be resistant to degradative enzymes if fusion with lysosomes occurs).

Tissue injury

Bacteria cause tissue injury primarily by several

distinct mechanisms involving:

- Exotoxins
- Endotoxins and non-specific immunity
- Specific humoral and cell mediated immunity

3.5 Exotoxins

Many bacteria produce proteins (exotoxins) that modify, by enzymatic action, or otherwise destroy certain cellular structures. Effects of exotoxins are usually seen acutely, since they are sufficiently potent that serious effects (e.g. death) often result. Examples of this are botulism, anthrax, cholera and diphtheria. If the host survives the acute infection, neutralizing antibodies (anti-toxins) are often elicited that neutralize the effect of the exotoxin. Classes of exotoxins include: Toxins that act on the extracellular matrix of connective tissue e.g. *Clostridium perfringens* collagenase, *Staphylococcus aureus* hyaluronidase.

Toxins that have a cell binding "B" component and an active "A" enzymatic component (A-B type toxins)

These include:

a) Those with ADP-ribosylating activity e.g. cholera toxin, E. coli heat labile toxin, Pseudomonas aeruginosa and diphtheria toxins.

b) Those with a lytic activity on 28S rRNA e.g. *shiga* and *shiga*-like (vero) toxins.

c) Those with a partially characterized site of action e.g. *botulinum* toxin, tetanus toxin and anthrax lethal toxin.

Membrane Damaging Toxins e.g. Staphylococcus aureus delta toxin

Toxins which act extracellularly. These include proteases, collagenases and hyaluronidases. For example, *Clostridium perfringens* produces a potent collagenase, whilst *Staphylococcus aureus* produces a hyaluronidase. Damage to the connective tissue matrix (by hyaluronidase and collagenase) can "loosen up" the tissue fibers allowing the organism to spread through the tissues more readily. Also included in this group is the exfoliatin of *Staphylococcus aureus* which causes separation of the layers within the epidermis and is the causative agent of scalded skin syndrome in the new born.

A - B Toxins. Such toxins consist of two components. One binds to cell surfaces and the other passes into the cell membrane or cytoplasm where it acts. The classical toxins demonstrated to act in this fashion are those of cholera and diphtheria.

(i) ADP-ribosylating exotoxins
Diphtheria toxin (produced by *Corynebacterium diphtheriae*) is coded by the phage tox gene. The toxin is synthesized as one polypeptide chain and readily nicked into two chains held together by a disulfide bond. B binds to cells and A has the enzymatic activity. A is endocytosed and from the endosome passes into the cytosol. Diphtheria toxin ADP-ribosylates elongation factor (EF2) in ribosomes, thus inhibiting protein synthesis. Pseudomonas exotoxin A has an similar mode of action to diphtheria toxin.

Cholera toxin has several subunits which form a ring with one A subunit inserted in the center. B binds to gangliosides on the cell surface and appear to provide a channel through which A penetrates. A1 is formed by proteolytic cleavage and after internalization ADP-ribosylates a cell membrane regulator complex (using NADH as a substrate), in turn causing activation of adenylate cyclase. Activation of adenylate cyclase causes an increase in cyclic AMP

production with resulting decrease in sodium chloride uptake from the lumen of the gut and active ion and water secretion with a watery diarrhoea resulting. E. coli labile toxin has a similar mode of action.

(ii) Toxins that act on 28S rRNA

Shiga toxins (chromosomally encoded) are involved in the pathogenesis of shigellosis, whilst shiga-like toxins (phage encoded) are primarily produced by enterohemorraghic E. coli. They share a common mode of action. A fragment of the A subunit passes to the ribosome where it has N-glycosidase activity on a single adenosine residue; i.e. the bond between the base and ribose is lysed. Diarrhoea results not from active ion/water secretion, but poor water absorption due to death of epithelial cells from inhibition of protein synthesis.

(iii) Partially characterized site of action

Botulinum neurotoxins, tetanospasmin and the lethal toxin of B. anthracis appear to be A-B type exotoxins. Botulinum toxin acts by causing inhibition of release of acetylcholine at the neuromuscular junction. Tetanus toxin is taken up at neuromuscular junctions and transported in axons to synapses. It then acts by inactivating inhibitory neurons. The exotoxins of tetanus and botulism appear to have B components, but the mode of action of their A subunits are not known. The B component of lethal toxin of B. anthracis is the protective antigen; interestingly, this also serves as the B subunit for oedema toxin.

Membrane Damaging Toxins: These toxins enzymatically digest the phospholipid (or protein) components of membranes or behave as detergents. In each case holes are punched in the cell membrane and the cytoplasmic contents can leach out. The phospholipase ("toxin") of C. perfringens is an example of a membrane damaging toxin. It destroys blood vessels stopping the influx of inflammatory cells. This also helps create an anaerobic environment which is important in the growth of this strict anaerobe. The delta toxin of *S. aureus* is an extremely hydrophobic protein that inserts into cell membranes and is believed to have a detergent-like action.

3.6 Endotoxins

Despite the advances of the antibiotic era, around 200,000 patients will develop Gram negative sepsis each year of whom around 25-40% will ultimately die of septic shock. Septic shock involves hypotension (due to tissue pooling of fluids), disseminated intravascular coagulation and fever and is often fatal from massive system failure. This includes lack of effective oxygenation of sensitive tissues such as the brain. There is no effective therapy to reverse the toxic activity of lipid A or peptidoglycan in patients.

Endotoxins are toxic components of the bacterial cell envelope. The classical and most potent endotoxin is lipopolysaccharide. However, peptidoglycan displays many endotoxin-like properties. Certain peptidoglycans are poorly biodegradable and can cause chronic as well as acute tissue injury. Endotoxins are "non-specific" inciters of inflammation. For example, cells of the immune system and elsewhere are stimulated to release cytokines (including interleukin 1 and tumour necrosis factor). Endotoxins also activate the alternate complement pathway. The production of these cytokines results in attraction of polymorphonuclear cells into affected tissues. PG and LPS and certain other cell wall components (e.g. pneumococcal teichoic acid) are also activators of the alternate complement cascade. Thus many bacteria will bind complement encouraging their uptake and killing by phagocytes in the absence of antibody. Certain complement by-products are also chemo attractants for neutrophils. Endotoxins are also potent B cell mitogens, polyclonal B cell activators and adjuvants (for both antibodies and cell mediated immunity); this plays a role in the development of a suitable chronic immune response in handling the microbes if they are not eliminated acutely.

In a "primary" infection during the acute phase "non-antigen specific" immunity will be of utmost importance in eradicating the infection. If the organism persists (or in a reinfection at a later date), specific immunity will be of greater significance in slowing growth of the organisms or in eliminating infection. This is important in chronic infections such as tuberculosis, leprosy, Lyme disease and syphilis.

3.7 Immunopathology

The infected tissue often serves as an innocent bystander and immunopathology results. This can occur in acute and chronic infections. Over stimulation of cytokine production and complement activation by endotoxins can cause tissue injury in the absence of an immune response. Continuously

generated antigens released from persisting viable microbes will subsequently elicit humoral antibodies and cell mediated immunity resulting in chronic immunopathology. Certain poorly degradable antigens (e.g. pneumococcal polysaccharide and group A streptococcal cell walls) can maintain immunopathology even in the absence of persistence of live agents. Other bacterial antigens cross-react with host tissue antigens causing the development of autoimmunity (e.g. the M protein of S. pyogenes cross-reacts with mammalian myosin). Thus immunopathology can persist even after the infection and microbial antigens are eliminated.

The immune system in resistance to infection - examples

1. Extracellular parasites. Antibodies cause lysis of the organism and/or their opsonization by phagocytes at which point they are rapidly killed.

2. Intracellular parasites are primarily killed by cell mediated immunity.

3. Exotoxins can be neutralized by antitoxins. These can be elicited using toxoid vaccines (toxoids are antigenic but not toxic). This occurs, for example, in vaccination against diphtheria.

4. Certain organisms produce IgA proteases (including *H. influenzae, S. pneumoniae, N. gonorrhoeae* and *N. meningitidis*) this helps survival on external surfaces.

4.0 **Conclusion**: Pathogenesis which is intricately linked with exotoxins, endotoxins, transmission, adhesion - is a multi-factorial process which depends on the immune status of the host, the nature of the species or strain (virulence factors) and the number of organisms in the initial exposure. You must be mindful of the fact that the current development of antibiotic resistant organisms, is rapidly changing the earlier success of vaccination, antibiotics and effective public health measures.

5.0 **Summary**

In this unit, we have learnt about pathogenesis in bacteria, whose link are:

- Transmission, that occurs through different routes, air, food, water;

- Exotoxins are bacteria proteins that are modified by enzymatic action and could destroy certain hosts cellular structures;

- Endotoxins, which are toxic components of the bacterial cell envelope; the classical and most potent being lipopolysaccharide;

- Adhesion, which is non-specific "stickiness". Specific interactions between external constituents on the bacterial cell (adhesins) and on the host cell (receptors) resulting in an adhesin-receptor interaction;

- Immunopathology; Continuously generated antigens released from persisting viable microbes elicits humoral antibodies and cell mediated immunity;

6.0 Tutor-Marked Assignment

a. In outlines only, give examples of how the immune system provide resistance to microbial infection.
b. Briefly discuss the following in line with pathogenesis in bacteria

- Transmission
- Exotoxins
- Endotoxins
- Adhesion
- Immunopathology

7.0 References/Further Reading

Schmidt H. Hensel M.: Pathogenicity islands in bacterial pathogenesis. *Clinical Microbiological Review* 17: 14, 2004**.**

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MODULE TWO

UNIT 1: ENTEROBACTERIACEAE

1.0 INTRODUCTION

This group of organisms includes several that cause primary infections of the human gastrointestinal tract. Thus, they are referred to as enterics (regardless of whether they cause gut disorders). Bacteria that affect the gastrointestinal tract include certain strains of *Escherichia coli* and *Salmonella*, all 4 species of *Shigella,* and *Yersinia entercolitica*. The rheumatic disease, Reiter's syndrome (associated with HLA-B27), can result from prior exposure to *Salmonella*, *Shigella*, or *Yersinia*. Other organisms that are not members of the *Enterobacteriacae*, including *Campylobacter* and *Chlamydia*, are also causative agents of Reiter's syndrome. *Yersina pestis* (the cause of "plague") will be considered separately with other zoonotic organisms.

Members of this family are major causes of opportunistic infection (including septicaemia, pneumonia, meningitis and urinary tract infections). Examples of genera that cause opportunistic infections are: *Citrobacter, Enterobacter, Escherichia, Hafnia, Morganella, Providencia* and *Serratia*. Selection of antibiotic therapy is complex due to the diversity of organisms.

Some of the organisms additionally cause community-acquired disease in otherwise healthy people. *Klebsiella pneumoniae* is often involved in respiratory infections. The organism has a prominent capsule aiding pathogenicity. The commonest community acquired ("ascending") urinary tract infection is caused by *E. coli*. The vast majority of urinary tract infections are ascending, often from faecal contamination. Proteus is another common cause of urinary tract infection; the organism produces a urease that degrades urea producing an alkaline urine.

9.0 OBJECTIVES

At the end of this unit you should be able to describe

Isolation and identification of Enterobacteriaceae, Vibrio, Campylobacter, and Helicobacter species of bacteria.

10.0 CONTENTS

3.1 *ENTEROBACTERIACEAE* are Gram-negative facultative anaerobic rods. They lack cytochrome oxidase and are referred to as oxidase-negative. They are often isolated from faecal matter on agar containing lactose and a pH indicator. Colonies that ferment lactose will produce sufficient acid to cause a colour shift in the indicator (Figure 2.1.1).

Escherichia coli is a fermenter of lactose, while *Shigella, Salmonella* and *Yersinia* are non-fermenters. "Non-pathogenic" strains of *E. coli* (and other lactose-positive enterics) are often present in normal faeces. Since they are difficult to differentiate from "pathogenic" E. coli, lactose-negative colonies are often the only ones identified in faeces. All Enterobacteriaceae isolated from other sites (which contain low numbers of bacteria (e.g. urine) or are normally sterile (e.g. blood)) are identified biochemically, for example using the API 20E system. Important serotypes can be differentiated by their O (lipopolysaccharide), H (flagella) and K (capsular) antigens. However, serotyping is generally not performed in the routine clinical laboratory.

Figure 2.1.1A Reactions in TSI agar slants.

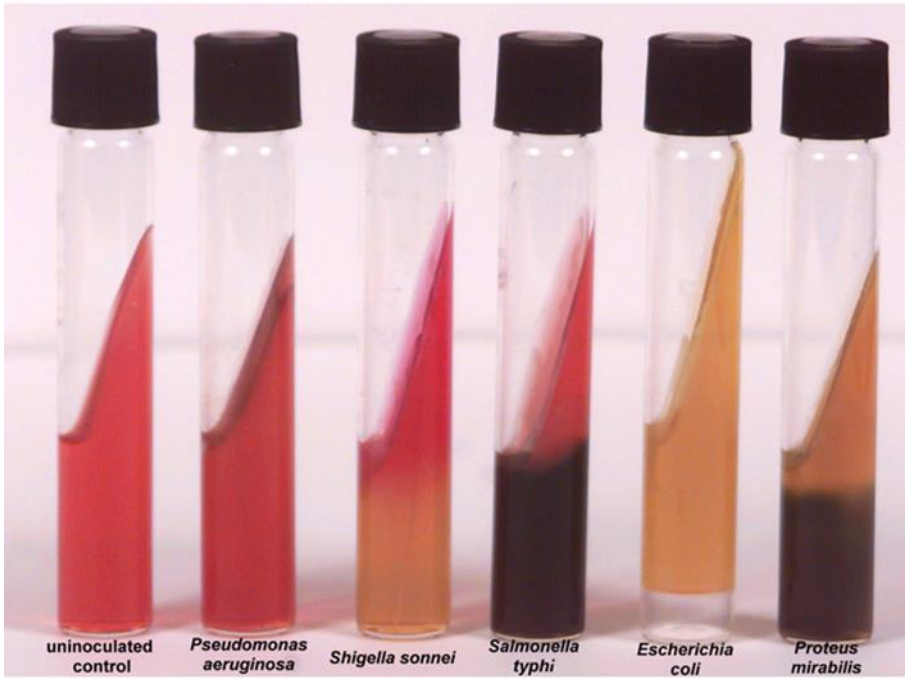
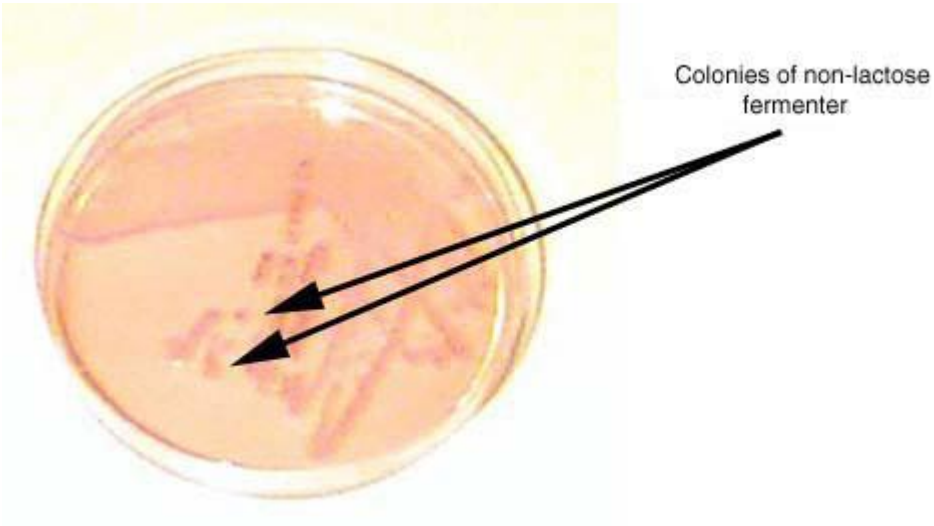


Figure 2.1.1B Nonlactose fermenter on Hektoen agar which contains bile salts and acid indicators (bromthymol blue and acid fuchsin).

The gram-positive bacteria are inhibited so the agar is selective for gram-negative bacteria. The lactose fermenters form orange colonies while the nonfermenters appear green to blue-green. This is especially helpful in distinguishing potential pathogens from normal flora in stool specimens. However, it is difficult to tell the non-fermenters from each other. The organism on this plate could be Salmonella, Proteus, or Shigella.

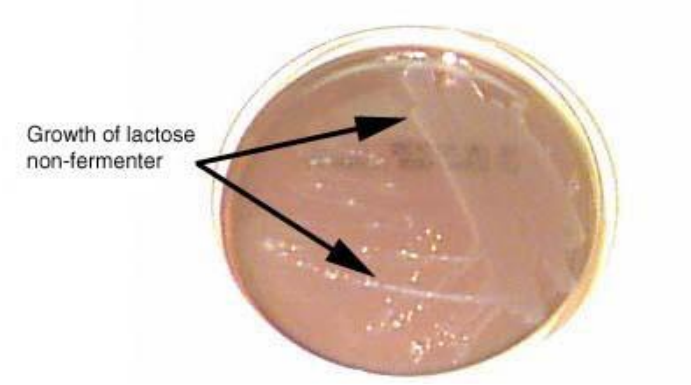
Figure 2.1.1B Growth of a nonlactose fermenter on MacConkey agar which contains bile salts and crystal violet which inhibit the growth of gram-positive bacteria.



Courtesy: Microbiology Department National Hospital Abuja

The agar also contains lactose and a red dye that differentiates the lactose fermenters from the non-fermenters. Colonies of lactose fermenting bacteria are pink to red while the nonfermenters are colourless or transparent. This agar does not distinguish between the non-lactose fermenters; this growth could indicate several organisms - Proteus, Salmonella or Shigella, for example. In a stool specimen, it would be enough evidence to continue with further identification.

Figure 2.1.1C Growth of gram-negative bacteria that cannot ferment lactose on eosin methylene blue (EMB) agar which contains bile salts and dyes which inhibit growth of gram-positive bacteria.



Courtesy: Microbiology Department National Hospital Abuja , 2017.

Growth on EMB agar is a useful diagnostic tool to distinguish between lactose fermenters and non-fermenters which will appear colourless. *Salmonella* and *Shigella*, both non-lactose fermenting pathogens, can be distinguished from the more common intestinal flora which ferment lactose.

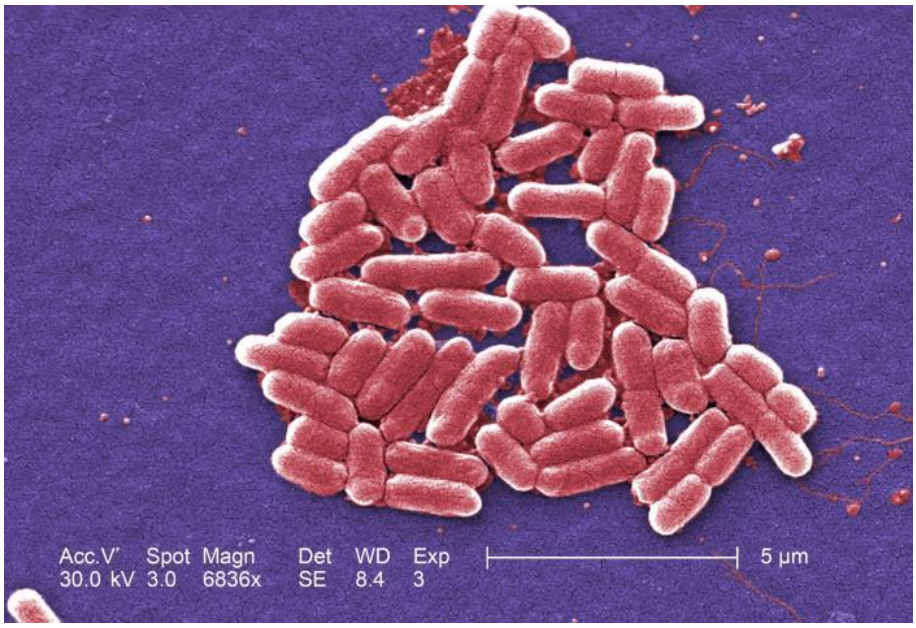


FIGURE 2.1.2 DIAGRAM: **COLORIZED SCANNING ELECTRON MICROGRAPH. GRAM-NEGATIVE ESCHERICHIA COLI BACTERIA OF THE STRAIN**

COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

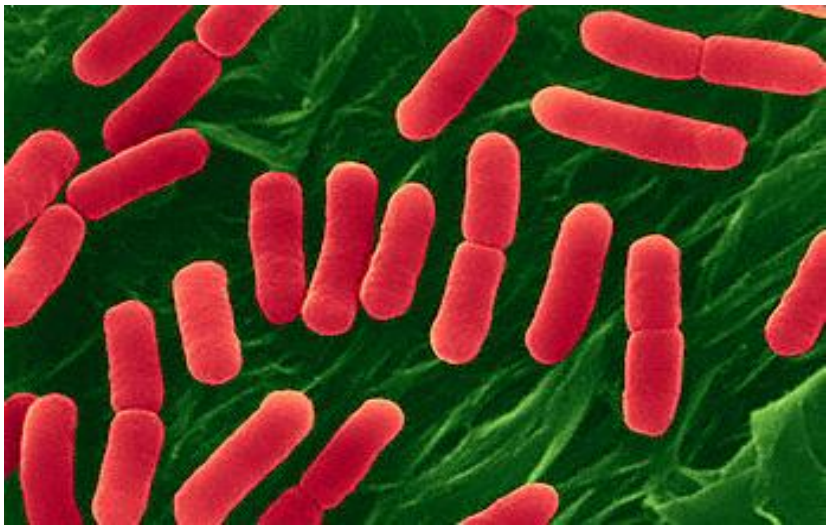


FIGURE 2.1.3 E. COLI HAEMORRHAGIC TYPE. GRAM-NEGATIVE, ENTERIC, FACULTATIVELY ANAEROBIC, ROD PROKARYOTE. POTENTIALLY FATAL TO HUMANS, CONTRACTED WHEN CONTAMINATED MEAT IS COOKED INADEQUATELY.

COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

GASTROENTERITIS, DIARRHOEA AND DYSENTERY

Escherichia coli

E. *coli* (figure 2.1.3) live in the human gut and are usually harmless but some are pathogenic causing diarrhoea and other symptoms as a result of ingestion of contaminated food or water.

At the species level, *E. coli* and *Shigella* are indistinguishable. For practical reasons (primarily to avoid confusion), they are not placed in the same genus. Not surprisingly there is a lot of overlap between diseases caused by the two organisms.

1) Enteropathogenic *E. coli* (EPEC). Certain serotypes are commonly found associated with infant diarrhoea. The use of gene probes has confirmed these strains as different from other groups listed below. There is a characteristic morphological lesion with destruction of microvilli without invasion of the organism which suggests adhesion is important. Clinically, one observes:

•fever

•diarrhoea

•vomiting

•nausea usually with non-bloody stools

2) Enterotoxigenic *E. coli* (ETEC) produce diarrhoea resembling cholera but much milder in degree. They also cause "travellers' diarrhoea". Two types of plasmid-encoded toxins are produced.

•Heat labile toxins which are similar to choleragen (see cholera section below). Adenyl cyclase is activated with production of cyclic AMP and increased secretion of water and ions.

•Heat stable toxins. Guanylate cyclase is activated which inhibits ionic uptake from the gut lumen. Watery diarrhoea, fever and nausea result in both cases.

3) Enteroinvasive *E. coli* (EIEC) produce a dysentery (indistinguishable clinically from shigellosis, see bacillary dysentery below).

4) Enterohemorraghic *E. coli* (EHEC). These are usually serotype O157:H7 (figure 2.1.2, 2.1.3, 2.1.4a). Other kinds of EHEC are sometimes called "non-O157 EHEC". *E. coli* sero groups O26, O111, and O103 are those that most often cause illness in people in the United States. Most non-O157 EHECs cause less severe disease than O157:H7 but a few can cause more severe symptoms. Very often, non-O157 EHECs are not identified and much less is known about them.

Figure 2.1.4A Transmission electron micrograph of *Escherichia coli*

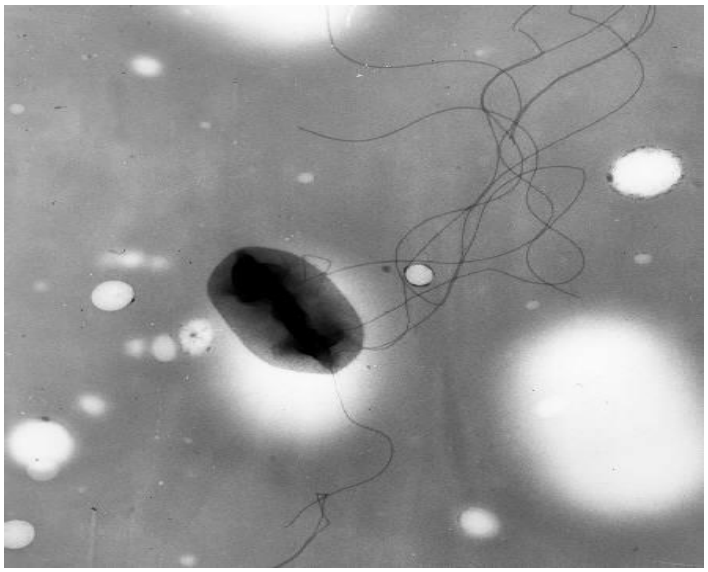
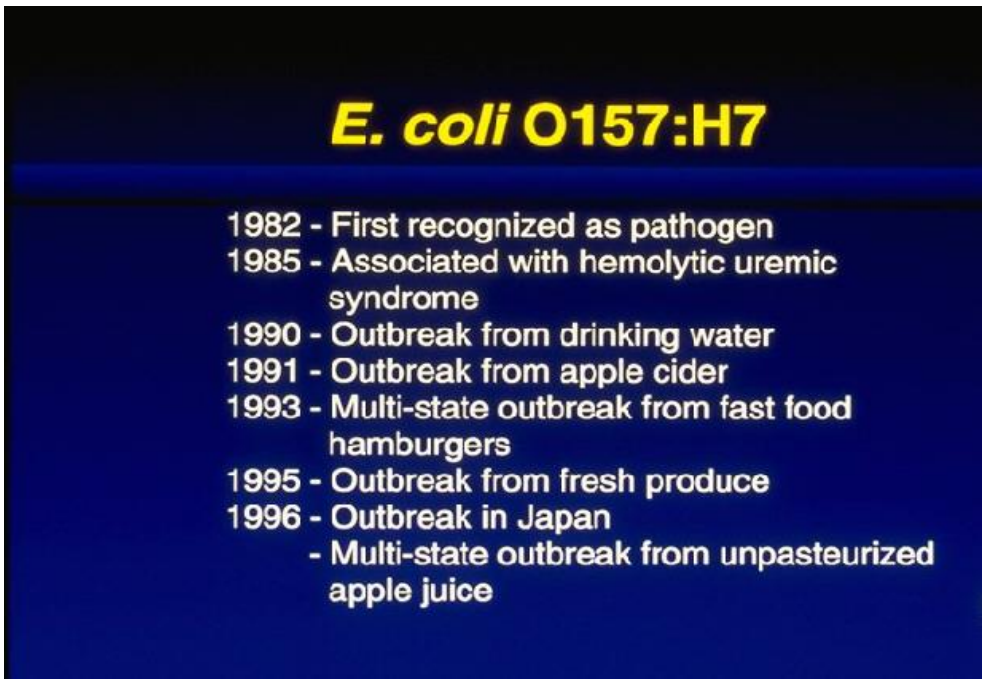


Figure 2.1.4B Chronology of *E. coli* infections, an emerging type of foodborne illness.



CASE REPORT

Multistate Outbreak of *E. coli* O157:H7 Infections Associated with Lebanon *Bologna*

***Courtesy**: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA*

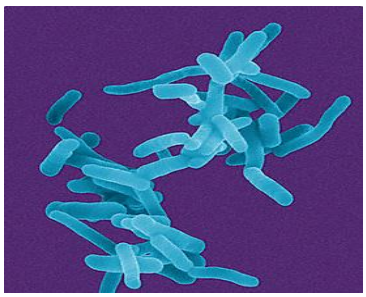
These organisms can produce a haemorrhagic colitis (characterized by bloody and copious diarrhoea with few leukocytes in afebrile patients). However, they are taking on increasing importance (figure 2.1.4b) with the recognition of outbreaks caused by contaminated hamburger meat. The organisms can disseminate into the bloodstream producing systemic haemolytic-uremic syndrome (haemolytic anaemia, thrombocytopenia and kidney failure) which is often fatal. Around 5–10% of those who are diagnosed with EHEC infection develop a potentially life-threatening haemolytic uremic syndrome.

Production of Vero toxin (biochemically similar to Shiga toxin - thus also known as "Shiga-like") is highly associated with this group of organisms. The toxin is encoded by a lysogenic phage. Haemolysins (plasmid-encoded) are also important in pathogenesis.

Since these bacteria make Shiga-like toxins, they are often called “Shiga toxin-producing” *E. coli*, or STEC for short. They are also called Vero cytotoxic *E. coli* (VTEC); these all refer to the same group of bacteria.

As noted above, there are at least four etiologically distinct diseases. However, in the diagnostic laboratory, the groups are not generally differentiated and treatment is based on symptomatology. Usually, fluid replacement is the primary treatment. Antibiotics are generally not used except in severe disease or disease that has progressed to a systemic stage (e.g. haemolytic-uraemia syndrome).

Two major classes of pili are produced by *E. coli*: mannose-sensitive and mannose-resistant pili. The former bind to mannose containing glyocoproteins and the latter to cerebrosides on the host epithelium, allowing attachment. This aids in colonization by *E. coli*.



Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Figure 2.1.5. *Shigella dysenteriae* - Gram-negative, enteric, facultatively anaerobic, rod prokaryote; causes bacterial dysentery. This species is most often found in water contaminated with human faeces.

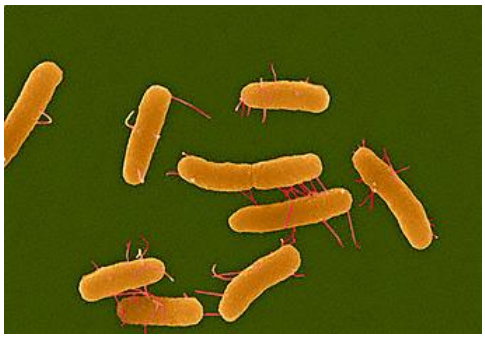
Shigella

There are about 14,000 reported cases of shigellosis in the United States each year but because many milder cases are not diagnosed, the actual number of infections is thought to be at least twenty times greater. Shigellosis is particularly common and causes recurrent problems in settings with poor hygiene where epidemics can occur. It is diagnosed more often in summer than winter with children between the ages of 2 to 4 years being the most prone to infection. Often the disease is seen in child care facilities and many cases are the result of the spread of the illness in families with small children. *Shigella* pass from an infected person to another as they are present in the diarrheal stools. Stools can be infectious while the patient is sick and for up to two weeks after. Most *Shigella* infections are the result of the bacterium passing from stools or soiled fingers of one person to the mouth of another person. Thus, hygiene is important in containing an outbreak. In the developing world, shigellosis is far more common and is present in most communities most of the time.

Shigella (4 species; *S. flexneri, S. boydii, S. sonnei, S*. *dysenteriae* (figure 2.1.5)) all cause bacillary dysentery or shigellosis, (bloody faeces associated with intestinal pain). The organism invades the epithelial lining layer but does not penetrate. Usually within 2 to 3 days, dysentery results from bacteria damaging the epithelial layers lining the intestine, often with release of mucus and blood (found in the faeces) and attraction of leukocytes (also found in the faeces as "pus"). However, watery diarrhoea is frequently observed with no evidence of dysentery. *Shiga* toxin (chromosomally-encoded), which is neurotoxic, enterotoxic and cytotoxic, plays a role. Its enterotoxicity can make the disease clinically appear as a diarrhoea. The toxin inhibits protein synthesis (acting on the 70S ribosome and lysing 28S rRNA). This is primarily a disease of young children occurring by faecal-oral contact. Adults can catch this disease from children, although it can be transmitted by infected adult food handlers who contaminate food. The source in each case is unwashed hands. Man is the only "reservoir".

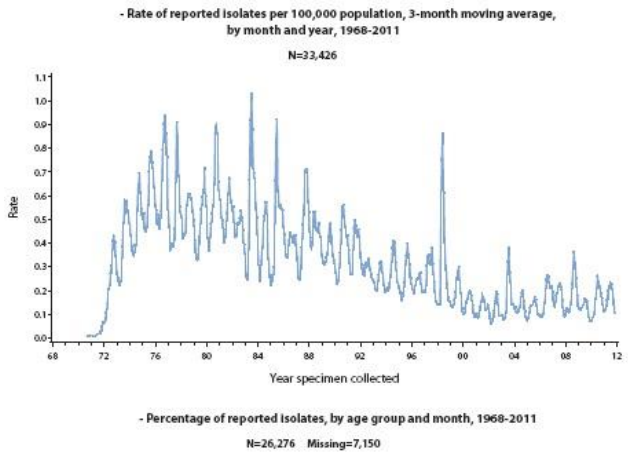
Managing of dehydration is of primary concern. Indeed, mild diarrhoea is often not recognized as shigellosis. Patients with severe dysentery are usually treated with antibiotics (e.g. ampicillin). In contrast to salmonellosis, patients respond to antibiotic therapy and disease duration is diminished.

Figure 2.1.6a. *Salmonella* - rod prokaryote (dividing); note the *flagella*. Causes salmonellosis (food poisoning).



Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Figure 2.1.6b.Rate of reported *Salmonella* isolates in US per 100,000 populations, 3-month moving average by month and year 1968-2011 CDC



Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Figure 2.1.7a Computer-generated image of five drug-resistant *Salmonella* serotype Typhi bacteria based upon scanning electron micrographic image. Note the presence of numerous thin, short fimbriae emanating from the

organisms' cell wall, imparting a furry appearance to these bacteria, and the multiple peritrichous *flagella*.

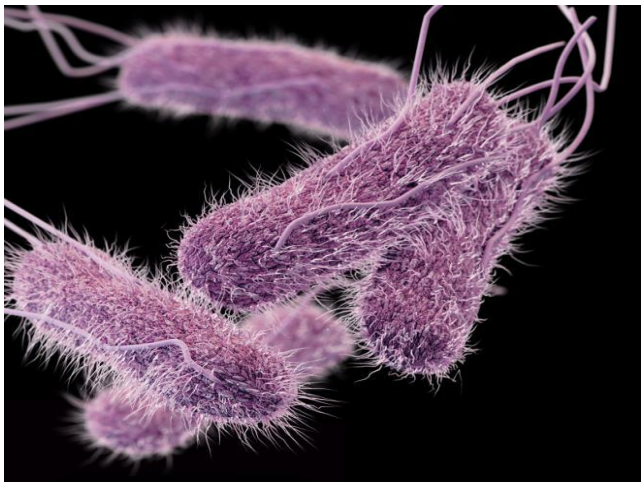


FIGURE 2.1.7A

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Rose spots on the chest of a patient with typhoid fever due to the bacterium *Salmonella typhi.*

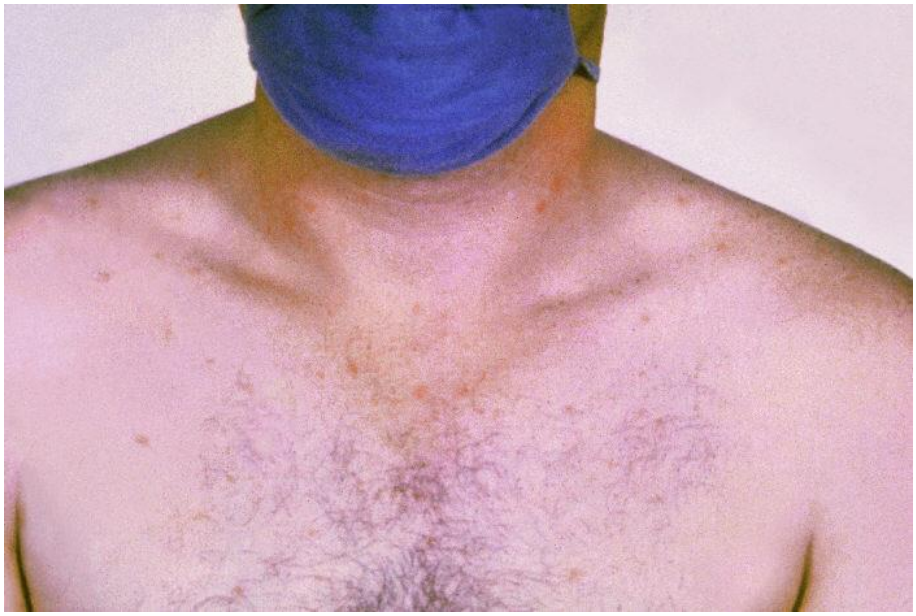


FIGURE 2.1.7B

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Salmonella

It is estimated that *Salmonella* cause more than 1.2 million illnesses each year in the United States, resulting in more than 23,000 hospitalizations and 450 deaths. The overall rate of Salmonellosis is falling in the United States although outbreaks periodically occur (figure 2.1.6b).

Salmonella infections most often cause vomiting or diarrhoea, sometimes severe. In rare cases, *Salmonella* illness can lead to severe and life-threatening bloodstream infections.

Based on genetic studies, there is a single species of *Salmonella* (*Salmonella enterica*) (figure 2.1.6a). At the other extreme using appropriate antibodies, more than 2000 antigenic "types" have been recognized. There are, however, only a few types that are commonly associated with characteristic human diseases (most simply referred to as *Salmonella enteritidis*, *Salmonella cholerae-suis* and *Salmonella typhi*).

Salmonellosis

Salmonellosis, the common *salmonella* infection, is caused by a variety of serotypes (most commonly *S. enteritidis*) and is transmitted from contaminated food (such as poultry and eggs). It does not have a human reservoir and usually presents as a gastroenteritis (nausea, vomiting and non-bloody stools). The disease is usually self-limiting (2 - 5 days). Like *Shigella*, these organisms invade the epithelium and do not produce systemic infection. In uncomplicated cases of salmonellosis, which are the vast majority, antibiotic therapy is not useful. *S. cholerae-suis* (seen much less commonly) causes septicaemia after invasion. In this case, antibiotic therapy is required.

Typhoid

The severest form of *salmonella* infections, "typhoid" (enteric fever), caused by *Salmonella typhi* (figure 2.1.7a), is not often seen in the United States but common in Nigeria and some other sub Saharan African countries, although it is one of the historical causes of widespread epidemics and still is in the third world. It is estimated that about 5,700 cases occur annually in the United States. Most cases (up to 75%) are acquired while traveling internationally. Typhoid fever affects about 21.5 million persons each year in the developing world.

The organism is transmitted from a human reservoir or in the water supply (if sanitary conditions are poor) or in contaminated food. It initially invades the

intestinal epithelium and, during this acute phase, gastrointestinal symptoms are noted. The organisms penetrate (usually within the first week) and passes into the bloodstream where it is disseminated in macrophages. Symptoms of typhoid include a fever up to 103° to 104° F (39° to 40° C). The patient may also feel weak and have stomach pains, headache, and/or loss of appetite. In some cases, patients have a rash of flat, rose-coloured spots. Diagnosis of typhoid fever is carried out from stool or blood samples that are tested for the presence of *Salmonella typhi*.

Typical features of a systemic bacterial infection are seen. The septicaemia usually is temporary with the organism finally lodging in the gall bladder. Organisms are shed into the intestine for some weeks. At this time, gastroenteritis (including diarrhoea) is noted again. The Vi (capsular) antigen plays a role in the pathogenesis of typhoid. A carrier state is common; thus one person (e.g. a food handler) can cause a lot of spread. Antibiotic therapy is essential. Unfortunately, there is increasing resistance to antibiotics, including fluoroquinolones and, as a result there may be increases in case-fatality rates. Epidemics and high endemic disease rates occur in the Central Asian Republics, the Indian subcontinent, and across Asia and the Pacific Islands.

Most people in the United States are not vaccinated against typhoid but those traveling to a country where typhoid is common, should consider being vaccinated against typhoid. There are two vaccines available: Ty21a, taken orally in a capsule and ViCPS, taken by injection. Both need boosters after a number of years.

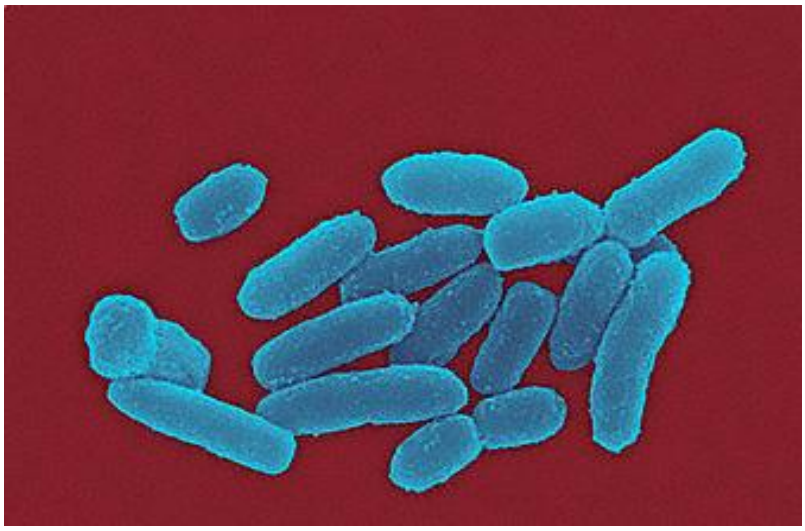


FIGURE 2.1.7C: ***YERSINIA ENTEROCOLITICA*** **- GRAM-NEGATIVE, FACULTATIVELY ANAEROBIC, ROD PROKARYOTE (DIVIDING). THIS BACTERIUM RELEASES A TOXIN THAT CAUSES ENTERITIS WITH PAIN RESEMBLING APPENDICITIS.**

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Yersinia entercolitica

Yersinia entercolitica (figure 2.1.7c) infection (Yersiniosis) is a major cause of gastroenteritis (the main clinical symptom) in Scandinavia and elsewhere and is seen in the United States. The organisms are invasive (usually without systemic spread). Typically, the infection is characterized by diarrhoea, fever and abdominal pain.

Y. enterocolitica infections are seen most often in young children in whom symptoms include:

•fever

•abdominal pain

•diarrhoea, which is often bloody.

These symptoms usually develop 4 to 7 days after infection and can last for one to three weeks or more. In older children and adults' predominant symptoms include:

•right-sided abdominal pain (this may lead to confusion with appendicitis)

•fever

In a few cases, complications including skin rash, joint pains, or bacteraemia can occur. Uncomplicated cases of diarrhoea due to *Y. enterocolitica* usually

resolve without antibiotics but in more severe or complicated infections the use of antibiotics such as aminoglycosides, doxycycline, trimethoprim-sulfamethoxazole, or fluoroquinolones is recommended.

Y. enterocolitica can be transmitted by faecal contamination of water or milk by domestic animals or from eating meat products. It is best isolated by "cold" enrichment: when refrigerated this organism survives while others do not.

Yersinia pseudotuberculosis

A similar, but less severe, disease is caused by *Y. pseudotuberculosis*. The disease is characterized by

•fever

•acute abdominal pain due to

mesenteric lymphadenitis that mimics appendicitis.

Secondary symptoms include

• erythema nodosum

•reactive arthritis (Reiter's Syndrome)

Outbreaks have been reported in Canada, Japan, Finland and Russia (among others). Only in a few of the outbreaks has the vector or source of the infection been identified. Unwashed vegetables including iceberg lettuce and carrots have been implicated by epidemiologic investigations as a source of infection; however, the source of the contamination has not been identified.

3.2 *VIBRIO* SPECIES

Several species of vibrio are known to cause human disease and there are an estimated 80,000 illnesses, 500 hospitalizations and 100 deaths each year in the United States.

Vibrio cholerae

These are Gram-negative rods. They are comma shaped, facultative anaerobes which are oxidase positive. The most important vibrio, Vibrio cholerae (figure

3.1.8), is the causative agent of cholera. It has simple nutritional requirements and is readily cultivated. *V. cholerae* is found in the faeces of an infected individual and ends up in the water supply if sewage is untreated. The organism is thus transmitted by drinking contaminated water. The organism survives in fresh water and, like other vibrios, in salt water. Food, after water contamination, is another means of transmission. Thus, it is primarily a disease of the third world. In the United States, it is observed in the occasional international traveller (especially to parts of Africa, Southeast Asia, or Haiti), although it is sometimes seen after ingestion of seafood. Once in the gut, the organism adheres to the epithelium of the intestine without penetration. Adhesion to the microvilli is thus important in pathogenesis. Cholera toxin is then secreted.

Choleragen (cholera toxin) is chromosomally encoded and contains two types of subunit (A and B). The B subunit binds to gangliosides on epithelial cell surfaces allowing internalization of the A subunit. B subunits may provide a hydrophobic channel through which A penetrates. The A subunit catalyses ADP-ribosylation of a regulator complex which in turn activates adenylate cyclase present in the cell membrane of the epithelium of the gut. The overproduction of cyclic AMP in turn stimulates massive secretion of ions and water into the lumen. Dehydration and death (without treatment) result. Thus, fluid replacement is the major component of treatment. Antibiotic therapy (including tetracycline) is additionally used. Vaccination is only partially effective and not generally recommended. It is most commonly used by international travellers.

Cholera is usually an acute, diarrheal illness. It is often mild or asymptomatic, but sometimes it can be more severe. About 5-10% of infected patients develop severe cholera, the early symptoms of which include (CDC):

•profuse watery diarrhoea, sometimes described as “rice-water stools,”

•vomiting

•rapid heart rate

•loss of skin elasticity

•dry mucous membranes

•low blood pressure

•thirst

•muscle cramps

•restlessness or irritability

This can lead to:

•acute renal failure

•severe electrolyte imbalances

•coma

If untreated, severe dehydration can rapidly lead to shock and death.

As noted above, diarrhoea from people with cholera contains large amounts of infectious bacteria that can contaminate the environment (such as water supplies or food) and infect others, if ingested, thereby spreading the disease. Improved sanitary conditions can prevent the spread of cholera. Washing hands after touching anything that might be contaminated and properly disposing of contaminated items and human waste is essential.

In severe cases of cholera, CDC recommends:
•Oral or intravenous hydration is the mainstay of cholera treatment
•In conjunction with hydration, treatment with antibiotics is recommended for severely ill patients. It is particularly recommended for patients who are severely or moderately dehydrated and continue to pass a large volume of stool during rehydration treatment. Antibiotic treatment is also recommended for all patients who are hospitalized.

•Antibiotic choices should be informed by local antibiotic susceptibility patterns. In most countries, Doxycycline is recommended as first-line treatment for adults, while azithromycin is recommended as first-line treatment for children and pregnant women. During an epidemic or outbreak, antibiotic susceptibility should be monitored through regular testing of sample isolates from various geographic areas.

•None of the guidelines recommend antibiotics as prophylaxis for cholera prevention, and all emphasize that antibiotics should be used in conjunction with aggressive hydration.

•Education of health care workers, assurance of adequate supplies, and monitoring of practices are all important for appropriate dispensation of antibiotics.

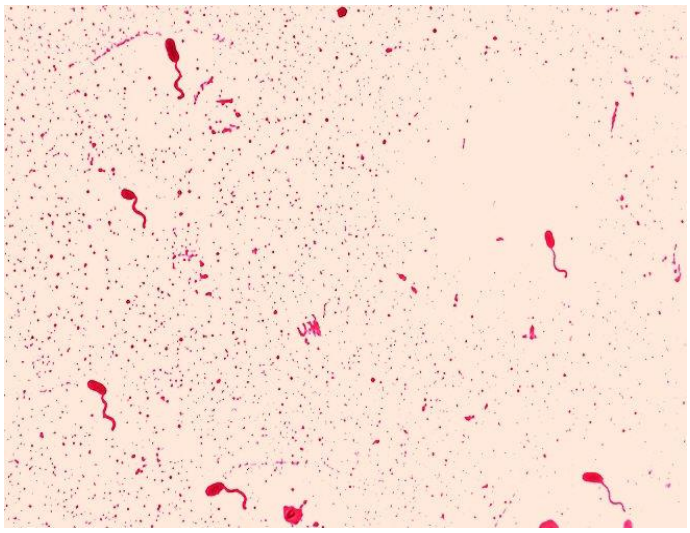


FIGURE 2.1.8A

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

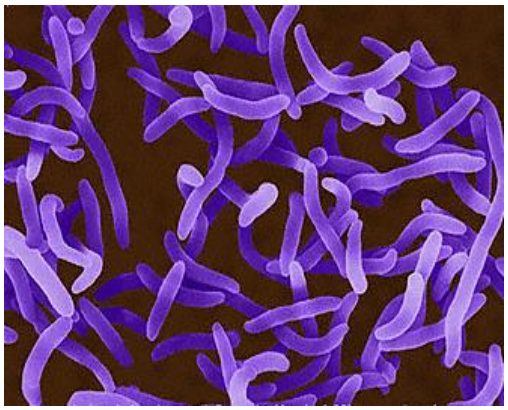


FIGURE 2.1.8B

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Vibrio parahemolyticus (Figure 2.1.9 A&B) is the agent that causes vibriosis and is usually transmitted by ingestion of raw seafood (especially oysters). An estimated 4,500 cases of vibriosis occur each year in the United States.

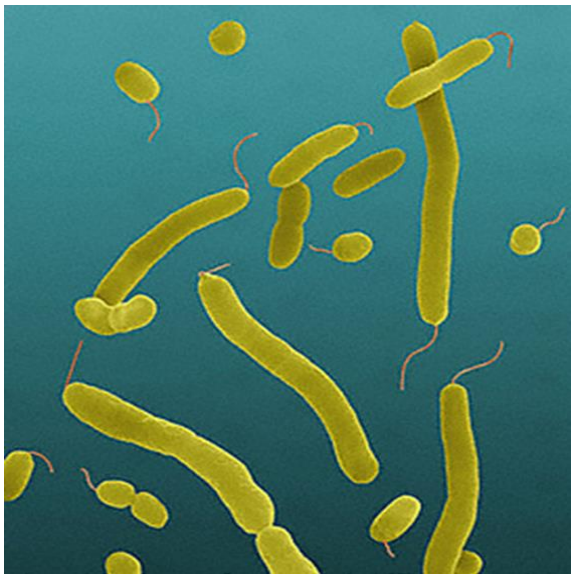


FIGURE 2.1.9A

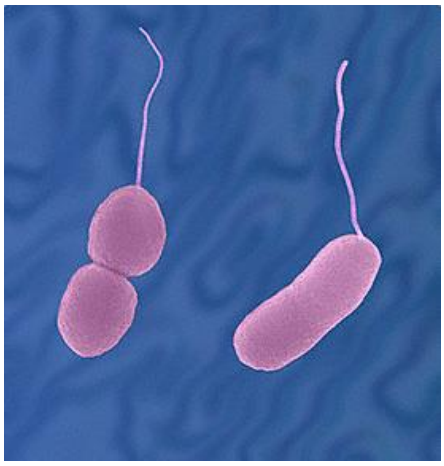


FIGURE 2.1.9B

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The organism lives in brackish saltwater and causes gastrointestinal illness in humans. Vibrio parahemolyticus inhabits coastal waters in Nigeria and Chana and is present in higher concentrations during summer; it grows best in high concentrations of salt (i.e. it is halophilic). A non-bloody diarrhoea is observed but it is not as severe as cholera.

The symptoms of vibriosis are (CDC):

•watery diarrhoea

•frequently with abdominal cramping

•nausea

•vomiting

•fever and chills

Usually these symptoms occur within 24 hours of ingestion of the bacterium. The disease is usually self-limiting and lasts 3 days. Severe disease is rare and occurs more commonly in persons with weakened immune systems. V. parahemolyticus can also cause an infection of the skin when an open wound is exposed to warm seawater.

Diagnosis is by isolation of the bacterium from cultures of stool, wound, or blood. For isolation from stool, the use of a selective medium that has thiosulfate, citrate, bile salts, and sucrose (TCBS agar) is recommended by CDC. Usually treatment is not necessary and there is no evidence that antibiotic treatment decreases the severity or the length of the illness. The patient should be encouraged to drink plenty of water to replace fluids lost through diarrhoea. In severe or prolonged illnesses, antibiotics such as tetracycline or ciprofloxacin can be used.

Vibrio vulnificus

Vibrio vulnificus (figure 2.1.9C) is another salt loving (halophilic) bacterium that causes cases of vibriosis and again disease is caused by eating contaminated raw seafood or exposure of an open wound to contaminated sea water. In the latter case, infection can lead to skin breakdown and ulceration. Between 1988 and 2006, CDC received reports of more than nine hundred V. vulnificus infections from the Gulf Coast states, where most cases occur.

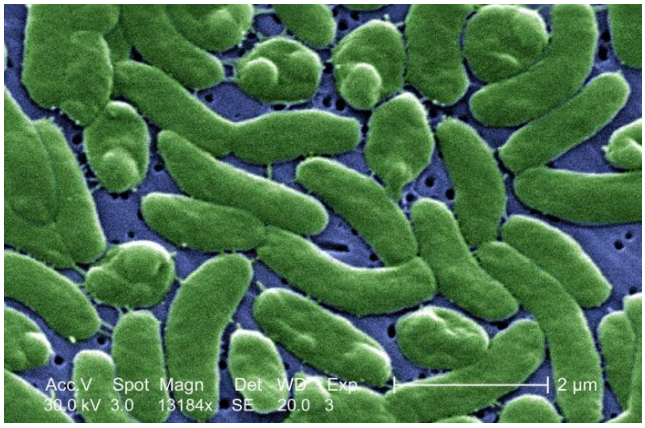

FIGURE 2.1.9C

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Infection by V. vulnificus can cause, in non-immunocompromised people:
•vomiting
•diarrhoea
•abdominal pain
In immunocompromised people, especially those with chronic liver disease, V. vulnificus can infect the bloodstream (bacteraemia), resulting in a severe disease that can be life-threatening. It is characterized by:
•fever and chills
•decreased blood pressure (septic shock)
•blistering skin lesions
About half of *V. vulnificus* bloodstream infections result in death.
Diagnosis is by stool, wound, or blood cultures. If *V. vulnificus* is suspected, treatment should be initiated immediately because antibiotics improve survival with aggressive treatment of the wound site; debridement of infected necrotic tissue or amputation of the infected limb is sometimes necessary. Doxycycline and cephalosporin are indicated or a fluoroquinolones such as levofloxacin, ciprofloxacin or gatifloxacin. Children, in whom doxycycline and fluoroquinolones are contraindicated, can be treated with trimethoprim-sulfamethoxazole plus an aminoglycoside.

3.3 CAMPYLOBACTER

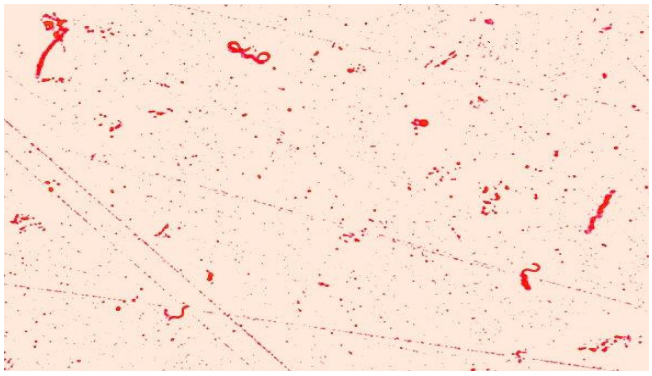

Figure 2.1.10a. *Campylobacter foetus*. Leifson *flagella* stain (digitally colorized).

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

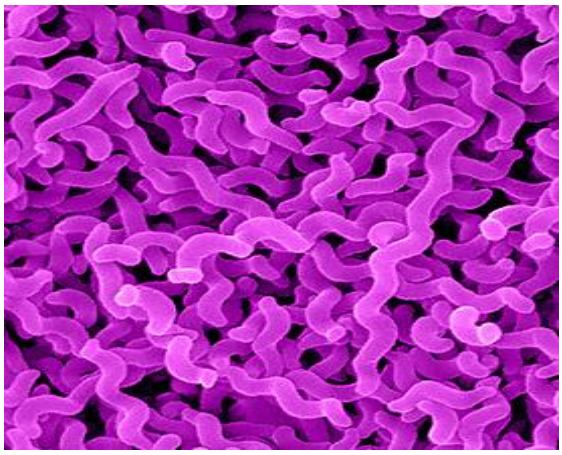

Figure 2.1.10b

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Campylobacter jejuni is an enteric, curved-rod prokaryote (bacterium). It is the bacterium that causes campylobacteriosis, one of the most common bacterial causes of diarrheal illness in the United States. It is a relatively fragile bacterium that is easily killed by cold or hot temperatures. Birds are carriers due to their body temperature being just right to host the bacteria. Improper handling of raw poultry or undercooked fowl is usually the source of infection in humans.

Campylobacter and *Helicobacter*

These two groups of Gram-negative organisms are both curved or spiral shaped and are genetically related.

Campylobacter jejuni

Campylobacteriosis is one of the commonest bacterial disease causing diarrhoea in the United States. There are approximately 14 cases each year per 100,000 populations.

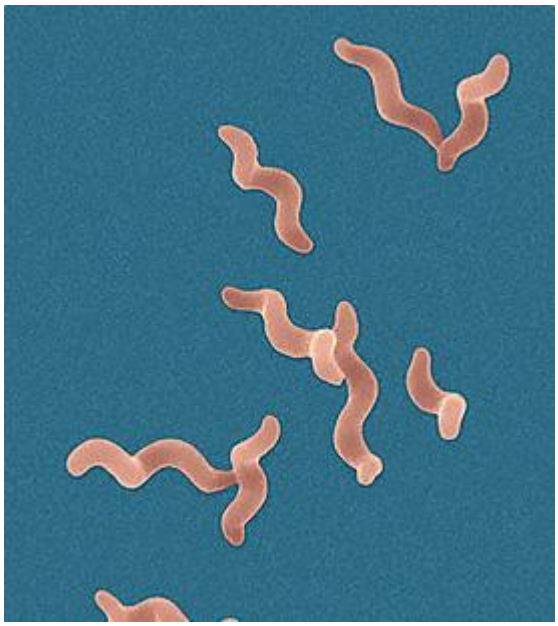

Figure 2.1.10

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However, many cases are not diagnosed and it is estimated that there are over 1.3 million cases annually. Infections occur much more frequently in the summer than in winter and the disease occurs in infants and young adults more often than in older people. It is more often seen in males than females. Campylobacteriosis is rarely fatal but there are approximately 76 deaths in the United States among persons with *Campylobacter* infections each year.

The most common of the *Campylobacter* (figure 2.1.10) causing human disease are *C. jejuni*. The organism infects the intestinal tract of several animal species (including cattle and sheep) and is a major cause of cause of abortions. It is transmitted to man in milk and meat products. Watery diarrhoea predominates but dysentery is common. The organism is invasive but generally less so than Shigella. Malaise, fever and abdominal pain are other disease features. Bacteraemia is observed in a small minority of cases.

Campylobacter infection is diagnosed when a culture of a stool specimen yields the bacterium. The organism is microaerophilic and grows best at 42^{o}C. It is frequently isolated under these conditions using selective media. It can be treated with antibiotics but is usually a self-limiting disease. Patients should drink extra fluids as long as the diarrhoea lasts. Antibiotics are only used to treat patients with severe disease or those at high risk for severe disease. These include patients with immune systems severely weakened from

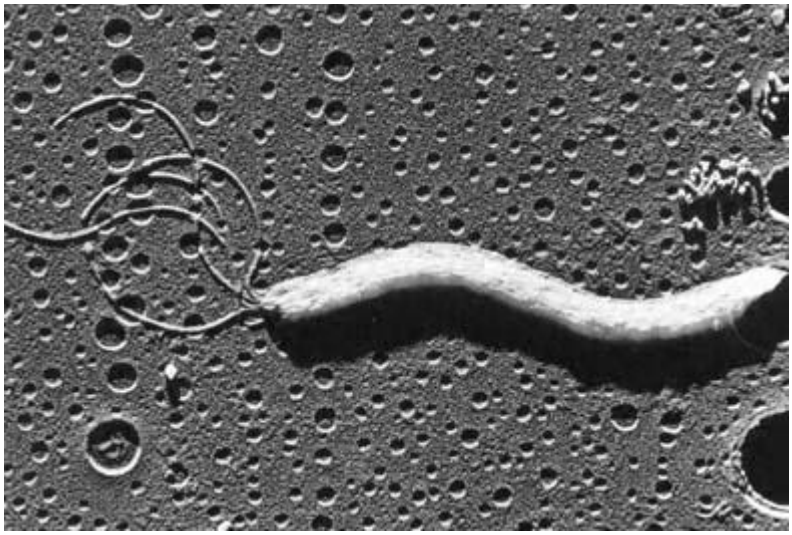
medications or other illnesses. Azithromycin and fluoroquinolones (e.g., ciprofloxacin) are commonly used for treatment of these infections, but resistance to fluoroquinolones is common.

Campylobacteriosis can sometimes have long-term sequelae. These include:
•arthritis.

•Guillain-Barre syndrome. This is a rare disease that affects the nerves of the body beginning several weeks after the diarrheal illness and results from an attack on body's nervous system by the immune system and can result in temporary paralysis for several weeks. It requires intensive medical care. It is estimated that about one in every 1,000 Campylobacter illnesses leads to Guillain-Barre syndrome. As many as 40% of Guillain-Barre syndrome cases in the United States may result from campylobacteriosis.

3.4 *HELICOBACTER PYLORI*

Helicobacter pylori (figure 2.1.11) has been accepted in the last few years as the major cause of stomach ulcers. The organism chronically lives in and on the stomach mucosa of man. Culture is the preferred method of diagnosis but may miss a number of cases. The organism characteristically produces a urease which generates ammonia and carbon dioxide. This aids in detecting and identifying the isolated organism. Urease is produced in such large amounts that it can be directly detected in mucosa sampled after endoscopy.



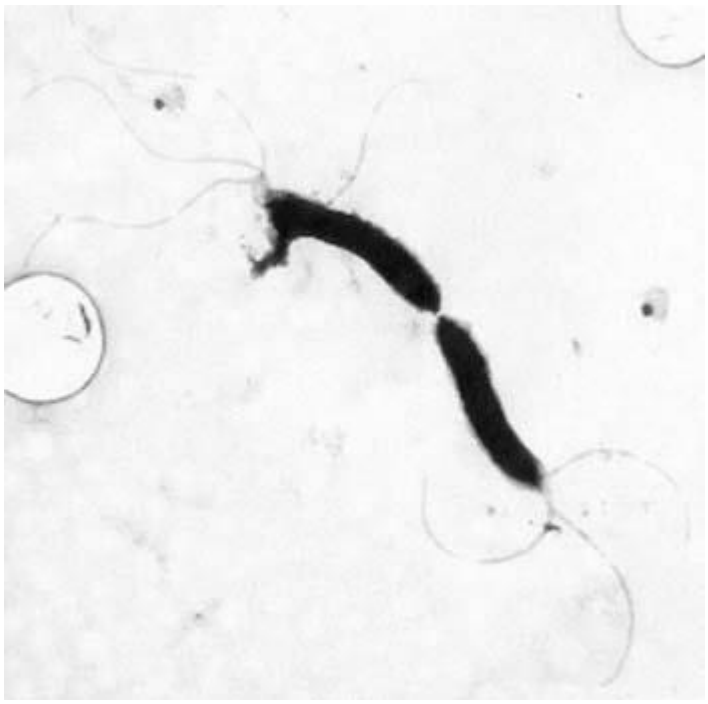


FIGURE 2.1.11

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Alternatively, 13C or 14C labelled CO_2 is detected in the breath after feeding labelled urea. Production of ammonia is a factor in pathogenesis (in locally neutralizing stomach acid). Antibiotic therapy eliminates the organism, peptic ulcers heal and relapses are generally avoided.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Figure 2.1.11b Helicobacter pylori - Gram-negative, spiral to pleomorphic, spiral rod prokaryote. It can move by means of tiny flagella at the end of the cell. There are many strains of H. pylori which are distinguished by the human disease with which they cause. H. pylori infection is the main cause of chronic superficial gastritis and it is associated with both gastric and duodenal ulcers. It lives in the interface between the surface of gastric epithelial cells (the lining of the stomach). It often clusters at the junctions of epithelial cells.

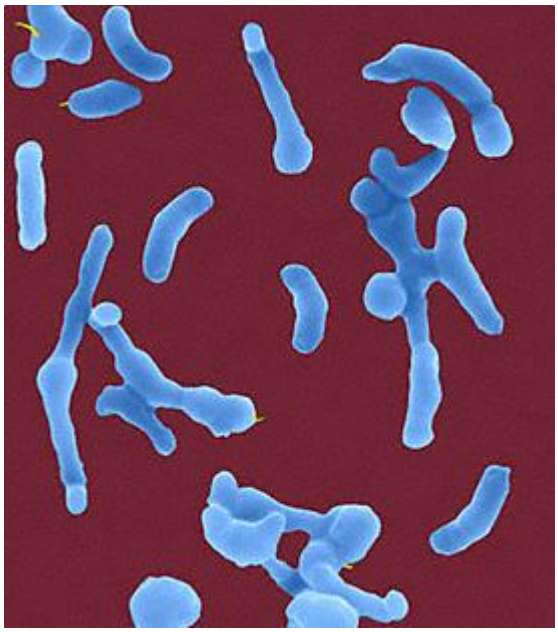


Figure 2.1.11c Helicobacter pylori - Gram-negative, spiral to pleomorphic, spiral rod prokaryote.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

4.0 CONCLUSION

Sanitary measures protect the water supply, avoiding contamination with sewage. This is the primary reason that epidemics with life-threatening pathogens (e.g. cholera and typhoid) are rarely seen in western countries but are commonly seen in the third world such as Nigeria. Other less severe diseases (e.g. salmonellosis, EHEC) are still common from eating contaminated animal products, which has been less well controlled. *Shigella*, which has a human host, would be even more difficult to eradicate. Vaccination is rarely used and, indeed, is an expensive way to go compared to sewage treatment. In severe diarrhoea, fluid replacement is essential. Antibiotic therapy is used in severe local infection and always in systemic disease.

5.0 SUMMARY

In this unit we have learnt about the following Gram Negative bacteria of Public Health importance:

- *Enterobacteriaceae* which are Gram-negative, facultative anaerobic rods, lacking cytochrome oxidase and are referred to as being oxidase-negative. *Escherichia coli* is a fermenter of lactose, while *Shigella*, *Salmonella* and *Yersinia* are non-fermenters (They are mostly dysenteric pathogens found in faecal samples apart from some species of *E-coli*).

- *Vibrio cholerae* -these are Gram-negative rods, comma shaped, facultative anaerobes, oxidase positive, and the most important *vibrio*, *Vibrio cholerae*, is the causative agent of *cholera* characterized with severe rapid body electrolyte lost by stooling and vomiting.

- *Campylobacter* species are enteric, curved-Gram negative rods. Cause campylobacteriosis, one of the most common bacterial causes of diarrhoeal diseases, relatively fragile, killed by cold or hot temperatures; Birds are carriers due to improper handling of raw poultry or undercooked fowl being source of infection in humans.

- *Helicobacter pylori* is the major cause of stomach ulcers. The organism chronically lives in and on the stomach mucosa The organism is Gram negative, urease positive and generates ammonia and carbon dioxide

6.0 Tutor-Marked Assignment

Write a full account on any *Enterobacteriacae* of your choice.

a)Define faecal oral route of infection.

b)What diseases of public Health importance are caused by the following etiologic agents?

I) Vibrio cholerae
ii) Salmonella typhi
III)Shigella spp.
iv) Entero invasive *E-coli*

v) Entero pathogenic *E. coli*

7.0 References/Further Reading

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Unit 2: Groups A, B , D and Viridans Streptococci.

1.0 Introduction

2.0 Objectives

3.0 Contents

3.1 Groups A, B and D streptococcus, viridans streptococci

3.2 Enterococcus faecalis (formerly group D).

3.4 Streptococcus pneumonia

3.5 Staphylococcus infections; food poisoning, toxic shock, MRSA

3.6 Neisseria and Spirochetes

3.7 Anaerobes and Pseudomonas in Opportunistic

3.8 Mycobacteria and Corynebacteria

4.0 Conclusion

5.0 Summary

6.0 Tutor-Marked Assignment

7.0 References/Further Reading

1.0 INTRODUCTION

Streptococci are facultatively anaerobic, Gram-positive organisms that often occur as chains or pairs and are catalase-negative (in contrast, staphylococci are catalase positive). Streptococci are subdivided into groups by antibodies that recognize surface antigens. These groups may include one or more species. The most important group able streptococci are A, B and D.

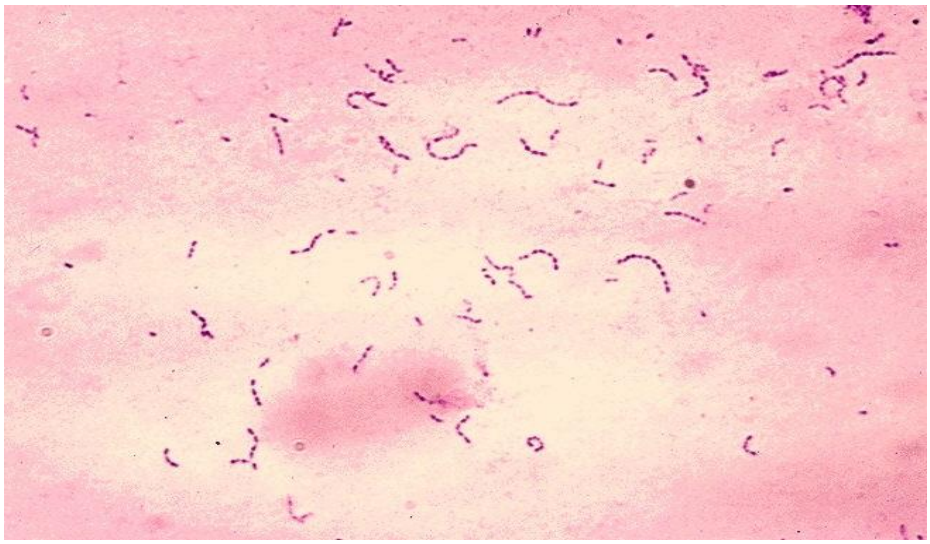

FIGURE 2.2.1

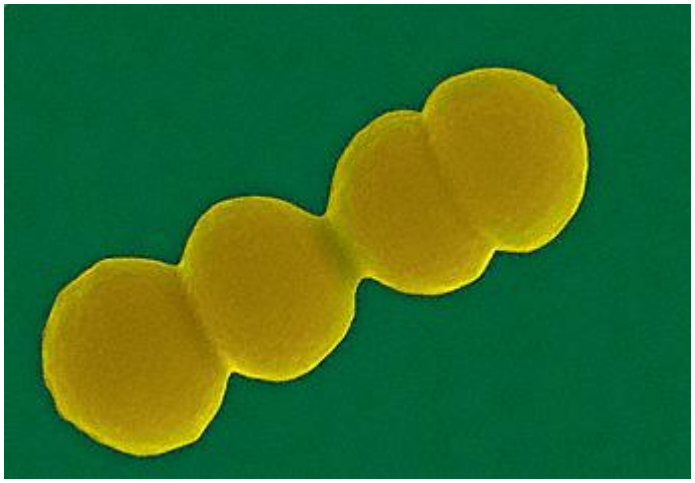

FIGURE 2.2.2

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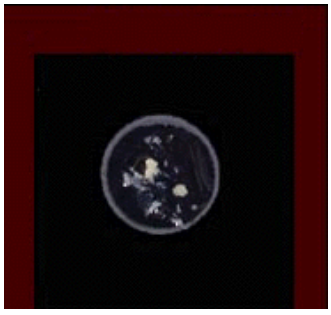

FIGURE 2.2.3

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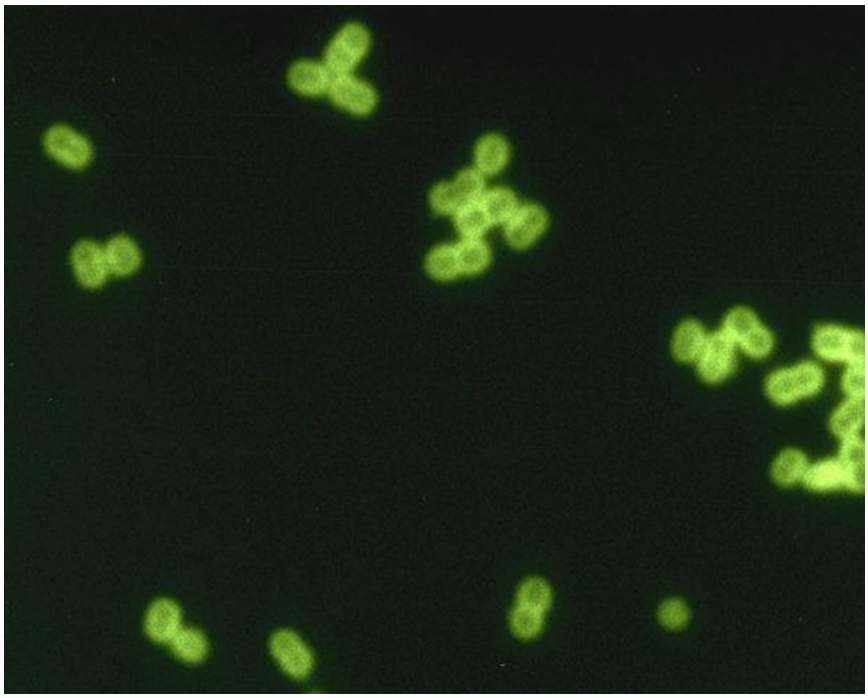

FIGURE 2.2.4:

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Among the groupable streptococci, infectious disease (particularly pharyngitis) is caused by group A which is thus emphasized here. Streptococcus pneumoniae (a major cause of human pneumonia) and Streptococcus mutans and other so-called viridans streptococci (among the causes of dental caries) do not possess group antigens. Three types of haemolysis reaction (alpha, beta, gamma) are seen after growth of streptococci on sheep blood agar. Alpha refers to partial haemolysis with a green coloration (from production of an unidentified product of haemoglobin) seen around the colonies; beta refers to complete clearing and gamma means there is no lysis. Group A and group B streptococci are beta haemolytic, whilst D are usually alpha or gamma.

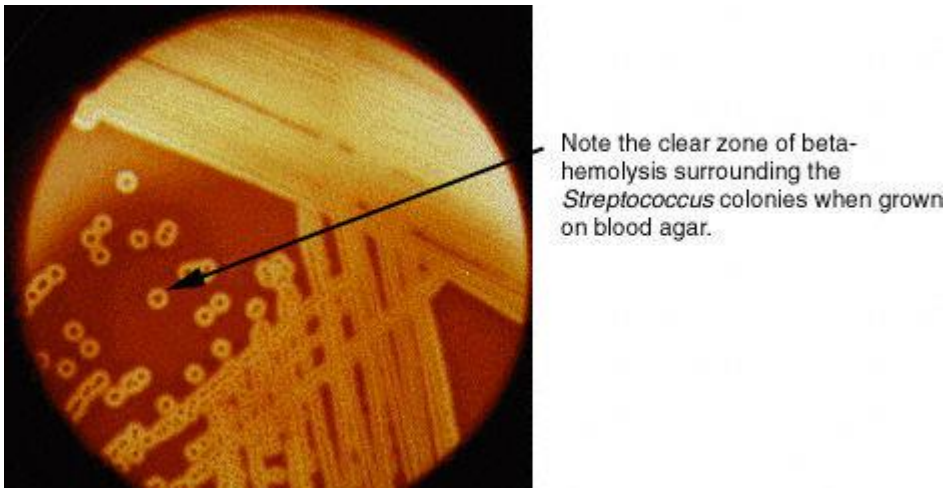


FIGURE 2.2.5 *COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA*

Streptococcus pneumoniae and viridans ("green") streptococci are alpha haemolytic. Thus, the haemolysis reaction is important in grouping streptococci. The haemolysis reaction along with one physiologic characteristic is sufficient for a presumptive clinical identification.

Streptococcus pneumoniae is a leading cause of pneumonia in all ages (particularly the young and old), often after "damage" to the upper respiratory tract (e.g. following viral infection). It also causes middle ear infections (otitis media). The organism often spreads causing bacteremia and meningitis. *S. pneumoniae* is α haemolytic and there is no group antigen.

Staphylococcus aureus is one of the commoner causes of opportunistic nosocomial and community infections. These infections include pneumonia, osteomyelitis, septic arthritis, bacteremia, endocarditis, abscesses/boils and other skin infections (figure 2.2.2 and 2.2.3). *S. aureus* has gained notoriety because of the increased incidence of Methicillin-resistant. Staphylococcus aureus (MRSA) Infections.

Mycobacteria; **In** the 1980's, many experts felt that the days of tuberculosis as a threat to the US population had passed and the incidence of new cases (around 20,000 a year) was slowly decreasing, even though it was still the leading infectious cause of death world-wide. The situation in the 1990's has changed dramatically. The incidence of tuberculosis has slightly increased and the disease is certainly not going away (This is primarily due to the AIDS epidemic). At the same time multiple drug-resistant strains of M. tuberculosis are appearing regularly. *The M. avium - M. intracellulare* complex, long considered a group of organisms that only rarely infects man, is now recognized as one of the leading opportunists associated with AIDS. M. leprae is the causative agent of leprosy which remains a major disease in the third world. Due to eradication of infected cattle and pasteurization of milk M. bovis (a zoonotic cause of tuberculosis) is rarely seen in the United States.

Spirochetes and ***Neisseria.*** The most important genera of spirochetes are *Treponema*, Borrelia and *Leptospira*. These are Gram negative bacteria that are long, thin, helical and motile. Axial filaments (a form of *flagella*) found between the peptidoglycan layer and outer membrane and running parallel to them, are the locomotory organelles. While *Neisseria* are Gram negative diplococci (pairs of cocci). These bacteria grow best on chocolate agar (so-called because it contains heated blood, brown in colour); a modified

(selective) chocolate agar commonly used is Thayer Martin. The colonies are oxidase positive (i.e. produce cytochrome oxidase) which is demonstrated by flooding the plate with a dye which on oxidation changes colour.

Anaerobes Obligate anaerobes are bacteria that cannot survive in the presence of a high oxidation-reduction potential (redox potential) / high oxygen content. During metabolism, bacteria can produce toxic bi-products from oxygen (including superoxide radicals and hydrogen peroxide). Strict anaerobes lack certain enzymes (including superoxide dismutase and catalase) that detoxify these products.

Aerobes such as Pseudomonads are aerobic, gram-negative rods with polar flagella. They are oxidase positive, in contrast to Enterobacteriaceae. These organisms are found in most environments including in water and soil and air.
Mycobacteria. In the 1980's, many experts felt that the days of tuberculosis as a threat to the US population had passed and the incidence of new cases (around 20,000 a year) was slowly decreasing, even though it was still the leading infectious cause of death world-wide. The situation in the 1990's has changed dramatically. The incidence of tuberculosis has slightly increased and the disease is certainly not going away (This is primarily due to the AIDS epidemic). At the same time multiple drug-resistant strains of M. tuberculosis are appearing regularly. The *M. avium* - *M. intracellulare* complex, long considered a group of organisms that only rarely infects man, is now recognized as one of the leading opportunists associated with AIDS. *M. leprae* is the causative agent of leprosy which remains a major disease in the third world. Due to eradication of infected cattle and pasteurization of milk *M. bovis* (a zoonotic cause of tuberculosis) is rarely seen in the United States.

Corynebacterium diphtheriae grows best under strict aerobic conditions It is Gram positive and pleomorphic. Colonization of the upper respiratory tract (pharynx and nose) and less commonly skin with *C. diphtheriae* can lead to diphtheria. The organism does not produce a systemic infection. However, in addition to a pseudo membrane being formed locally (which can cause choking), systemic and fatal injury results primarily from circulation of the potent exotoxin (diphtheria toxin). The latter begins over a period of a week. Thus treatment involves rapid therapy with anti-toxin. The gene for toxin synthesis is encoded on a bacteriophage (the tox gene). Corynebacteria that are not infected with phage, thus do not generally cause diphtheria. Diphtheria is now a disease of almost historic importance in the U.S. due to effective immunization of infants (in conjunction with pertussis and tetanus, DPT) with a

toxoid (inactive toxin) which causes production of neutralizing antibodies. However, colonization is not inhibited and thus C. diphtheriae is still found in the normal flora (i.e. a carrier state exists). Immunity can be monitored with the Schick skin test. Treatment in non-immune individuals primarily involves injection of anti-toxin. Antibiotics are also administered at this time.

2.0 OBJECTIVES

At the end of this unit you should be able to describe the microbiological features and other public health importance of the following groups of bacteria:

- Groups A, B and D streptococcus, viridans streptococci
- Enterococcus faecalis (formerly group D).
- Streptococcus pneumonia
- Staphylococcus infections; food poisoning, toxic shock, MRSA
- Neisseria and Spirochetes
- Anaerobes and Pseudomonas in Opportunistic
- Mycobacteria and Corynebacteria

3.0 CONTENT

3.1 GROUPS A, B AND D STREPTOCOCCUS, VIRIDANS STREPTOCOCCI

Group A streptococcus (S. pyogenes)

Most group A streptococcal infections are relatively mild illnesses but sometimes infection by these bacteria can result in severe and life-threatening diseases. There are several million cases of strep throat and impetigo each year.

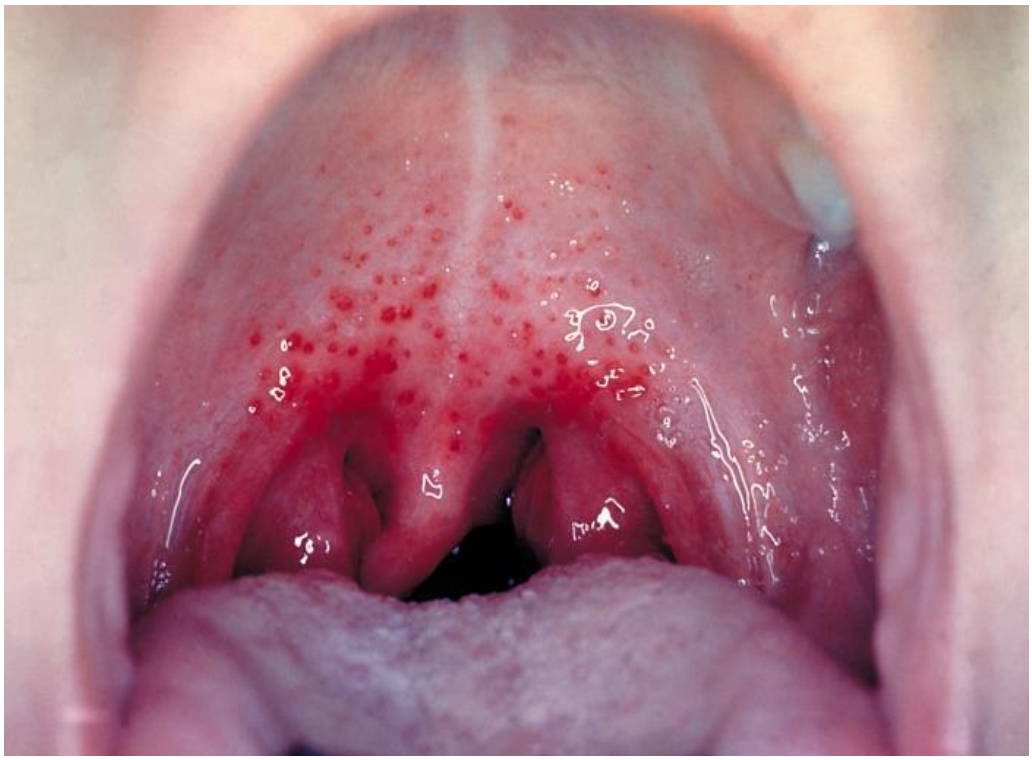


FIGURE 2.2.6

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Streptococcus pyrogenes frequently causes suppurative, but non-invasive pharyngitis (Strep Throat) (figure 3.2.6), and less frequently the skin infection, impetigo. In the middle part of the 1900's, the serious complications of group A streptococcal infections began to decline dramatically and had greatly decreased by the 1970's. Thus, interest waned. In the 1980's and 1990's, there was an upsurge in classical "rheumatic fever" (a non-suppurative disease of the heart) but also new forms of streptococcal disease which include both "invasive" bacteremia, a toxic shock-like syndrome (as seen with Staphylococcus aureus) and so-called "flesh eating" bacteria.

Group A streptococcal infections affect all ages with peak incidence at 5 to 15 years of age. The serious complications (including rheumatic fever and invasive bacteremia) were felt to affect primarily those with some underlying defect in their immune system (including infants, elderly people and those immunocompromised). However, it is clear now that previously healthy children and adults are definitely at risk of serious complications.

Strep Throat

Strep throat is an infection in the throat and tonsils caused by group A Streptococci. The disease is spread through contact with aerosols produced in a cough or sneeze of an infected person. It can also be spread by drinking or eating from a utensil used by an infected person. It is also possible to get strep throat from contact with sores from group A strep skin infection.

Common Symptoms of Strep Throat include (CDC):

•Sore throat, usually starting quickly

•Severe pain when swallowing

•Fever (101° F or above)

•Red and swollen tonsils, sometimes with white patches or streaks of pus

•Tiny red spots (*petechiae*, figure 2.2.6) on the soft or hard palate—the area at the back of the roof of the mouth

•Headache

•Nausea and/or vomiting

•Swollen lymph nodes in the neck

•Body aches

•Rash

Rheumatic fever

Rheumatic fever is an inflammatory disease affecting primarily the heart and joints. Although severe, it can take an extended period of time to develop. The mechanism of chronic immunopathology of rheumatic fever is not resolved. M protein cross-reacts with heart myosin leading to autoimmunity. Also the group A streptococcal cell wall is highly resistant to degradation in the host. These antigens persist for months in vivo and experimentally elicit diseases that resemble rheumatic arthritis and carditis. Rheumatic arthritis should not be confused with the most common rheumatic disease - rheumatoid arthritis. Early termination of throat infections with penicillin therapy decreases the incidence of the subsequent development of rheumatic carditis.

Acute glomerulonephritis.

This is an immune complex disease of the kidney.

Scarlet fever

Scarlet fever usually begins with a fever and sore throat which may be accompanied by:

•chills

•vomiting

•abdominal pain

•the tongue may have a whitish coating and appear swollen. It may also have a "strawberry"-like (red and bumpy) appearance

•the throat and tonsils may be very red and sore leading to pain in swallowing

One or two days after the onset of illness, a characteristic red rash appears (although the rash can appear before illness or up to 7 days later). The rash, which is caused by erythrogenic (pyrogenic) toxins that are phage encoded, gives the name: Scarlet Fever. Initially, the rash is seen on the neck, under the arms, and in the groin. It then spreads to other parts of the body. First the rash appears as flat red patches which gradually become fine bumps and feel like sandpaper (figure 2.2.7).

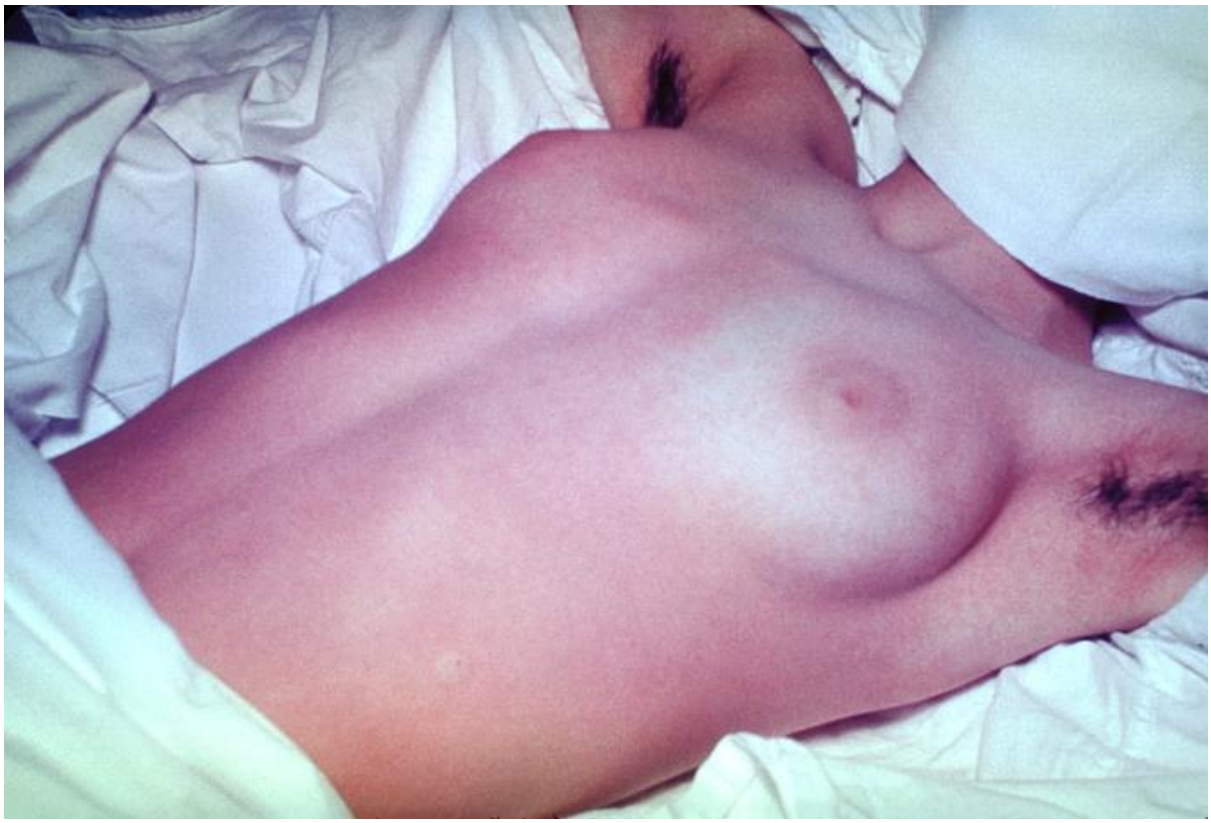


FIGURE 2.2.7:

COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

The cheeks may have a flushed appearance but sometimes there is a pale area around the mouth. Underarm, elbow and groin skin creases may become brighter red than the rest of the rash (Pastia's lines). The rash generally subsides in about a week and the skin may peel around the finger tips, toes, and groin area. This can last up to several weeks.

Treatment .

Bacteremia, toxic-shock syndrome and necrotizing fasciitis

Normally, infection by group A Streptococci results in mild symptoms. However, these bacteria can also cause a bacteremia resulting in a much more severe disease which can sometimes be fatal. Such diseases include:

•A toxic shock-like disease (including rash, fever and shifting of fluid from the bloodstream to peripheral tissues with resulting oedema). This causes blood pressure to drop rapidly and organs (e.g., kidney, liver, lungs) to fail.

•and/or necrotizing myositis and fasciitis. Necrotizing fasciitis (which has earned Group A Streptococci the name "the flesh-eating bacteria") rapidly destroys muscles, fat, and skin tissue.

Production of pyrogenic toxins (A, B and C) are a hallmark of these strains. Pyrogenic toxin is a super antigen (a mitogen) for T cells causing non-specific activation of the immune system. This may be involved in the pathogenesis. This disease is still uncommon but can progress very quickly (a few days) and is life-threatening.

Approximately 9,000 to 11,500 cases of invasive Group A Streptococcal disease occur each year in the United States and lead to 1,000 to 1,800 deaths annually. Thus death occurs in 10%-15% of all invasive cases, approximately 40% of patients with streptococcal toxic shock syndrome and approximately 25% of necrotizing fasciitis cases die from the infection.

CDC list the early signs and symptoms of necrotizing fasciitis. These include:

•Severe pain and swelling, often rapidly increasing

•Fever

•Redness at a wound site

Early signs and symptoms of toxic shock syndrome include:

•Sudden onset of generalized or localized severe pain, often in an arm or leg

•Dizziness

•Flu-like symptoms such as fever, chills, muscle aches, nausea, vomiting

•Confusion

•A flat red rash over large areas of the body (only occurs in 1 in 10 cases)

Treatment is by antibiotics high dose penicillin and clindamycin are used for treatment of necrotizing fasciitis and toxic shock syndrome along with supportive care in an intensive care unit in very severe cases. Early and aggressive surgery, which may reduce the fatality rate, is often needed to remove damaged tissue and stop disease spread.

General features in pathogenesis

The identity of the adhesin allowing adhesion to the respiratory epithelium (via fibronectin) is somewhat controversial. Lipoteichoic acid is localized in the cell membrane of many bacteria. For group A streptococci, much is also present in the fimbriae on the cell exterior. Classical work suggests lipoteichoic acid is the group A streptococcal adhesin although more recently a role for an "F (fibronectin-binding) protein" has been suggested.

Group A streptococci in the absence of fibrinogen fix complement to the peptidoglycan layer and, in the absence of antibodies, are not phagocytosed. The M protein (also found in fimbriae) binds fibrinogen from serum and blocks the binding of complement to the underlying peptidoglycan. This allows survival of the organism by inhibiting phagocytosis. However, in immune individuals, neutralizing antibodies reactive with M protein elicit phagocytosis which results in killing of the organism. This is the major mechanism by which immunity is able to terminate group A streptococcal infections. M protein vaccines are thus a major candidate for use against rheumatic fever. The capsule of group A streptococci classically was stated to have limited anti-phagocytic activity. Many of the newly described virulent strains are highly mucoid and the capsules are important in pathogenesis.

Unfortunately, certain M protein types cross-react antigenically with the heart and may be responsible for rheumatic carditis. The fear of autoimmunity has rightly inhibited the use of group A streptococcal vaccines. However, distinct protective versus cross-reactive epitopes have been defined and the availability of a vaccine appears likely. M proteins vary antigenically between strains; thus immunity to one M protein does not imply general immunity to all S. pyogenes strains. M typing along with other antigens (T and R) are used for serotyping.

Laboratory diagnosis

1. Direct detection - the antigen is extracted from a throat swab. The antigen extract will then bind with antibody specific to the group A streptococcal carbohydrate. This has classically involved agglutination of antibody coated beads. However, simpler tests have been recently introduced. Results are available within minutes.

2. Lancefield grouping of isolated beta haemolytic colonies (see above).

3. Colonies are beta haemolytic (figure 2.2.5) and their growth is inhibited by bacitracin (presumptive diagnosis) (figure 2.2.7a).

4. Patient serum shows antibodies to streptomycin O or other streptococcal antigens. This is important if delayed clinical sequelae occur.

Beta haemolysis is caused by two haemolysins O and S; the former is inactive in the presence of oxygen. Thus, stabbing of the plate increases the intensity of the haemolysis reaction.

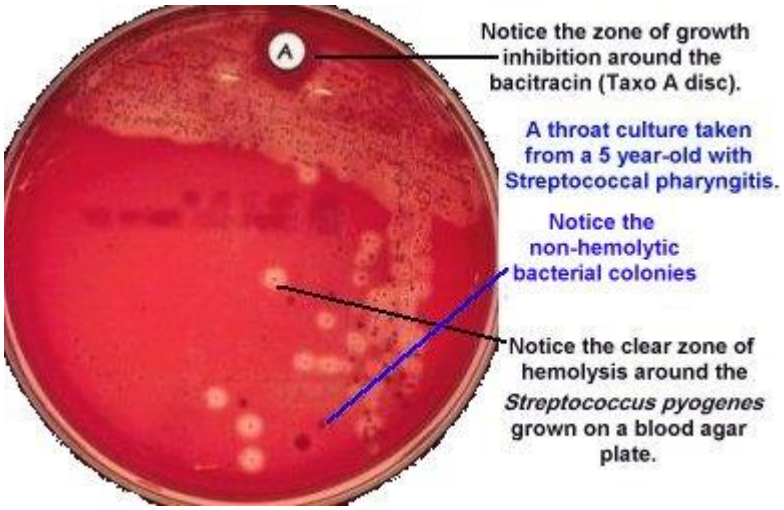


Figure 2.2.7a: β-haemolysis on a sheep blood agar from Bingham University Microbiology Department Nasarawa State Nigeria.

One way to differentiate beta-haemolytic group A Streptococcus from other beta-haemolytic streptococci is by determination of their sensitivity to bacitracin. Streptococcus pyogenes (group A beta-haemolytic) is sensitive to bacitracin and will not grow around the antibiotic- containing disc. The other beta-haemolytic streptococci are not sensitive to bacitracin and will grow next to the antibiotic-containing disc.

Group B streptococcus

Streptococcus agalactiae

Group B Streptococci, which are common in the alimentary tract, cause illness in people of all ages. In adults, group B streptococci most commonly cause invasive bloodstream infections (bacteremia), pneumonia, skin and soft-tissue infections, and bone and joint infections.
In newborns, these bacteria can cause sepsis (septicaemia), pneumonia and sometimes neonatal meningitis. The neonatal meningitis and septicaemia occur after transmission from the normal vaginal flora of the mother. Antibiotics given during labour can be very effective at preventing transmission.

According to CDC, about 19,800 cases occur each year in the United States in all age groups; approximately 7,600 cases occurred in newborns before recent prevention strategies. The rate of early-onset infection decreased from 1.7 cases per 1,000 live births in 1993 to 0.28 cases per 1,000 live births in 2008. Since active prevention began in the mid-1990s, the rate of group B strep disease among newborns in the first week of life has declined by 80%. The incidence among blacks approximately twice that of non-blacks for all age groups.

Adult infections

The rate of invasive disease is about 7 cases per 100,000 non-pregnant adults and increases with age with an average age in non-pregnant adults of about 60 years. The rate is highest among adults 65 years and older (20 to 25 cases per 100,000). Most adult group B disease occurs in adults with other medical conditions including:

- diabetes mellitus
- cardiovascular disease
- congestive heart failure
- cancer
- obesity

Serious group B strep infections in adults can be fatal. On average, 8% of adults with invasive group B strep infections (infections where the bacteria have

entered a part of the body that is normally not exposed to bacteria) die. Risk of death is lower among younger adults, and adults who do not have other medical conditions.

The most common problems caused by group B streptococci in adults are:

- Bloodstream infections
- Pneumonia
- Skin and soft-tissue infections
- Bone and joint infections

Group B streptococci can also lead to rare cases of meningitis.

The cause of adult infections is unknown but it may be from fecal contamination. Diagnosis is as used with newborns and treatment is with antibiotics (penicillin). On some occasions, infections of bone and soft tissue require surgery.

New-borns

Most new-borns with early-onset disease (less than 7 days old) have symptoms on the day of birth. Babies who develop late-onset disease (7 to 90 days old) may appear healthy at birth and develop symptoms of group B strep disease after the first week of life.
Symptoms include (CDC):

- Fever
- Difficulty feeding
- Irritability, or lethargy (limpness or hard to wake up the baby)
- Difficulty breathing
- Bluish colour to skin

Late-onset disease sometimes also results from mother to baby transmission, but sometimes the bacteria come from another source. For a baby whose mother does not test positive for group B strep, the source of infection for late-onset disease is often unknown. The fatality rate in newborns is about 5%.

Diagnosis

The disease is diagnosed when the bacteria are grown from samples of the infants blood or spinal fluid. The organism can be identified on the basis of beta haemolysis, hydrolysis of hippurate and the CAMP reaction (figure 3.2.8). CAMP is an abbreviation for the names of the four individuals who originally described the test. Group B streptococci produce a factor that increases beta haemolysis of an *S. aureus* indicator strain.

Risk Factors

Some pregnant women are at higher risk of having a baby with early-onset disease. Risk factors include (CDC):

•Testing positive for group B strep late in the current pregnancy (35 to 37 weeks gestation)

•Detecting group B strep in urine during the current pregnancy

•Delivering early (before 37 weeks gestation)

•Developing fever during labour

•Having a long period between water breaking and delivering

•Having a previous infant with early-onset disease

Late-onset disease is more common among premature babies (less than 37 weeks). Babies with group B strep positive mothers also have a higher risk of late onset disease.

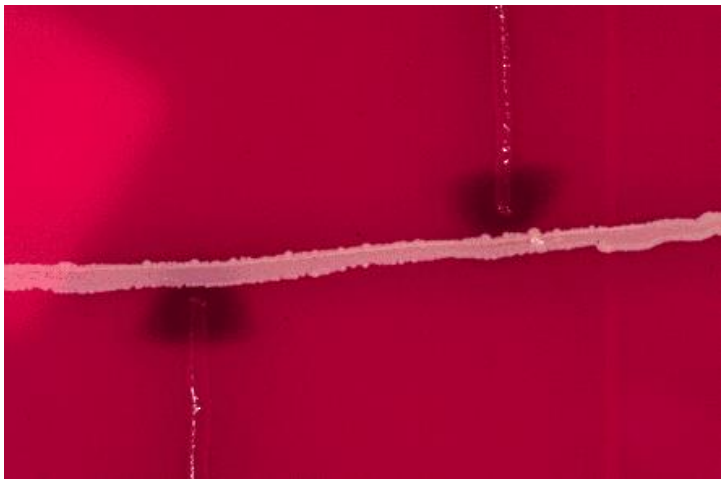


Figure 2.2.8a: CAMP positive reaction

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

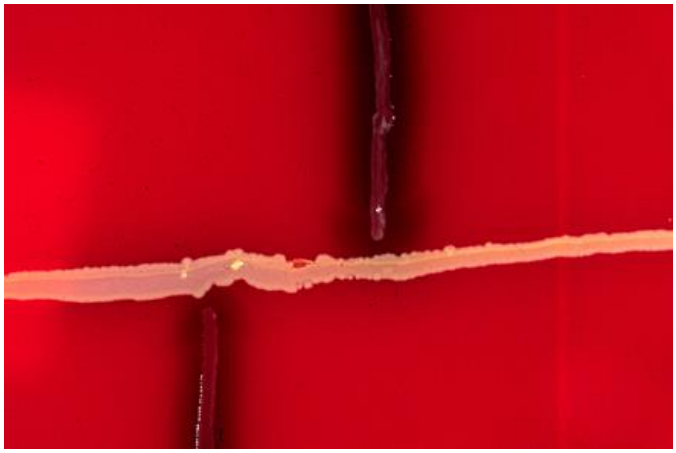
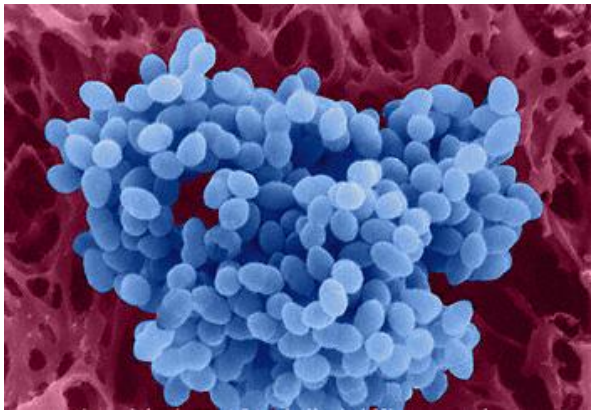


Figure 2.2.8b: CAMP negative reaction



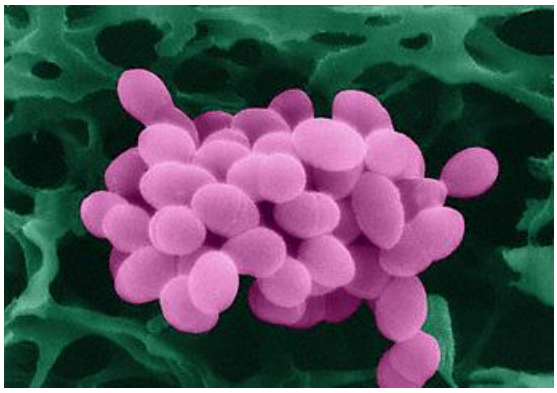
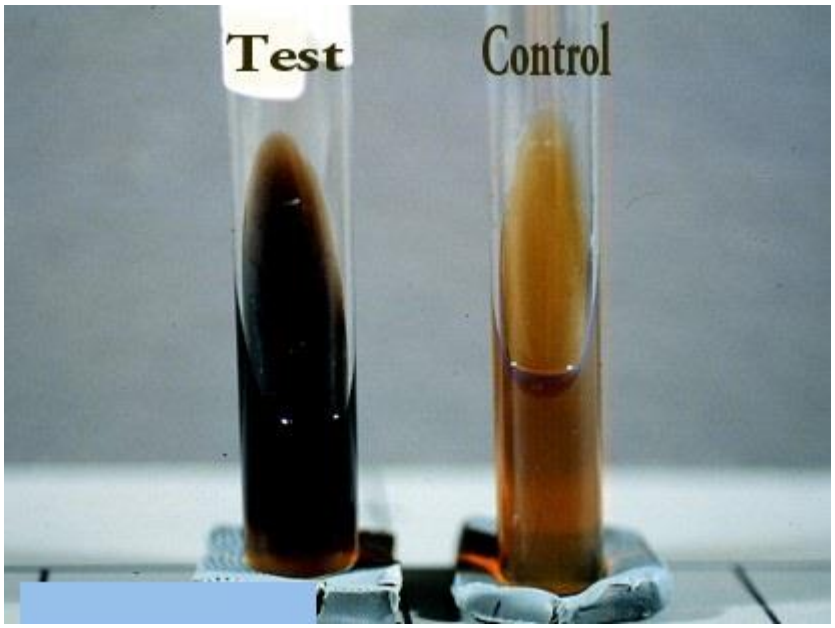


Figure 2.2.9: Streptococcus faecalis - coccoid prokaryote (dividing); a pathogen causing skin and wound infections

Figures 2.2.8-2.2.9 :

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA



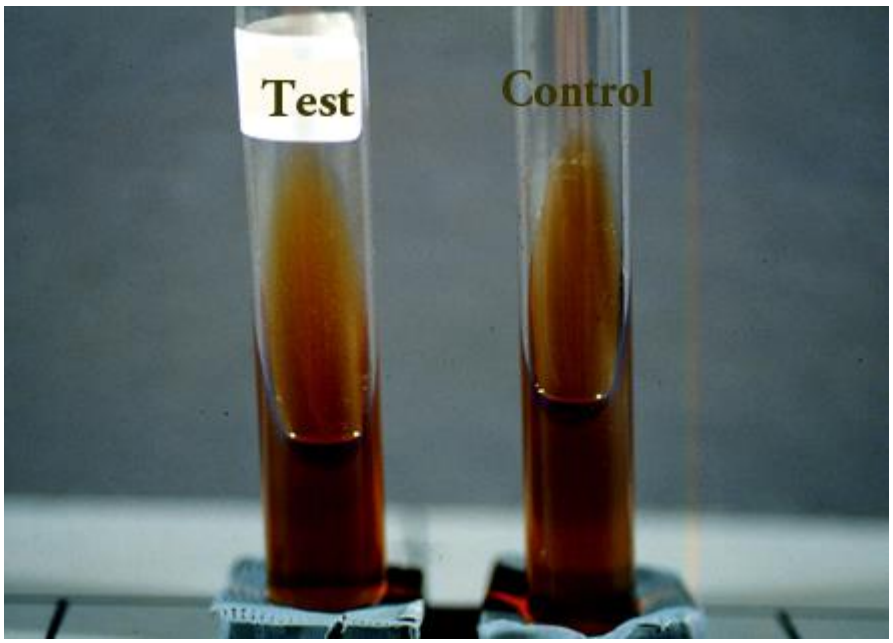
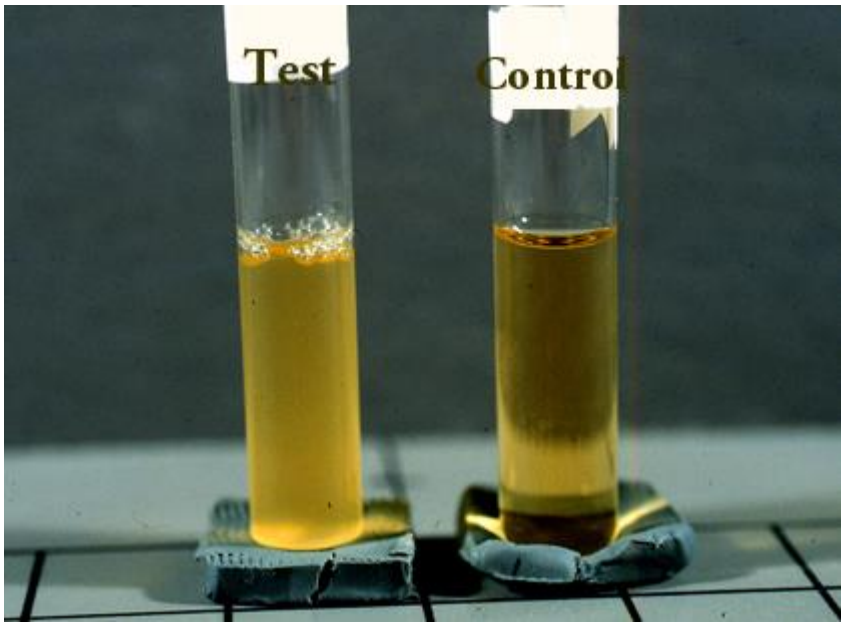


Figure 2.2.10: Bile esculin test. Group D streptococci are positive in this test (Above: Positive. Below: Negative)

Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby,USA



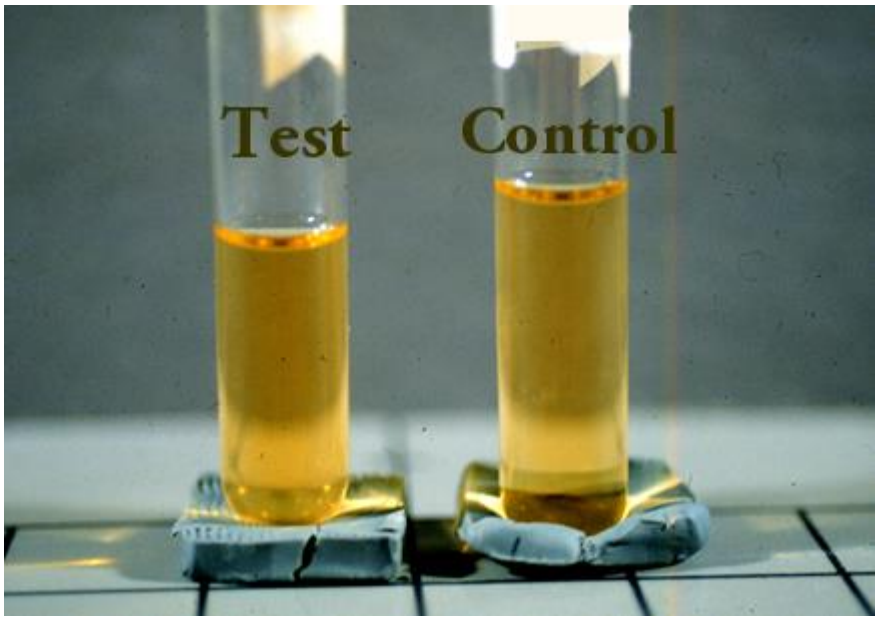


Figure 2.2.11: Positive growth in 6.5% sodium chloride (top) and no growth in a similar medium (bottom)

Figures 2.2.10- 2.2.11 Courtesy: Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby,USA

3.*2* *ENTEROCOCCUS FAECALIS* (FORMERLY GROUP D).

Streptococcus identification scheme

Now classified as an Enterococcus. The most common is *E. faecalis*. Enterococci are distantly related to other streptococci and have been moved into the genus Enterococcus; the most commonly isolated is E. (S*.) faecalis* (figure 2.2.9). As the name implies enterococci are found in the gut flora where they are usually harmless commensals and infection often follows from faecal contamination. They are a significant cause of urinary tract infections (but much less common than E. coli) and also of opportunistic infections (including intra-abdominal, septicaemia and endocarditis). There are a number of virulence factors that may contribute to E. faecalis infections.

- A plasmid-encoded haemolysin (cytolysin)
- A plasmid-encoded factor (aggregation substance)

The cytolysin in combination with high-level gentamicin resistance is associated with a five-fold increase in risk of death in human bacteremia patients.

E. faecalis can cause serious human nosocomial infections in humans. This is because the organisms shows high levels of antibiotic resistance. It is often found in teeth after root canal operations with a prevalence from 30% to 90% of the cases. It is resistant to many common antibiotics such as aminoglycosides, aztreonam, cephalosporins, clindamycin, the semisynthetic penicillins nafcillin and oxacillin, and trimethoprim-sulfamethoxazole. Resistance to vancomycin is becoming more common.

When the bacteria are vancomycin-resistant, the patient with a urinary tract infection may be treated with nitrofurantoin. Other options include ampicillin, linezolid and daptomycin. In root canal treatments sodium hypochlorite and chlorhexidine are used before isolating the canal.

Colonies are usually alpha or gamma haemolytic. Growth on bile-esculin produces a black precipitate derived from esculin; many other bacteria will not grow in the presence of bile. Group D *streptococci* are divided into those that will grow in 6.5% saline (*enterococ*ci) and those that will not (non-enterococci) (figure 2.2.11).

OTHER BETA HEMOLYTIC GROUPS

Groups C and G (and rarely group F) occasionally cause human disease (particularly pharyngitis).

Group C *streptococci* includes:

•*Streptococcus equi*, which causes a disease in horses
•*S. zooepidemicus* which causes infections in cattle and horses among other animals
•*S. dysgalactiae*

Group G *streptococci* includes
•*S. canis*. This is normally found in a number of animals but can also cause infection in humans.

Group H streptococci cause infections in dogs and rarely cause illness in humans unless the person has direct contact with the mouth of an infected dog. This can occur by "kissing" a dog or from saliva after being licked by an infected dog.

Minute colony streptococci

The normal human flora contains organisms that may be group A, C, F or G or are non-groupable (Streptococcus anginosus, Streptococcus milleri). Their role in human disease is unclear but Streptococcus anginosus can cause diseases including brain and liver abscesses under certain circumstances, particularly in immuno-deficient individuals.

Viridans streptococci

These are a diverse group of commensal species commonly found orally (including S. mutans) and cause endocarditis after release into the bloodstream from tooth extraction (figure 2.2.12).

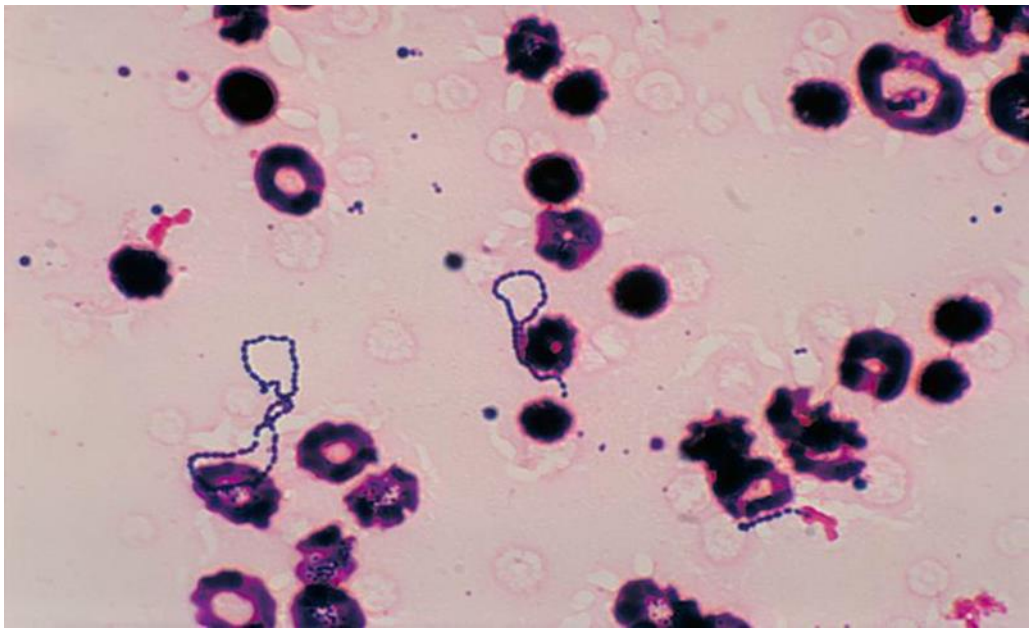


Figure 2.2.12: The bacterium Streptococcus viridans, is responsible for approximately half of all cases of bacterial endocarditis, but is found in the mouth as normal oral bacterial flora.

Courtesy: Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby,USA

S. mutans is responsible for approximately half of all cases of bacterial endocarditis. They can synthesize dextrans from glucose. This allows them to adhere to fibrin-platelet aggregates at damaged heart valves. Thus, they have the ability to cause sub-acute valvular heart disease following their introduction into the bloodstream (such as by tooth extraction).

These bacteria are also involved in dental caries and pericoronitis, an inflammation of the soft tissues surrounding the crown of a partially erupted tooth.

They are either alpha or non- haemolytic and negative for other tests described above. They produce a green colour on blood agar plates (Viridis, Latin: Green). Viridans streptococci can be differentiated from S. pneumoniae using an optochin test. Viridans streptococci are optochin-resistant. They are non-groupable.

3.4 STREPTOCOCCUS PNEUMONIAE

Pneumococcal Disease

S. pneumoniae (figure 2.2.13) is a leading cause of pneumonia in all ages (particularly the young and old), often after "damage" to the upper respiratory tract (e.g. following viral infection). It also causes middle ear infections (otitis media). The organism often spreads causing bacteremia and meningitis. S. pneumoniae is α haemolytic and there is no group antigen.

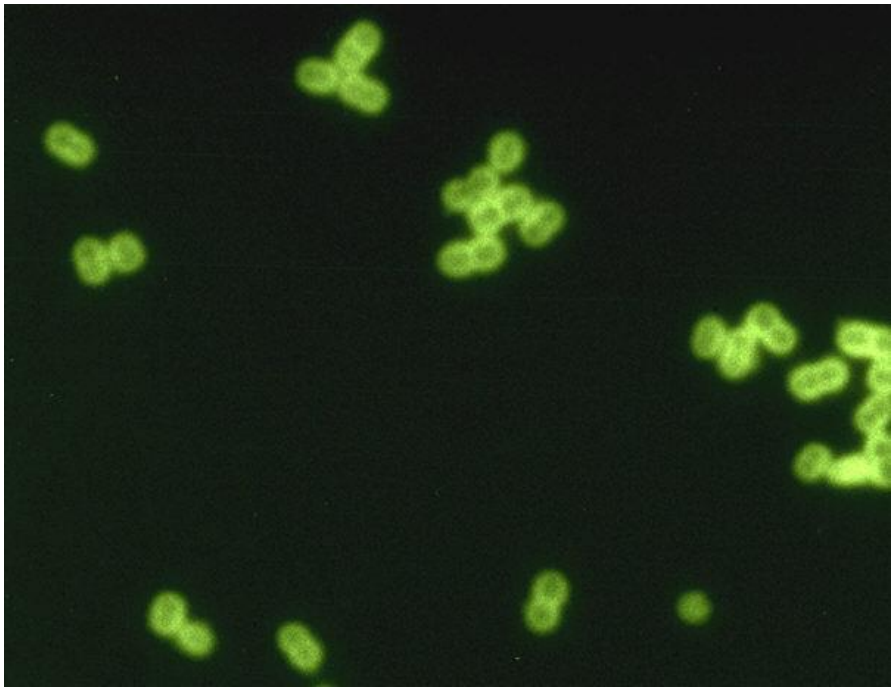


FIGURE 2.2.13A: *STREPTOCOCCUS PNEUMONIAE* IN SPINAL FLUID. FA STAIN (DIGITALLY COLORIZED).

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

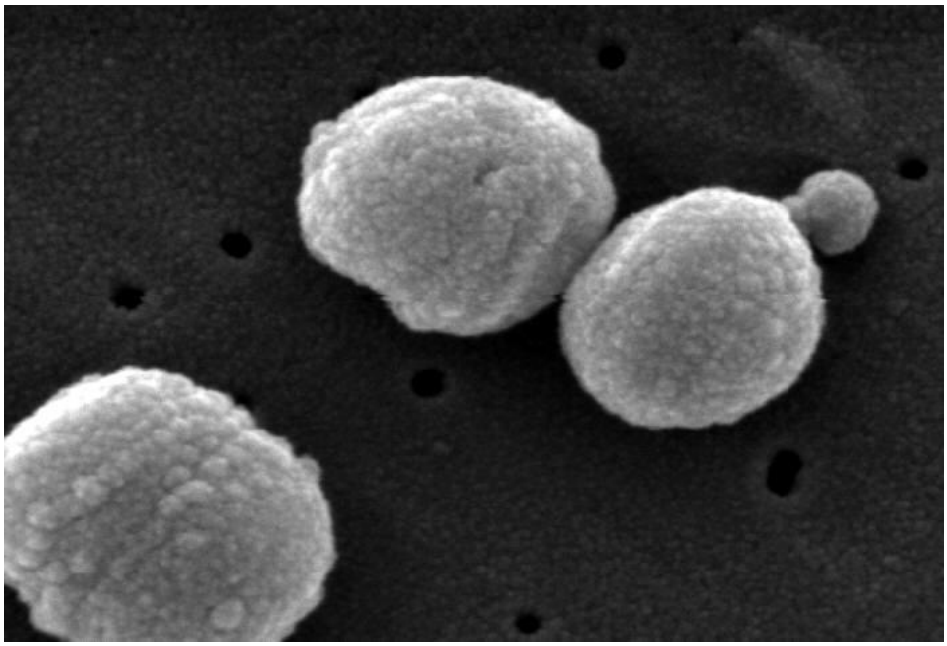


FIGURE 2.2.13B: SCANNING ELECTRON MICROGRAPH OF *STREPTOCOCCUS PNEUMONIAE*.

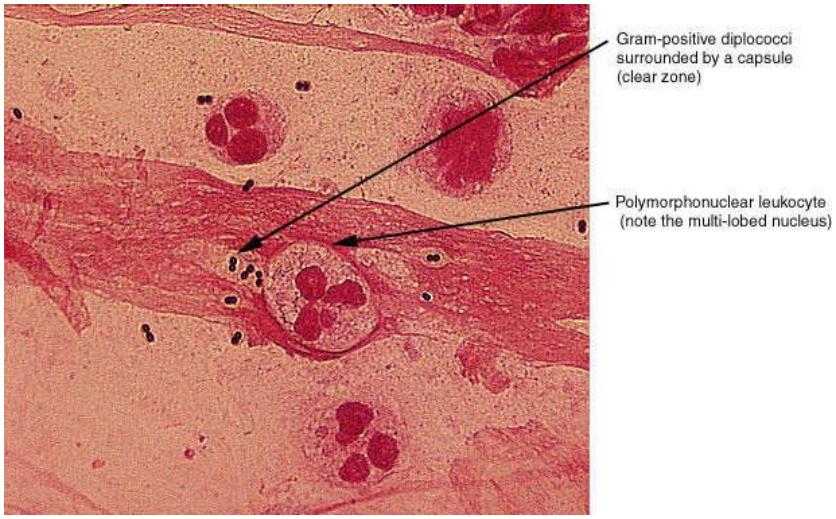


FIGURE 2.2.13C: ENCAPSULATED *STREPTOCOCCUS PNEUMONIAE*

Figures 2.2.13 B&C
Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Risk factors for pneumococcal disease in children (CDC)

•Younger than 2 years of age
•In group child care
•Certain illnesses (sickle cell disease, HIV infection, and chronic heart or lung conditions)
•Cochlear implants or cerebrospinal fluid (CSF) leaks (escape of the fluid that surrounds the brain and spinal cord)

Some American Indian, Alaska Native, and African American children may also be at increased risk.

Risk factors for pneumococcal disease in adults (CDC)

•Chronic illnesses (lung, heart, liver, or kidney disease; asthma; diabetes; or alcoholism)
•Conditions that weaken the immune system (HIV/AIDS, cancer, or damaged/absent spleen)
•Living in nursing homes or other long-term care facilities
•Cochlear implants or cerebrospinal fluid (CSF) leaks (escape of the fluid that surrounds the brain and spinal cord)
•Smoking

Serotypes: There are more than 90 strains of pneumococcus bacteria. Seven serotypes (6A, 6B, 9V, 14, 19A, 19F, and 23F) accounted for most drug-resistant S. pneumoniae. These serotypes are covered by the PCV7 vaccine.

Disease and Symptoms

Pneumococcal pneumonia
According to CDC, as many as 400,000 hospitalizations from pneumococcal pneumonia occur each year in the United States. Pneumococci account for about 30% of adult community-acquired pneumonia.

Pneumococcal pneumonia is the most common serious form of pneumococcal disease and can be mild to severe in all age groups. Complications include infection of the space between pleural membranes (empyema), inflammation of the pericardium, the sac surrounding the heart (pericarditis), and blockage of the airway that allows air into the lungs (endobronchial obstruction), with lung collapse (atelectasis) and collection of pus (abscess) in the lungs. It is fatal in about five per cent of patients with non-invasive pneumococcal pneumonia, but the rate may be higher among elderly patients.

Symptoms include (CDC):

•Fever and chills

•Cough

•Rapid breathing or difficulty breathing

•Chest pain

•Confusion or low alertness in older patients, rather than the more common symptoms listed above

Pneumococcal meningitis

Pneumococcal infection causes 13 to 19% of all cases of bacterial meningitis in the United States. An estimated 3,000 cases of pneumococcal meningitis occur each year. This is the most severe type of invasive pneumococcal disease. Ten per cent of children younger than 5 years old with pneumococcal meningitis die. Those that survive may have long-term problems, including hearing loss or developmental delay. The chance of death increases among elderly patients.

Symptoms include (CDC):

•Stiff neck

•Fever and headache

•Pain when looking into bright lights

•Confusion

•In babies, meningitis may cause poor eating and drinking, low alertness, and vomiting.

Pneumococcal bacteremia and sepsis

About 12,000 cases of pneumococcal bacteremia occur each year in the United States. Asplenic patients who develop bacteremia may deteriorate very rapidly.

Bacteremia occurs in up to 25 to 30% of patients with pneumococcal pneumonia. The case-fatality rate is 5 to 7% and may be higher than 60% among elderly persons. About 4% of children with pneumococcal bacteremia die of the infection. The death rate increases among elderly patients.

Symptoms include (CDC):

•Fever

•Chills

•Low alertness

Pneumococcal otitis media
Pneumococci commonly cause of acute otitis media. They are found in 28 to 55% of middle ear aspirates. By age 12 months, more than 60% of children have had at least one episode of acute otitis media. The sinuses can also be infected. These infections are usually mild. Some children develop repeated ear infections and may need ear tubes. It is likely that pneumococcal ear infections account for more than 10 million visits to doctors per year in the United States.

Symptoms include (CDC):
•Ear pain
•Red, swollen ear drum
•Sometimes fever and sleepiness

Diagnosis:
Direct Gram staining or detection of capsular antigen in sputum can be diagnostic. The organism grows well on sheep blood agar.

Autolysin

Pneumococci are identified by solubility in bile. An autolysin (peptidoglycan-degrading enzyme) is released by bile from the cell membrane and binds to a choline-containing teichoic acid attached to the peptidoglycan. The autolysin then digests the bacterial cell wall resulting in lysis of the cell. If the cells are grown in ethanolamine instead of choline, ethanolamine is incorporated into the teichoic acid. The autolysin then cannot lyse the cell wall. Understanding how the autolysin works has led to the suggestion that antibiotics (including penicillin) work together with the autolysin in killing of pneumococci in vivo.

The organisms are also identified by susceptibility to optochin (ethyl hydrocupreine) (figure 2.2.14)

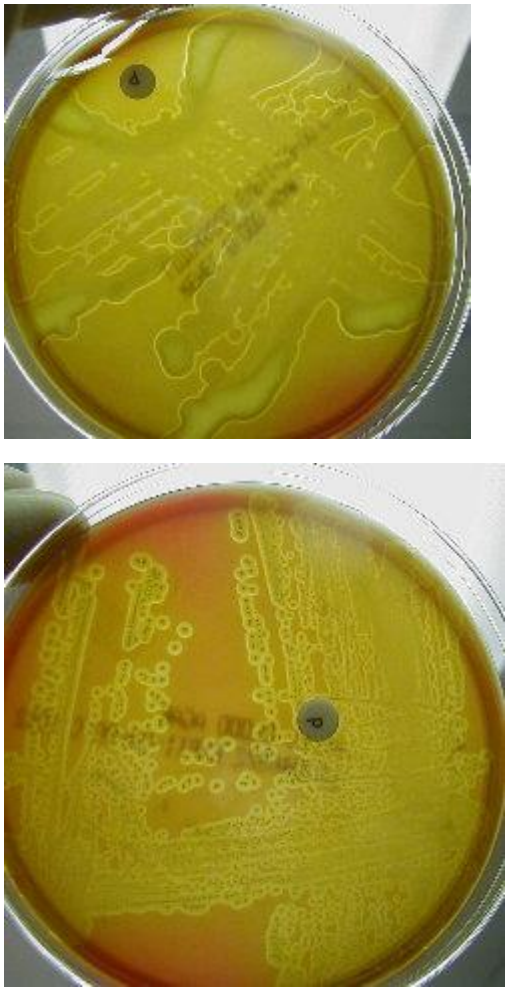


Figure 2.2.14: It is difficult to distinguish normal alpha streptococci found in the mouth from the pathogenic *Streptococcus pneumoniae*. Both are alpha-haemolytic on blood agar and so must be distinguished using the "P" disk (optochin).
S. pneumoniae (A) is sensitive while *S. mitis* (B) is resistant

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Capsule

This is highly prominent in virulent strains (figure 2.2.13c) and its carbohydrate antigens vary greatly in structure among strains. The capsule is anti-phagocytic and immunization is primarily against the capsule. Capsular vaccines are available for susceptible individuals; immunity is serotype-specific. Using appropriate type-specific antisera, the capsule on isolated bacteria can be "fixed" and becomes visible microscopically (the Quellung reaction) which is useful in microbial identification (figure 2.2.15).

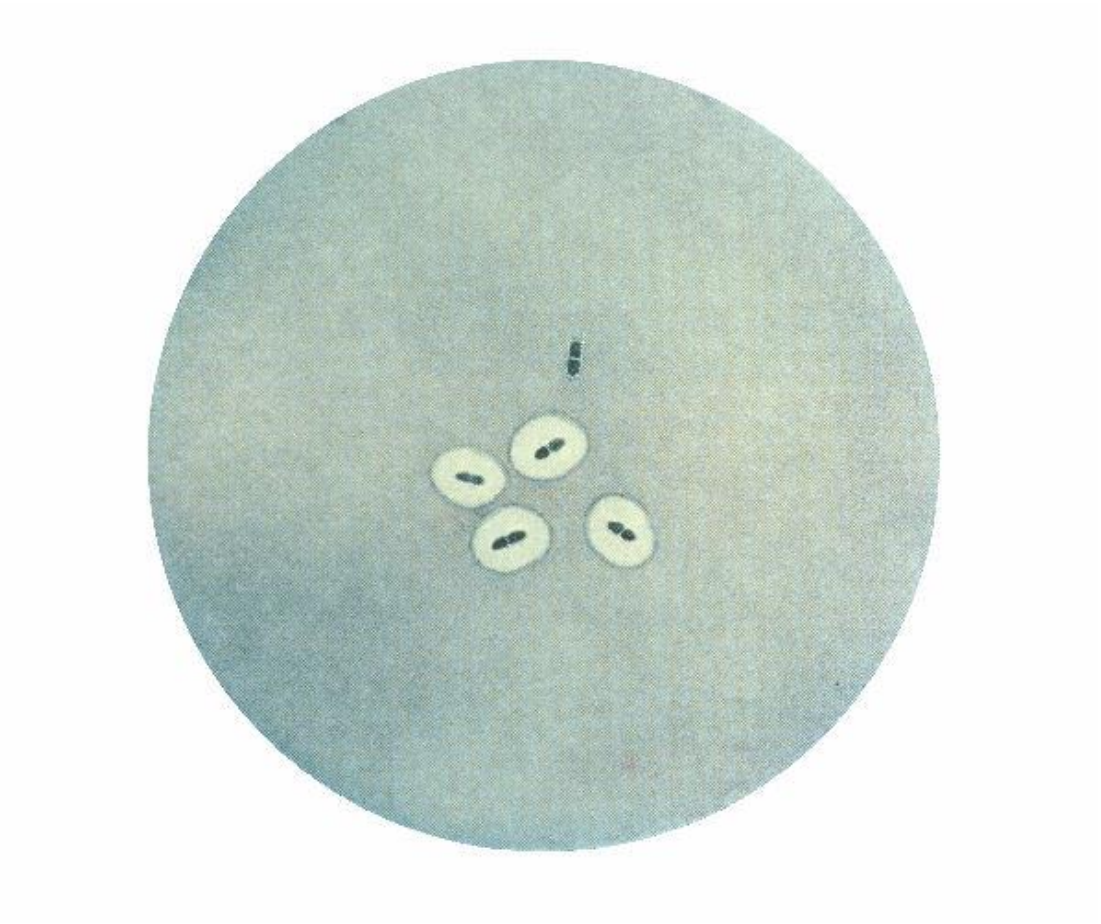


FIGURE 2.2.15: PHOTOMICROGRAPH OF STREPTOCOCCUS PNEUMONIAE BACTERIA REVEALING CAPSULAR SWELLING USING THE NEUFELD-QUELLUNG TEST.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

The organism also produces pneumolysin that degrades red blood cells under anaerobic conditions (observed as alpha haemolysis). Complement activation by teichoic acid may explain the attraction of large numbers of inflammatory cells to the focal site of infection.

Transmission

S. pneumoniae is transmitted person to person by contact with saliva and mucus.

Treatment

Most strains of S. pneumoniae are susceptible to penicillin. However, resistance is quite common and 15% of invasive pneumococcal isolates are resistant to penicillin in some parts of the United States.

Vaccine

Pneumococcal vaccines, of which there are several types, are very good at preventing severe disease, hospitalization and death. Before the vaccine, there were about 700 cases of meningitis, 13,000 blood infections, and 200 deaths from pneumococcal disease each year among children younger than 5 years old in the United States. After vaccination started, these numbers dropped dramatically. Before introduction of the first vaccine, rates of invasive pneumococcal disease among children under five were approximately 80 cases per 100,000 with 10 cases per 100,000 population being pneumococcal meningitis (figure 2.2.16).

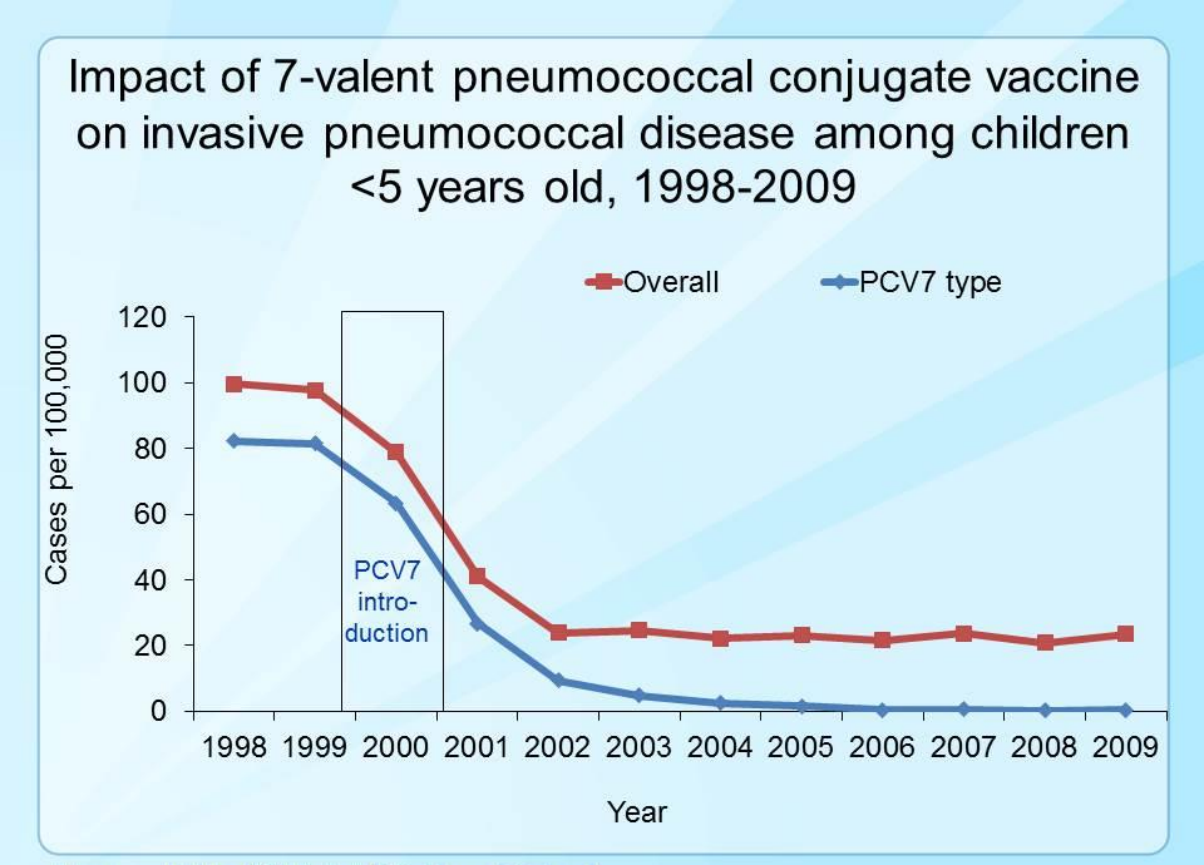


FIGURE 2.2.16: IMPACT OF SEVEN VALENT PNEUMOCOCCAL VACCINE ON INVASIVE PNEUMOCOCCAL DISEASE IN CHILDREN UNDER 5 YEARS OF AGE.

COURTESY; CONFERENCE RESOURCE 2017 ANNUAL GENERAL MEETING OF MEDICAL LABORATORY SCIENTIST OF NIGERIA KADUNA POSTER 56.

After the introduction of the PCV7 vaccine, rates of disease due to the seven serotypes in the vaccine dropped to less than 1 case per 100,000 by 2007.

The pneumococcal conjugate vaccine (PCV13 or Prevnar 13) provides protection against the 13 serotypes responsible for most severe illness in children.

Staphylococci are facultative anaerobes. They are Gram positive, occur in grape like-clusters and are catalase positive. They are major components of the normal flora of skin and nose in all people.

3.5 STAPHYLOCOCCUS INFECTIONS; FOOD POISONING, TOXIC SHOCK, MRSA

Staphylococcus aureus

Staphylococcus aureus (figure 2.2.17A) is one of the commoner causes of opportunistic nosocomial and community infections.

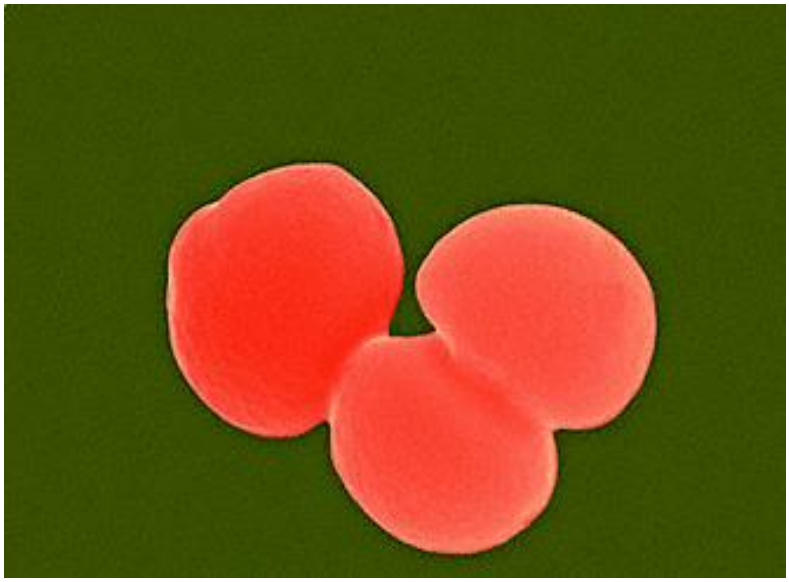


FIGURE 2.2.17 A *STAPHYLOCOCCUS AUREUS* - MRSA RESISTANT COCCOID PROKARYOTE (DIVIDING); CAUSES FOOD POISONING, TOXIC SHOCK SYNDROME AND SKIN AND WOUND INFECTIONS (SCALDED SKIN SYNDROME, SCARLET FEVER, ERYSIPELAS, IMPETIGO, ETC.)

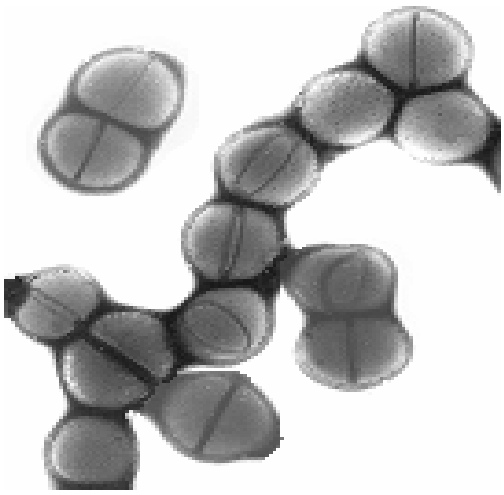


FIGURE 2.2.17B *STAPHYLOCOCCUS AUREUS* (GRAM-POSITIVE)

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

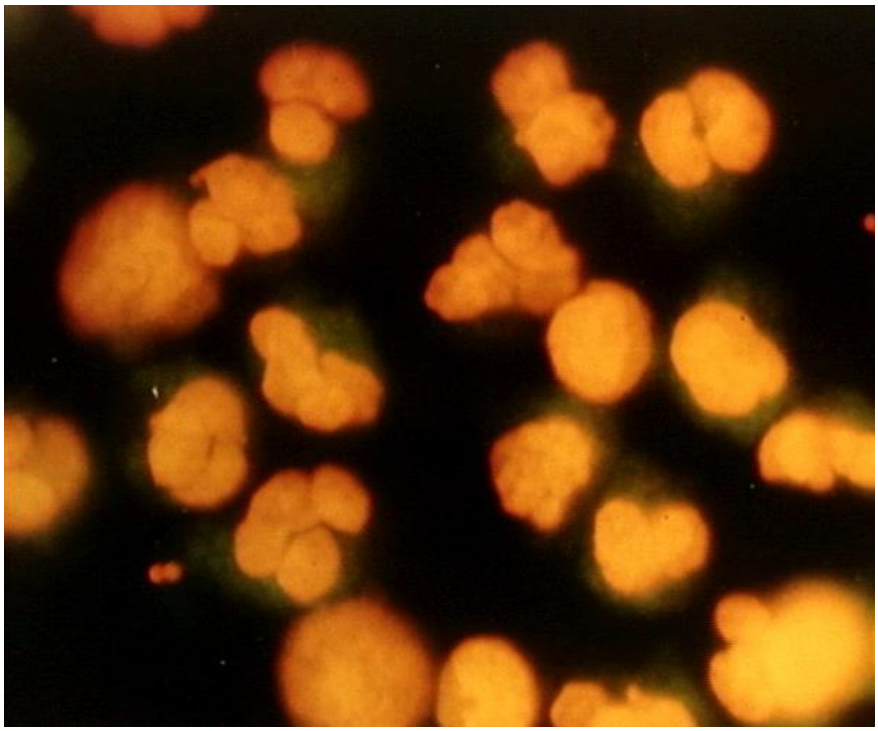


FIGURE 2.2.17C *STAPHYLOCOCCUS AUREUS* - ACRIDINE-ORANGE LEUCOCYTE CYTOSPIN TEST

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

These infections include pneumonia, osteomyelitis, septic arthritis, bacteremia, endocarditis, abscesses/boils and other skin infections (figure 3.2.18 and 3.2.19). S. aureus has gained notoriety because of the increased incidence of Methicillin-resistant *Staphylococcus aureus* (MRSA) Infections.

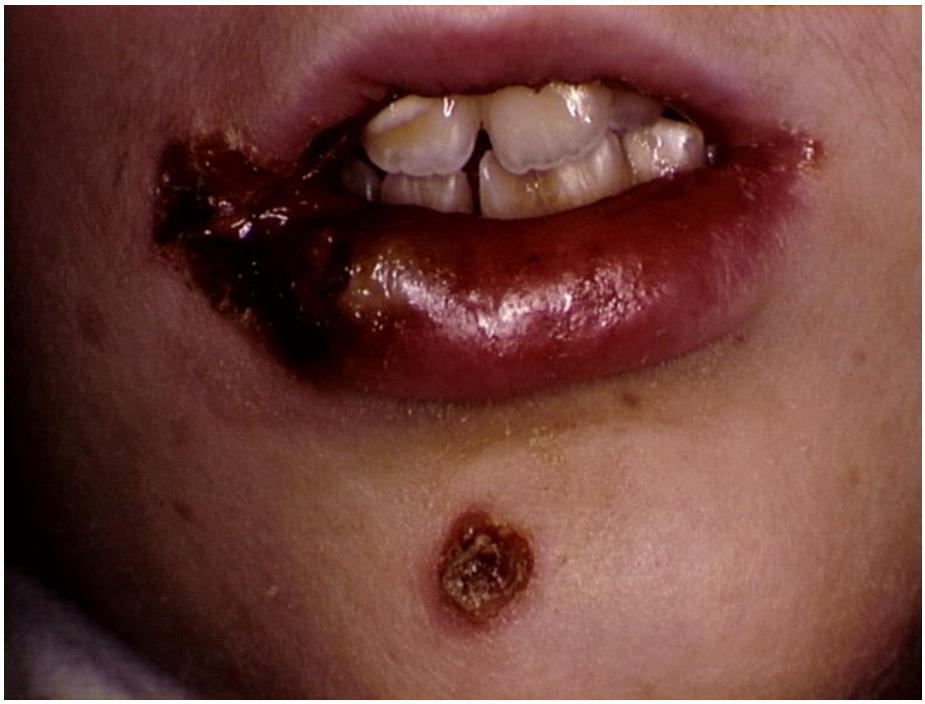


FIGURE 2.2.18 STAPHYLOCOCCAL INFECTION: IMPETIGO

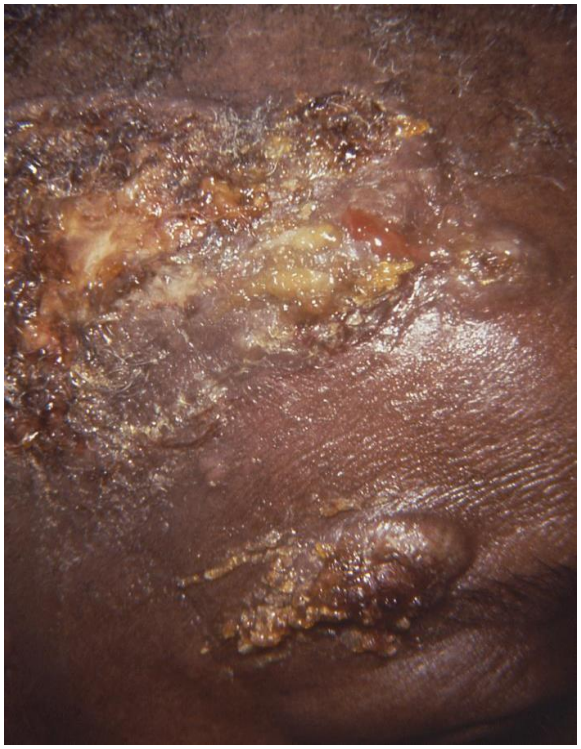


FIGURE 2.2.19 IMPETIGO LESIONS ON FOREHEAD CAUSED BY *STAPHYLOCOCCUS AUREUS* BACTERIA.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Pathogenesis

Food poisoning

S. aureus produces a number of toxins, of which the enterotoxins (A, B, C and D) cause food poisoning. About a third to a half of S. aureus strains produce enterotoxins which are heat stable and thus survive cooking (boiling for 30minutes). They are also resistant to proteolysis by intestinal proteases.

Food becomes contaminated with the organism from human contact, grows and produces enterotoxin. The organism does not "infect" the patient on ingestion of contaminated food; rather the pre-existing toxin causes the symptoms which include:

•vomiting

•nausea

•diarrhoea (watery and non-bloody, leading to dehydration)

•abdominal pain

Fever is not observed.

Because only the toxin is involved, onset of symptoms occurs within a few hours and recovery occurs within a day. Antibiotic treatment is not indicated because the bacteria are not directly involved in causing the symptoms (and may, anyway, have been killed by cooking).

Enterotoxins are super antigens that lead to cytokine production, T cell activation, neutrophil infiltration with loss of small intestine brush border cells. The release of inflammatory mediators may be the cause of the characteristic S. aureus food poisoning-associated vomiting.

Enterocolitis

The symptoms of enterocolitis are somewhat similar to food poisoning (watery diarrhoea and abdominal pain) but also include fever. They are also produced by enterotoxin A and leukotoxin. The cause is the treatment of patients with broad spectrum antibiotics that allow S. aureus (which infects almost everyone) to grow in the intestine in preference to the normal bacterial flora. The bacteria can be detected in faecal samples.

Toxic shock syndrome

Toxic shock syndrome is caused by infection with strains of S. aureus that produces toxic shock syndrome toxin. It may be associated with a wound in which the bacteria multiply rapidly but became particularly prominent to the public in the 1980's when S.aureus infection was found to cause the toxic shock syndrome that was seen after the use of certain tampons such as "Rely" (figure 2.2.20).

FIGURE 2.2.20: BOX OF RELY TAMPONS. ASSOCIATED WITH OUTBREAK OF TOXIC SHOCK SYNDROME.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

The bacteria were able to divide rapidly within the tampon; they do not disseminate but remain in the vagina. However, the toxin does disseminate and is responsible for the clinical features. This syndrome includes:

- fever
- macular erythrematous rash
- desquamation (all over the body)
- vomiting

•diarrhoea

Toxic shock syndrome toxin has the properties of a superantigen, resulting in the production of cytokines, vascular leak and cell toxicity. This results in hypervolemic shock and death as a result of multi-organ failure. Before the cause of toxic shock syndrome was discovered, the mortality rate was high but now is around 5%. There can be recurrent disease if the patient is not treated with the appropriate antibiotic.

Toxic shock syndrome toxin is involved in most menstruation-associated toxic shock syndrome. Enterotoxin B is involved in many non-menstruations associated cases of toxic shock syndrome.

Scalded skin syndrome (Ritter disease, pemphigus neonatorum) and bullous impetigo

A minority of S. aureus strains produce exfoliative toxins (A and B) and either toxin can cause scalded skin syndrome or bullous impetigo in babies and young children but rarely in adults. These toxins are serine proteases that can digest, among other proteins, some of the proteins found in desmosomes, the structures that link epithelial cells together. For example, the desmosomal protein called desmoglein is digested between the cells of the stratum granulosum epidermis. The process often resolves as the result of the formation of protective neutralizing antibodies. Exfoliative toxins are also superantigens.

Bullous impetigo is a mild form of S. aureus disease that usually occurs in newborn infants and young children. It is manifested by large, flaccid bullae and attributed to S. aureus strains belonging to phage group II capable of producing exfoliative toxins A and B that separate the stratum corneum from the rest of the epidermis. The more common and milder form of the disease (representing about 10% of all cases of impetigo) differs from non-bullous impetigo in that the vesicles enlarge into flaccid bullae before rupturing . The exposed skin surface is at first moist and red, resembling a small burn. A thin, light-brown, “varnish-like” crust then develops. Unlike the situation with scalded skin syndrome, bacteria can be cultured from the fluid of the bullae and Nikolsky's sign is absent.

The more severe form of disease with greater skin involvement caused by the same staphylococcal strains is known as the staphylococcal scalded skin

syndrome. This also usually affects younger children. The disease starts with local peri-oral erythema that spreads over the whole body and progresses to widespread, flaccid bullae that rupture causing exfoliation of the skin that resembles an extensive third-degree burn. There are no organisms that can be cultured from the fluid of the bullae, indicating that the bullae are caused by the toxin and not the bacteria themselves. Before the bullae form, slight pressure on the apparently normal epidermis may separate it at the basal layer. It may be rubbed off when pressed with a sliding motion. This is Nikolsky's sign.

This form of the disease can occur in epidemic form in nurseries, where it is known as pemphigus neonatorum or Ritter's disease. Fever and other systemic symptoms are usually absent in the more localized forms of the disease but are invariably present in patients with the staphylococcal scalded skin syndrome.

Localized bullous impetigo is self-limited due to the formation of neutralizing anti-toxin antibodies, and this is usually also the case with staphylococcal scalded skin syndrome. However, the latter carries a significant mortality rate (5%) that results from secondary bacterial infections of the areas where the skin surface has been lost. Staphylococcal scalded skin syndrome in adults is rare, and is usually associated with immunosuppression or kidney disease. In this case mortality can be as high as half of the patients.

Cytotoxins

As noted above, S. aureus causes a number of different disease entities associated with production of certain exotoxins. In addition to these "disease-specific" exotoxins, other cell lytic exotoxins (alpha, beta, gamma and delta toxins and leucocidins) may be produced. These are also called cytotoxins because they cause cytolysis as a result of plasma membrane damage. This leads to tissue destruction as a result of lysosomal enzyme release.

Alpha toxin

This singler polypeptide toxin interacts directly with the plasma membrane of many cells, embedding itself in the lipid bilayer and forming pores that allow ions to pass into and out of the cell. In particular, potassium ions are lost and sodium and calcium enter the cell. This leads to osmoticlysis. Alpha toxin is made by most S. aureus strains.

Beta toxin

Beta toxin also damages cell membranes by degrading specific lipids, sphingomyelin and lysophosptidyl choline. The toxin is a sphingomyelase C and is also a single polypeptide that is made by most S. aureus strains. It appears that the degree of toxicity depends on the concentrations of these lipids in the cell, both of which are found primarily in the outer monolayer of the plasma membrane bilayer.

Gamma toxins and P-V leukocidin

Gamma toxins and Panton-Valentine leukocidin consist of two polypeptide chains, an S chain and an F chain, which together form pores in the plasma membranes of susceptible cells. So far, three S chains and two F chains have been found which can combine to form a number of different toxins that are cytolytic to neutrophils and macrophages. The gamma toxins are also haemolytic whereas P-V leukocidin is not. The gamma toxins are also made by most S. aureus strains whereas P-V leukocidin is made by only a minority of strains. It has been particularly associated with virulent Methicillin-resistant Staphylococcus aureus (MRSA) infections.

Delta toxin

This is a small protein that is cytotoxic to many cells. It may act like a detergent, damaging cell membrane bilayers resulting in cytolysis. Other diseases caused by S. aureus

Respiratory disease

Aspiration pneumonia can result from entry of oral secretions into the lungs. The bacteria can cause local abscesses and infiltrates. The disease is found in the very young, the very old and patients with pulmonary disease. There can also be spread of blood-borne organisms to the lungs, causing haematogenous pneumonia. People with MRSA can get necrotizing pneumonia which has a very high fatality rate.

Empyema is an accumulation of pus in a cavity of the body such as the lungs and is sometimes seen in pneumonia patients. Many of these cases are the result of S. aureus infections.

Bacteremia

S. aureus is found on the skin of most people and can enter the body in wounds; however, many cases are nosocomial and result from surgery or catheter use. The bacteria may disseminate throughout the body.

Endocarditis

Endocarditis is an inflammation of the endocardium (the inner layer of the heart) and usually involves the heart valves (native or prosthetic valves). S. aureus-associated endocarditis can have a high mortality rate.

Urinary tract infections

Complicated urinary tract infections occur in specific clinical settings. Renal abscess can result from haematogenous seeding of the renal cortex (most often due to S. aureus) or from ascending infection leading to severe pyelonephritis (most often due to gram-negative rods).

Dissemination to other parts of the body

S. aureus bacteremia can disseminate via the bloodstream to other parts of the body causing disease. Such sites include bone giving rise to S. aureus osteomyelitis resulting in pain, fever and sometimes a Brodie abscess and septic arthritis.

Skin disease

Folliculitis

Folliculitis, by which is meant pyoderma involving the hair follicles and apocrine glands, affects nearly everyone at one time or another but is usually self-limited. Occasionally, folliculitis evolves into larger lesions known as furuncles and carbuncles.

S. aureus is the usual cause of folliculitis in non-immunocompromised patients, the infection probably arising from prior nasal colonization by this bacterium.

Furuncles, carbuncles and skin abscesses

The familiar furuncle or "boil" is thought to arise from folliculitis. The term furunculosis refers to multiple boils or to frequent recurrences. Carbuncles are more extensive and difficult-to-treat lesions that often require surgical intervention. Skin abscesses, although similar to carbuncles histologically, are usually deeper infections that do not originate in hair follicles.

S. aureus is the usual cause of both furuncles and carbuncles, and is also the sole or predominant pathogen in about 50% of skin abscesses. Predisposing factors to recurrent furuncles (furunculosis) include obesity, corticosteroid therapy, disorders of neutrophil function, and possibly diabetes mellitus. Immunoglobulin levels are usually normal in patients with furunculosis (low IgM levels have been demonstrated in some patients but this is of uncertain significance and, in contrast to IgG deficiency, replacement therapy is impractical). Most patients with recurrent furuncles have no obvious predisposing factors other than being nasal carriers of S. aureus nasal carriers. Outbreaks of furunculosis have been described in families, athletic teams, and in village residents who took steam baths together. Skin abscesses can result from minor trauma, injecting drug use (the practice of subcutaneous and intramuscular injection is known as "skin popping"), or bacteremia. Congenital immunodeficiency syndromes such as the hyper immunoglobulin E-recurrent infection syndrome (Job's syndrome) are sometimes present in patients with recurrent skin abscesses. Rarely, skin abscesses are self-inflicted (factitious abscess), in which case Gram's stain and culture may reveal "mouth flora" bacteria.

S. aureus strains secrete a number of tissue-degrading enzymes that may result in tissue damage. These include lipases, nucleases, hyaluronidase, coagulase and plasmin. One form of coagulase is bound to the S. aureus surface and

converts fibrinogen to fibrin. This insoluble protein causes the bacteria to aggregate. The other coagulase is secreted and combines with coagulase-reacting factor in the serum resulting in the formation of staphylothrombin that, like normal thrombin, also forms insoluble fibrin. This may also be anti-phagocytic.

Protection against phagocytosis

In addition, to the toxins and enzymes that directly damage cells and tissues described above, S. aureus strains produce other proteins involved in pathogenesis. For example, these bacteria have two mechanisms that protect them against phagocytosis by polymorphonuclear leukocytes and other phagocytic cells.

•Although the bacteria are opsonised by proteins in serum, the capsule and slime layer protect the cells against phagocytosis.

•Protein A is found on the surfaces of most S. aureus strains. It binds to immunoglobulin G and complement, blocking Fc and complement receptors and is thus anti-phagocytic.

Identification

•S. aureus is beta-haemolytic on sheep blood agar

•Ferments mannitol

•Is often golden pigmented (hence the name aureus)

•Is coagulase-positive

•Presence of protein A

In reference laboratories phage-typing is used.

Methicillin-resistant Staphylococcus aureus (MRSA) Infections

Methicillin-resistant Staphylococcus aureus (MRSA) is defined as any strain of Staphylococcus aureus that has developed resistance to beta-lactam antibiotics (such as penicillins) and cephalosporins. This results from the production of a phage-coded penicillinase that degrades beta lactam antibiotics. Some strains also have modified penicillin binding proteins.

Many healthy people carry MRSA asymptomatically. Patients with compromised immune systems are at a significantly greater risk of symptomatic infections. Apparently healthy people may have simple topical skin infections (noted above) but in some people MRSA may progress rapidly within a day or two of initial topical symptoms. In these patients, after about 72 hours, MRSA may invade tissues and become resistant to treatment.

The majority of community-associated MRSA infections are localized to skin and soft tissue and usually can be treated effectively but some strains exhibit enhanced virulence and spread into the tissues, causing illness much more severe than traditional nosocomial MRSA infections.

At first, MRSA is characterized by small red pimples and there may be fever and a rash. As the infection progresses over a period of a few days, the pimples increase in size and become more painful. Eventually, they form deep, pus-filled boils (figure 2.2.21a-d).

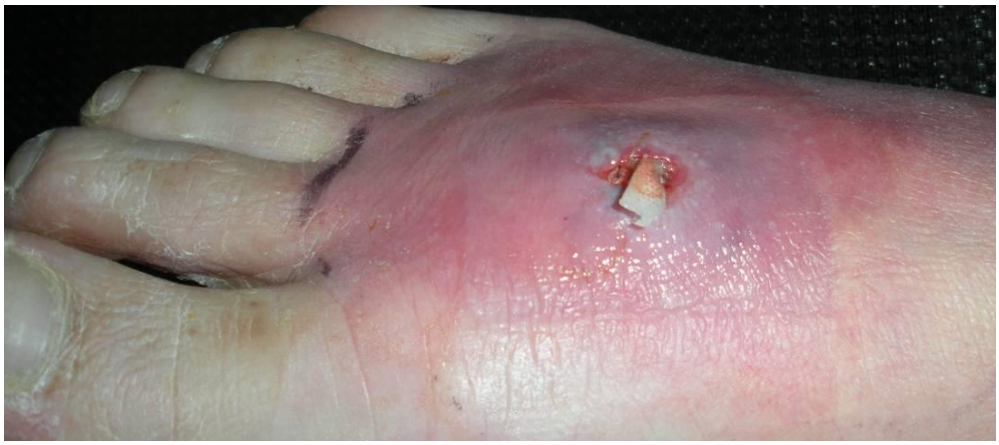


FIGURE 2.2.21A: A CUTANEOUS ABSCESS ON THE FOOT CAUSED BY METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*.

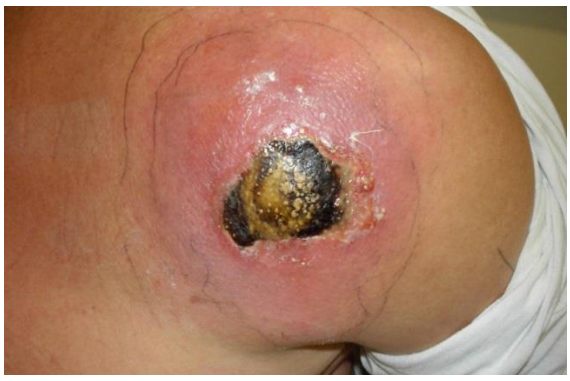


FIGURE 3.2.21B: CUTANEOUS ABSCESS CAUSED BY MRSA.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

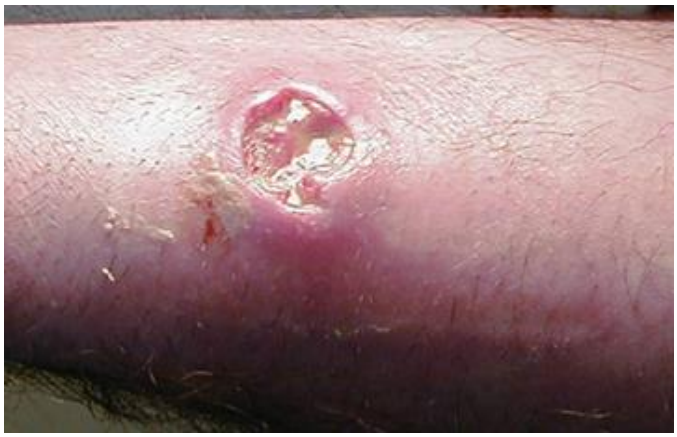


FIGURE 2.2.21C: CUTANEOUS ABSCESS CAUSED BY MRSA.

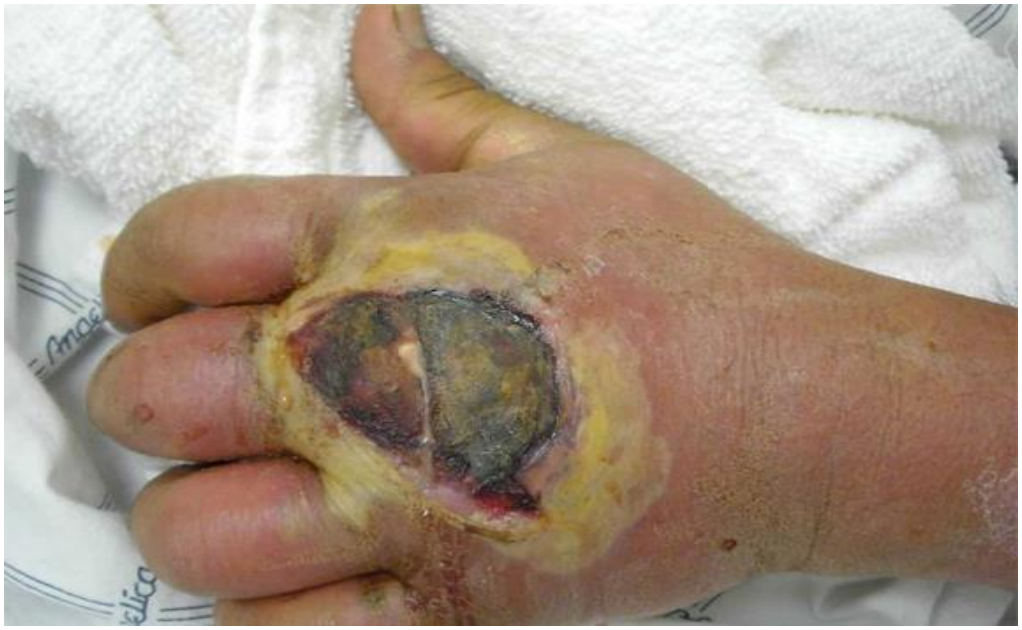


FIGURE 2.2.21D: CUTANEOUS ABSCESS CAUSED BY MRSA.

FIGURES 3.2.20-3.2.21: THE INFECTION CAN DISSEMINATE THROUGHOUT THE BODY (SEPSIS) AND VITAL ORGANS MAY BE AFFECTED. THIS CAN LEAD TO TOXIC SHOCK SYNDROME, AND NECROTIZING PNEUMONIA, SOME TIMES REFERRED TO AS "FLESH EATING" PNEUMONIA. IN HOSPITALS, THERE CAN BE SURGICAL SITE INFECTIONS.

COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

Epidemiology

Two per cent of people carry MRSA. Currently, in the United States, there are about 75,000 cases of invasive MRSA Infections per year, of which about 14,000 are in dialysis patients. Nosocomial invasive MRSA infections declined 54% between 2005 and 2011, with 30,800 fewer severe MRSA infections. In addition, there were 9,000 fewer deaths in hospital patients in 2011 versus 2005.

MRSA is usually spread by direct contact with an infected wound or from contaminated hands, usually those of healthcare providers. People who carry MRSA but do not have signs of infection can spread the bacteria to others and potentially cause an infection.

Diagnosis

This can be done by growth of the organism in the laboratory. There are more rapid tests available such as quantitative PCR.

Treatment

Intravenous vancomycin and teicoplanin are used to treat MRSA but some new MRSA strains are resistant to these antiboitcs also. Daptomycin is often used to treat these strains

Staphylococcus epidermidis

Staphylococcus epidermidis (figure 2.2.22) is a major component of the normal skin flora and thus commonly a contaminant of cultures in laboratories.

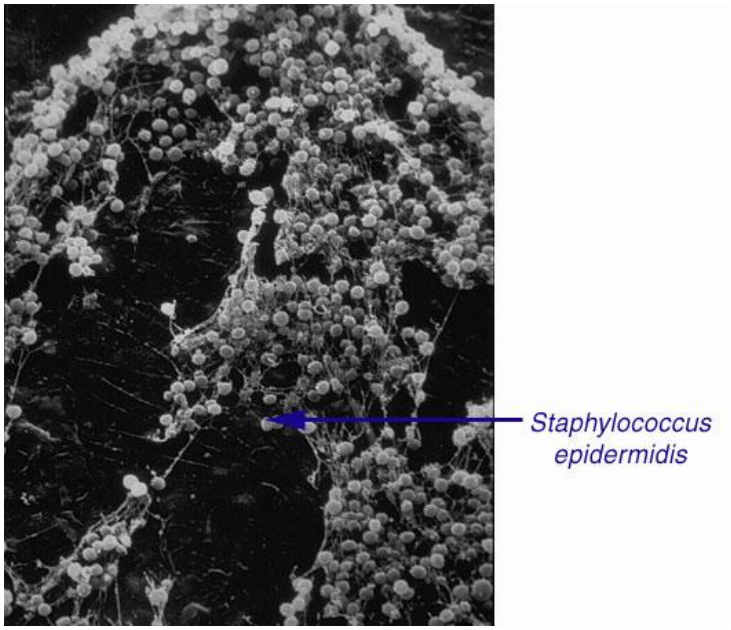


FIGURE 2.2.22: *S. EPIDERMIDIS*, THE MOST COMMON CAUSE OF BLOOD STREAM INFECTIONS IN PATIENTS WITH IVCS FIGURES 2.2.20-2.2.22 C *COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA*

It is a less common cause of opportunistic infections than S. aureus, but is still significant. Normally, infections are nosocomial. The bacteria form biofilms on catheters, shunts, artificial heart valves and other surgical devices and can cause endocarditis and sepsis.

The formation of biofilms is import in the virulence of the bacteria. It is likely that the bacteria bind blood proteins and extracellular matrix proteins to their surface. The bacteria also produce a sulfated polysaccharide extracellular coat called polysaccharide intercellular adhesion. Other bacteria bind to this surface coat making a multilayer biofilm. The cells within the biofilm become partially metabolically inactive and this, together with the difficulty in penetrating the biofilm with antibiotics, makes it difficult to treat the infection. In addition, S. epidermidis strains are often antibiotic-resistant (including penicillin, amoxicillin, and methicillin).

Since antibiotics are largely ineffective in clearing biofilms, the usual treatment is to change the infested medical device. The drug of choice is usually vancomycin, to which rifampin or aminoglycoside can be added.

Identification

- Staphylococcus epidermidis is non-haemolytic on growth on sheep blood agar
- Does not ferment mannitol (figure 2.2.23)
- Is non-pigmented
- Is coagulase-negative.

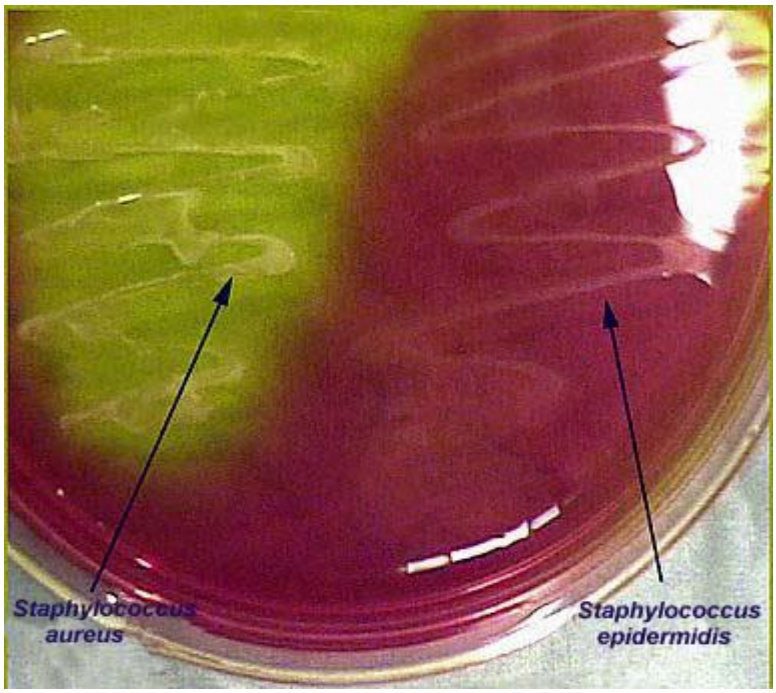


Figure 2.2.23 Two different species of Staphylococcus growing on mannitol salt agar (MSA).

Figures 2.2.23 Courtesy: Mannitol Salt agar from Bingham University Microbiology Department Nasarawa State Nigeria.

MSA is selective because it contains 7.5% salt–a high salt concentration that promotes the growth of some organisms while discouraging the growth of others. MSA is a differential medium because it contains the sugar mannitol and the pH indicator phenol red. Organisms that can ferment mannitol produce acid by-products, causing a colour change. Phenol red is a cherry red colour above pH 8.5, yellow-red from pH 6.9 to 8.5, and bright yellow at pH 6.9 or lower. Although both *Staphylococcus epidermidis* and *Staphylococcus aureus* can tolerate the high salt content of MSA, only *S. aureus* can ferment mannitol, causing the phenol red in the medium to turn yellow.

Staphylococcus saprophyticus

This organism is a Gram-positive, coagulase-negative bacterium and a significant cause of urinary tract infections, usually in young women who are sexually active. It is not usually differentiated from *S. epidermidis* clinically.

It occurs in the normal flora of the female genital tract and perineum and in females in the 17 to 27 years old age group, it is the second most common cause of urinary tract infection (after *E. coli*). Symptoms include dysuria and pyuria. Sexual activity increases the risk of infection of the urinary tract because bacteria are transferred from the vagina and perineum into the urethra. Most cases occur within a day of sexual intercourse and the infection is sometimes known as "honeymoon cystitis". *S. saprophyticus* has the capacity to selectively adhere to human urothelium. The adhesin for *S. saprophyticus* is a lactosamine structure. *S. saprophyticus* produces no exotoxins.

SPIROCHETES

The most important genera of spirochetes are Treponema, Borrelia and Leptospira. These are Gram negative bacteria that are long, thin, helical and motile. Axial filaments (a form of flagella) found between the peptidoglycan layer and outer membrane and running parallel to them, are the locomotory organelles.

3.6 *SPIROCHETES AND NEISSERIA*

Syphilis

Treponema pallidum

T. pallidum is the causative agent of syphilis, a common sexually-transmitted disease found world-wide (figure 2.2.24a).

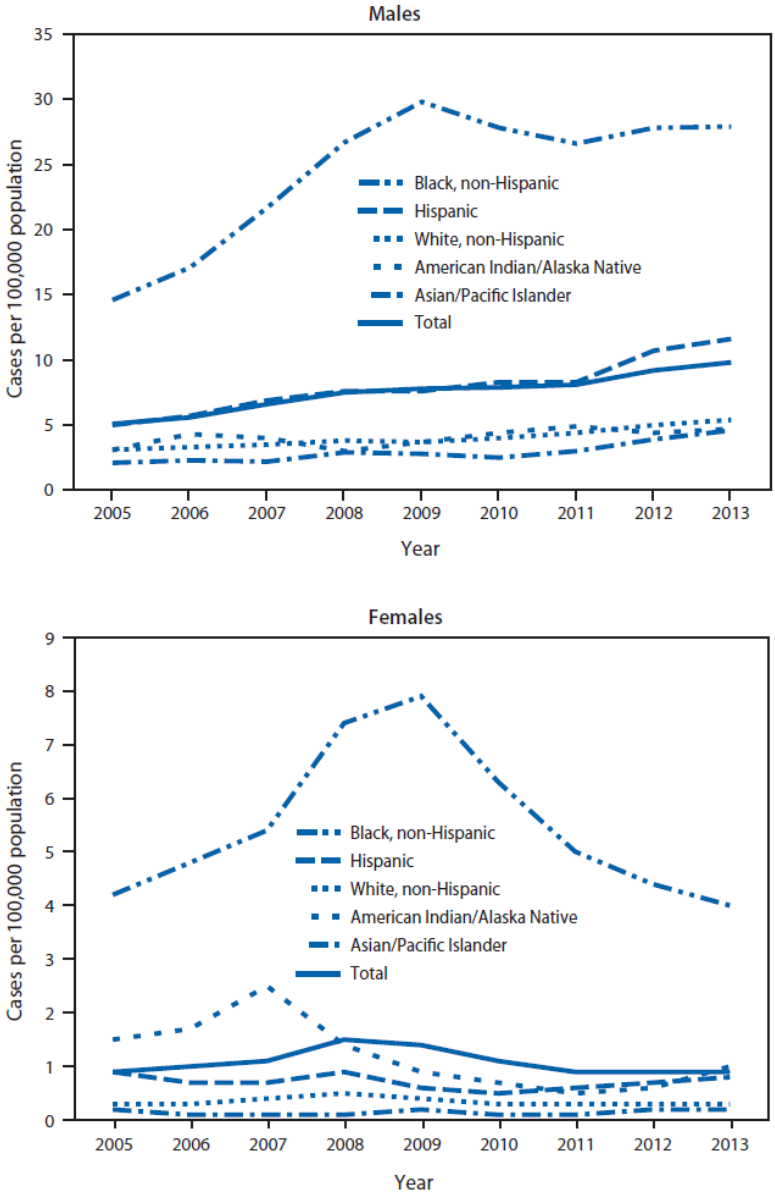


FIGURE 2.2.24A: ANNUAL RATE OF PRIMARY AND SECONDARY SYPHILIS CASES AMONG MALES AND FEMALES, BY RACE/ETHNICITY — NATIONAL NOTIFIABLE DISEASES SURVEILLANCE SYSTEM, UNITED STATES, 2005–2013

COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

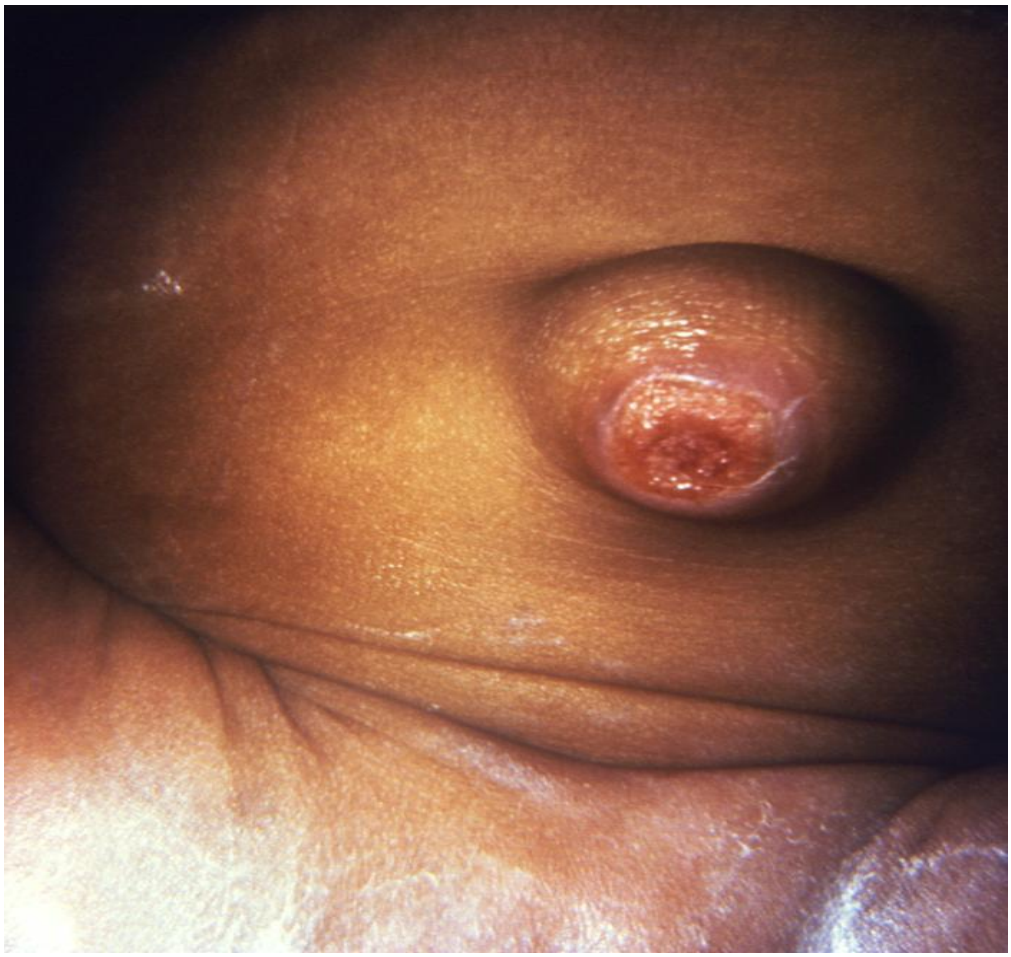


FIGURE 2.2.24B: UMBILICUS OF AN INFANT, WHICH DISPLAYED AN INFLAMED LESION THAT UNDER A DARKFIELD EXAMINATION REVEALED THE PRESENCE OF *TREPONEMA PALLIDUM* SPIROCHETES, AND HENCE, A DIAGNOSIS OF CONGENITAL SYPHILIS.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

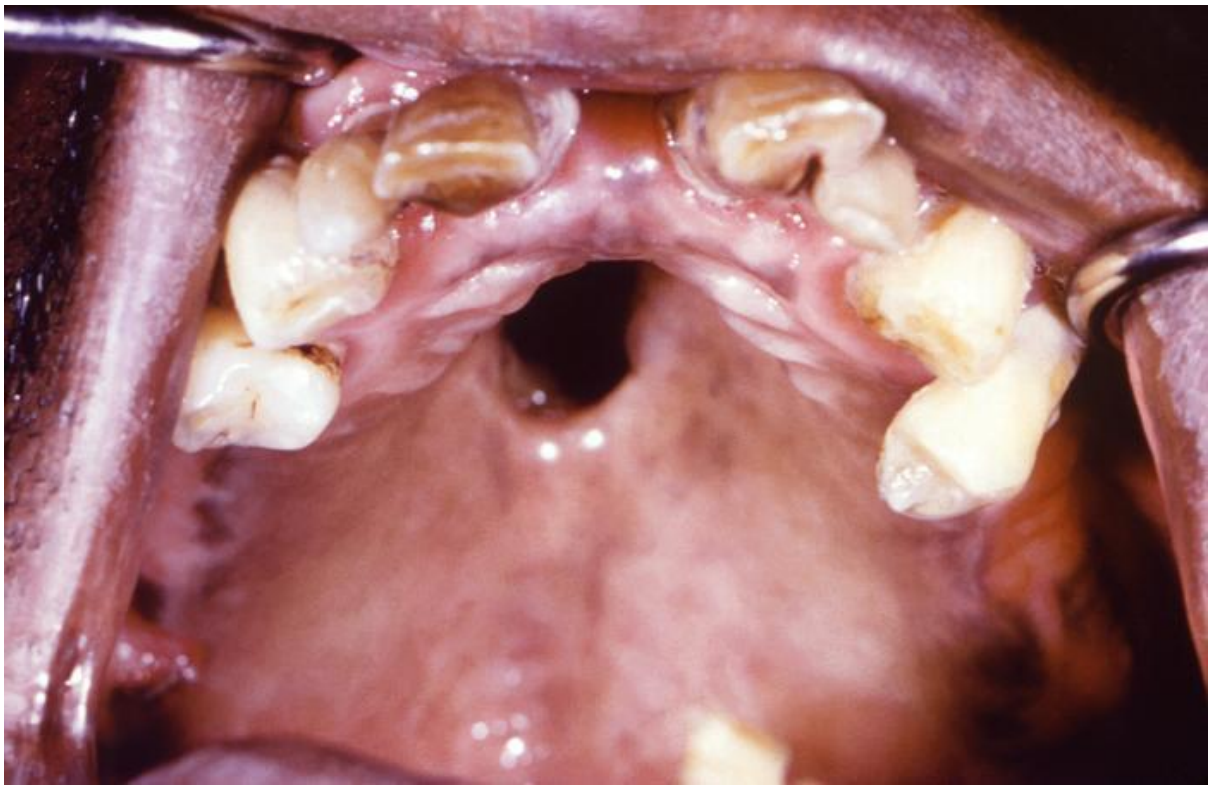


FIGURE 2.2.24C: THE INTERIOR ORAL CAVITY OF AN ELDERLY AFRICAN-AMERICAN MALE PATIENT, REVEALING A PERFORATED HARD PALATE DUE TO WHAT WAS A CONGENITAL SYPHILIS INFECTION. AT THE TIME OF THIS PHOTOGRAPH, THE PATIENT WAS BEING TREATED FOR BOTH ACTIVE SYPHILIS, AND GONORRHOEA INFECTIONS.

Courtesy: Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby,USA

It is generally transmitted by genital/genital contact. Transmission in utero or during birth can also occur (figure 2.2.24b). Syphilis, chronic and slowly progressive, is the third most common sexually transmitted disease.

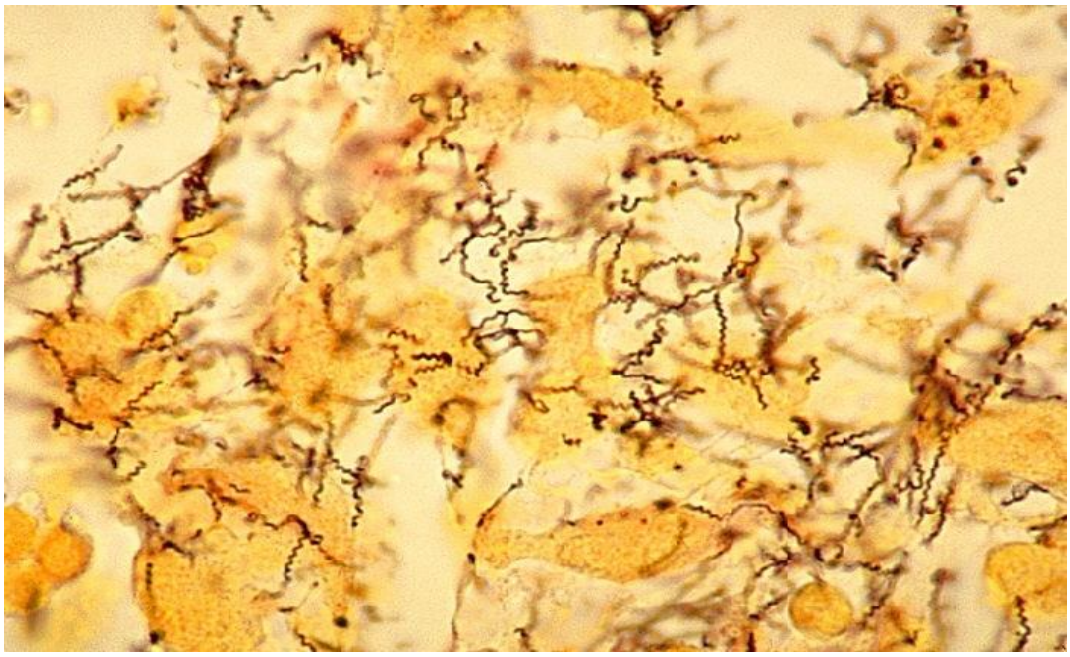


FIGURE 2.2.25: HISTOPATHOLOGY SHOWING *TREPONEMA PALLIDUM* SPIROCHETES IN TESTIS OF EXPERIMENTALLY INFECTED RABBIT. MODIFIED STEINER SILVER STAIN.

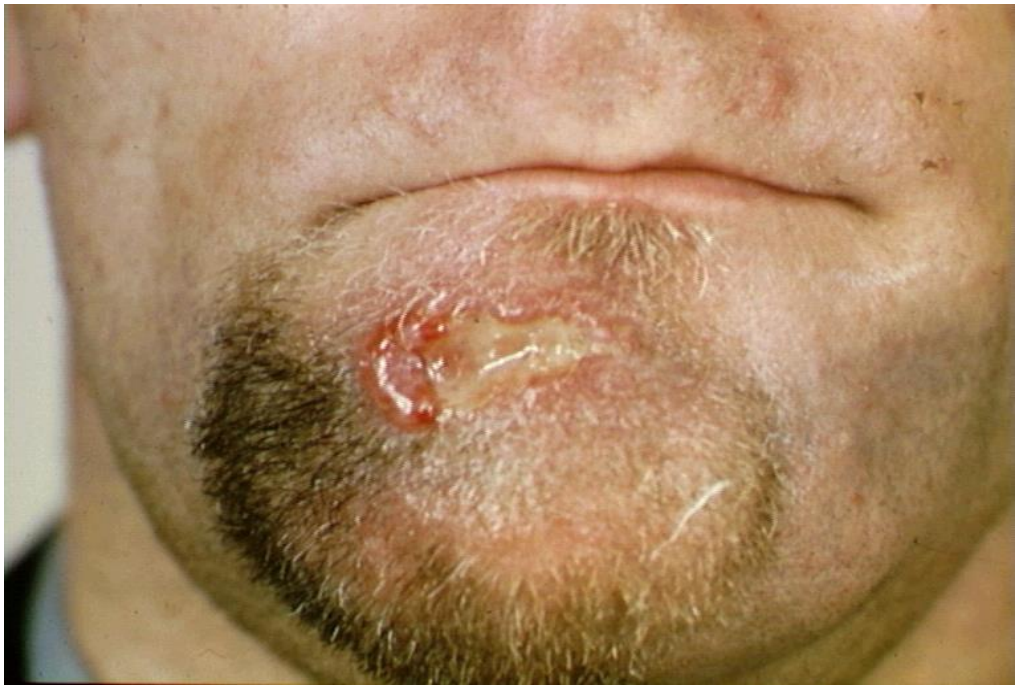


FIGURE 2.2.26: PRIMARY SYPHILIS

Figures 2.2.24-25 . *Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA*

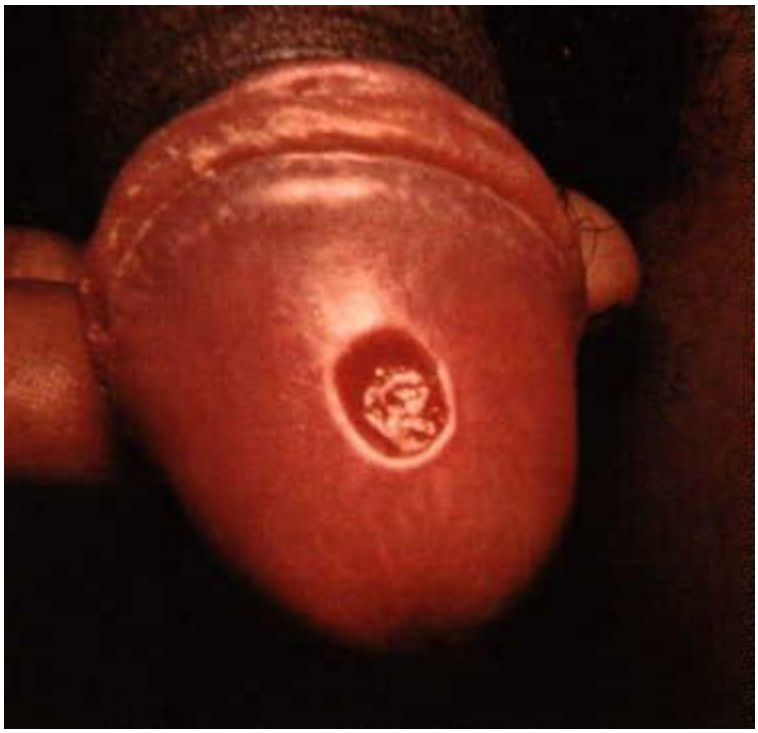


FIGURE 2.2.27: PRIMARY SYPHILIS. PRIMARY CHANCRE ON THE GLANS

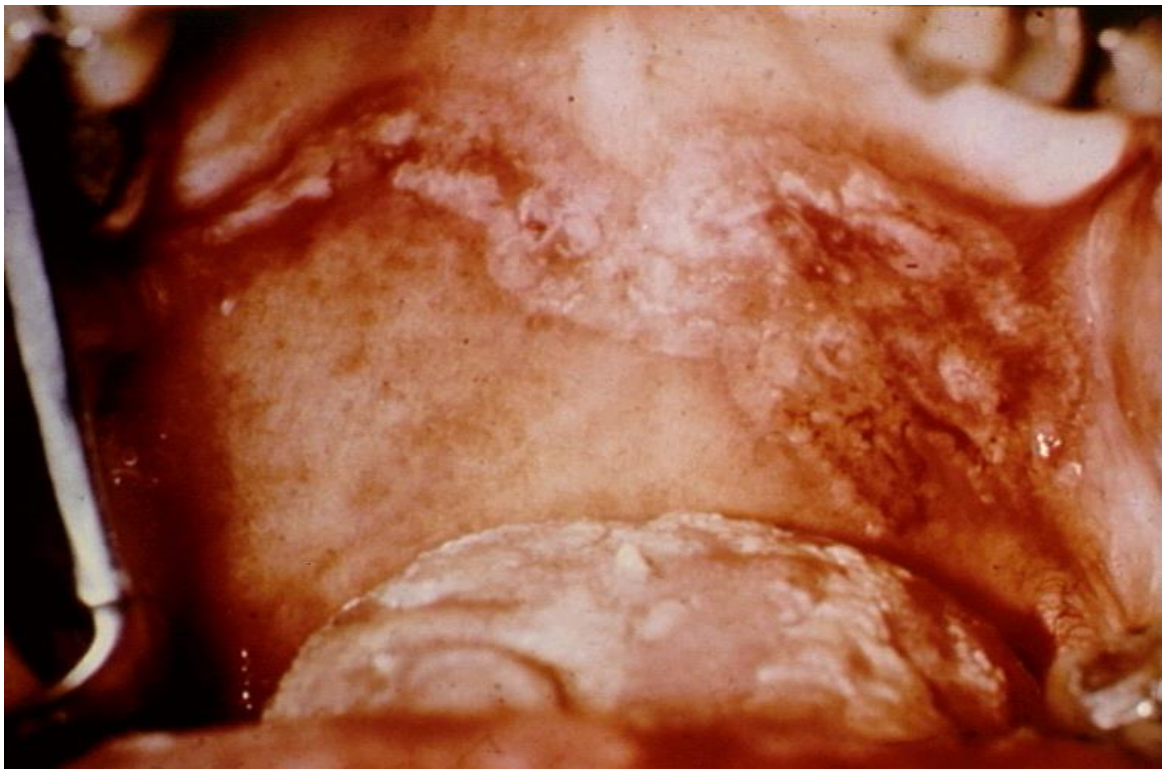


FIGURE 2.2.28: SECONDARY SYPHILIS - MOUTH MUCOSA

2.2.27-28 *Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA*

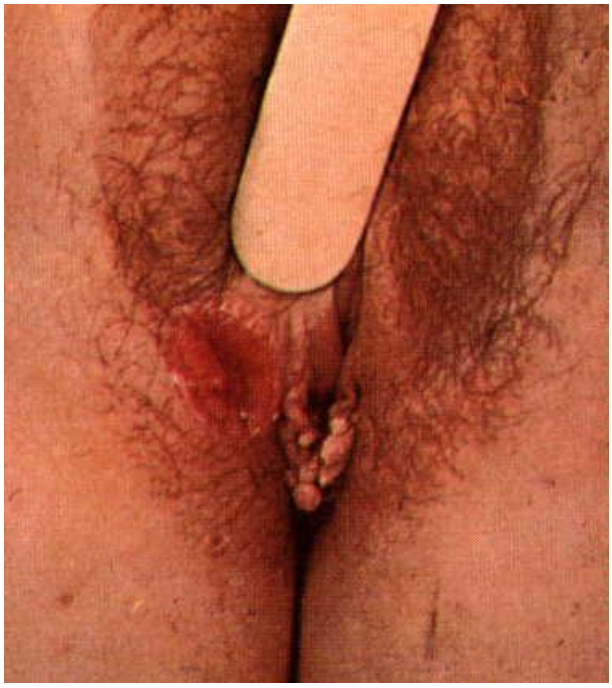


FIGURE 2.2.29: PRIMARY SYPHILIS. A VULVAR CHANCRE AND CONDYLOMATA ACUMINATA

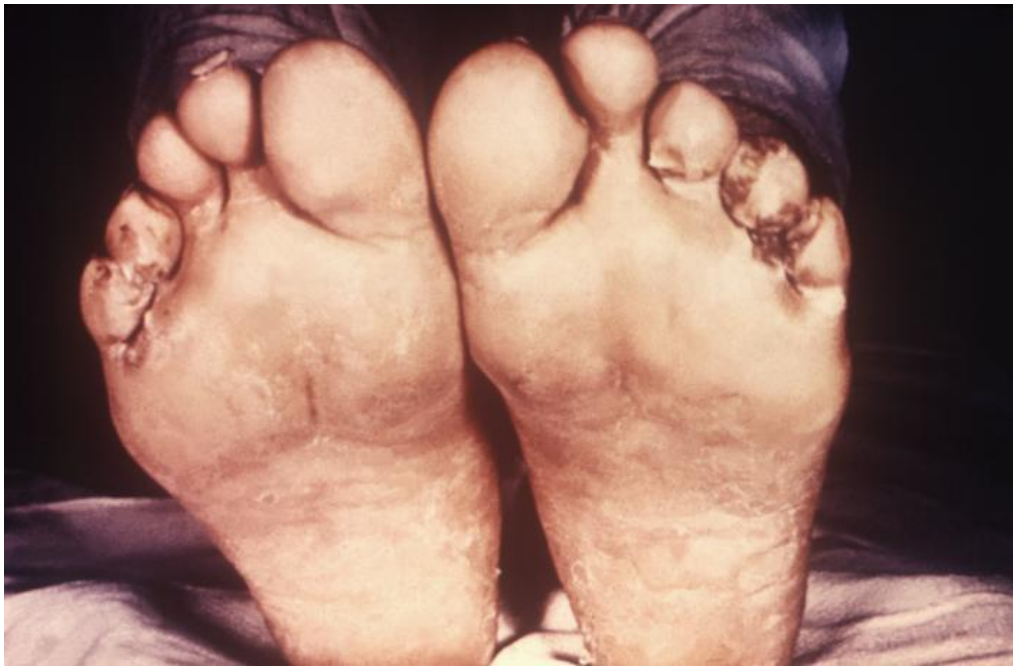


FIGURE 2.2.29A: SECONDARY SYPHILIS: SOLES OF BOTH FEET OF A SYPHILIS PATIENT REVEALING THE PRESENCE OF SECONDARY SYPHILITIC LESIONS CONSISTING OF EROSIVE DERMAL REGIONS OF THE TOES, MAINLY INVOLVING THE INTERTRIGINOUS SPACES BETWEEN THE TOES.

COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

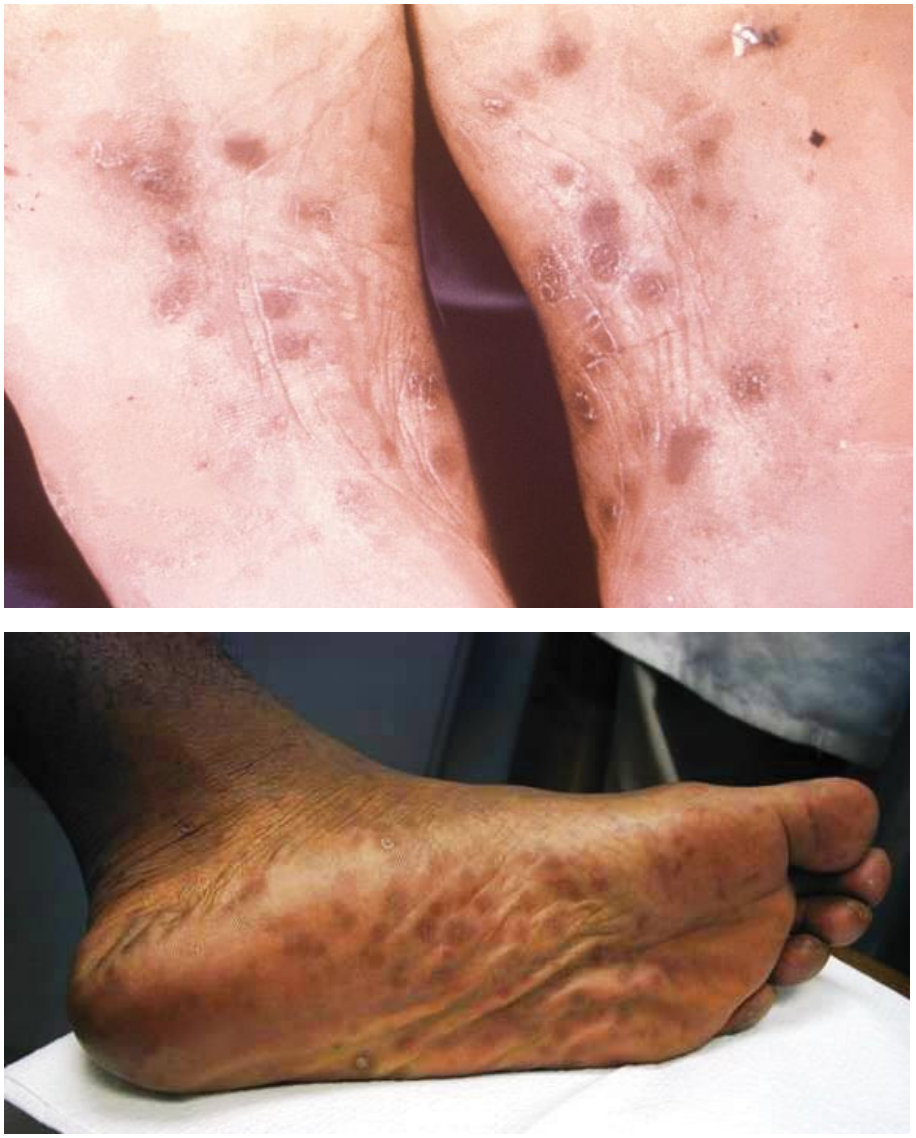


FIGURE 2.2.29B: SECONDARY SYPHILIS: SOLES OF FEET OF A SYPHILIS-INFECTED PATIENT (PLANTAR SYPHILIDS) IN A SECONDARY SYPHILITIC INFECTION.

COURTESY: *COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA*

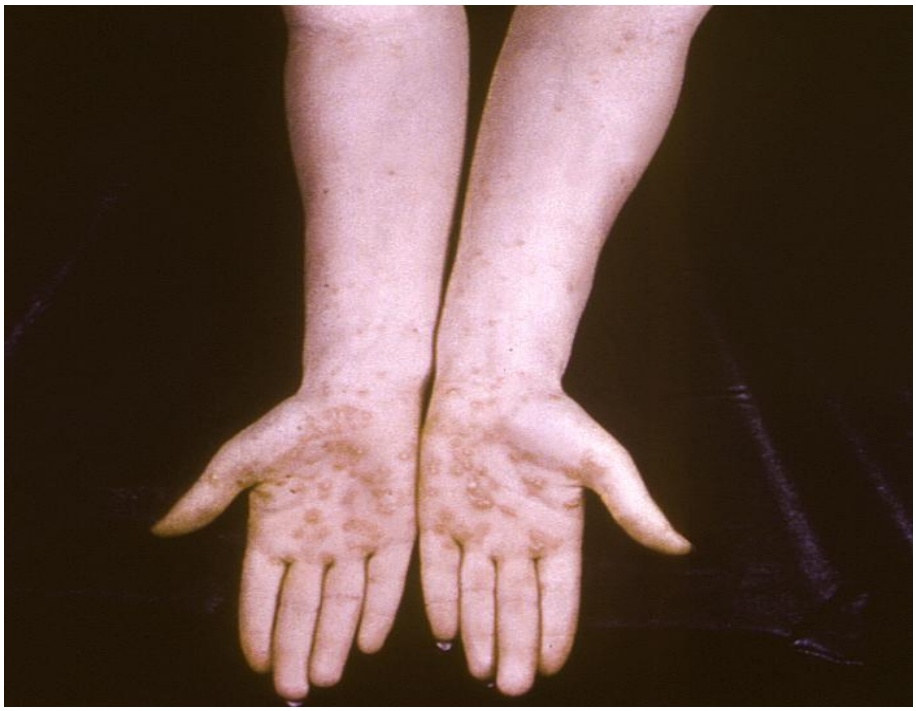
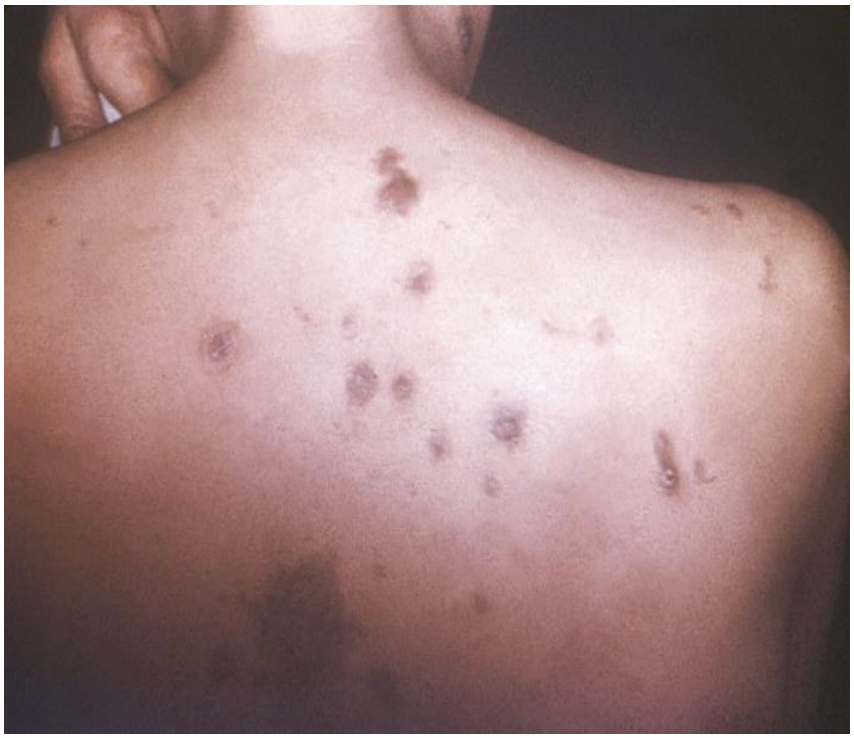


FIGURE 2.2.29C: SECONDARY SYPHILIS: PALMS OF HANDS SHOWING PALMAR SYPHILIDS, DUE TO SECONDARY SYPHILIS. RASH MAY INCLUDE FOREARMS.



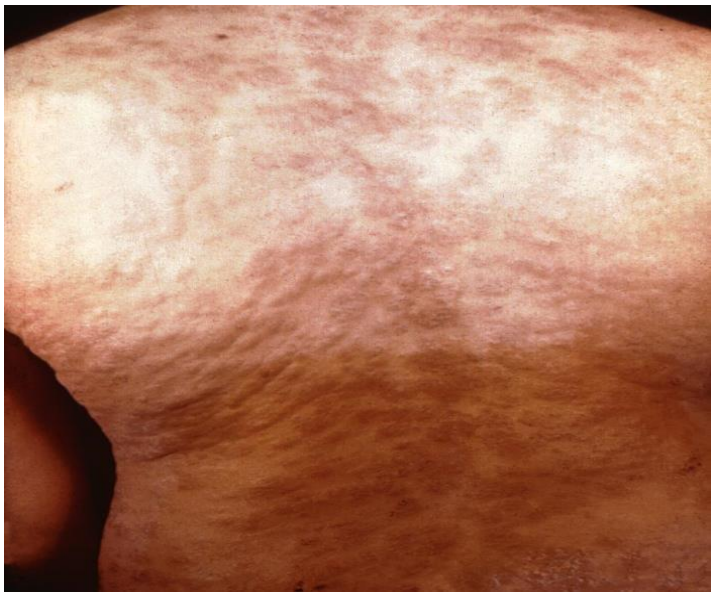


FIGURE 2.2.29D: SECONDARY SYPHILIS: UPPER BACK AND NECK OF PATIENT WITH A MACULOPAPULOSQUAMOUS OUTBREAK OF NODULAR SYPHILIDS.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

After initial infection, a primary chancre (an area of ulceration/inflammation) is seen in genital areas (figure 2.2.27 and 2.2.29) or elsewhere (figure 2.2.26) within 10 to 60 days. The organism, meantime, has penetrated and systemically spread. The patient has flu-like symptoms with secondary lesions particularly affecting the skin (figure 2.2.28). These occur 2 to 10 weeks later. The final stage (if untreated) is tertiary syphilis (several years later). In primary and secondary syphilis organisms are often present in large numbers. However, as the disease progresses immunity controls bacterial replication and fewer organisms are seen. It is extremely difficult to detect spirochetes in tertiary syphilis. The systemic lesions of skin, central nervous system and elsewhere are suggestive of a delayed hypersensitivity reaction.

The organism cannot be cultured from clinical specimens. Thus, experimentally, syphilis is commonly studied in animal models. Also microscopic and serological methods are the only means of clinical diagnosis.

In primary syphilis (before immunity develops), the organisms are often present in sufficient numbers in exudates to be detected by dark field microscopy. In conventional light microscopy, the light shines through the sample and thin treponemes cannot be visualized. In dark field microscopy, the light shines at an angle and when reflected from the organism will enter the objective lens. The actively motile organisms appears brightly lit against the dark backdrop. Alternatively fluorescent antibody staining is used.

In secondary and tertiary syphilis, serological methods are usually used to detect syphilis. Screening methods are based on detecting serum antibodies to cardiolipin in patients (including VDRL test). The antibodies result from tissue injury, with autoimmunity developing to self-components. Thus, there are many other diseases that result in anti-cardiolipin antibodies and false positives are common. However, these are cheap screening tests. More definitive diagnosis is achieved by detecting the presence of "specific" serum antibodies against treponemal antigens. These tests are more expensive and usually performed (as a definitive diagnosis) on sera previously shown to be positive after first detecting antibodies to cardiolipin.

Primary and secondary syphilis occur within a year of infection and are sometimes referred to as "early syphilis". Patients with early syphilis are highly infectious..

SUMMARY OF SYMPTOMS

Primary syphilis

•Usually a single firm, round sore (but there may be more). Usually on the genitals but can be elsewhere
•No pain at the site of the sore
The sore will heal without intervention

Secondary syphilis

•Rough red skin rash, often on the back (figure 2.2.29d) but can be elsewhere. The rash does not usually itch
•Sores on mucous membranes (seen in mouth, anus, vagina)
•Red spots (known as syphilids) on palms of hands and soles of feet (figure 3.2.29a, b and c)
•Fever
•Lymphadenopathy (swollen lymph glands)
•Sore throat
•Hair loss
•Headache
•Weight loss
•General malaise
Symptoms will resolve with or without treatment and the infection becomes latent.

Tertiary Syphilis

The disease, when untreated, can remain latent for years (even two to three decades) and most infected people do not develop further symptoms; however, if disease does reappear it can be very serious and sometimes fatal. The symptoms include:

- Failure to coordinate muscle movements
- Paralysis
- Numbness
- Blindness
- Dementia
- Organ failure

Epidemiology

From 2005 to 2013, the number of primary and secondary syphilis cases reported each year in the United States nearly doubled, from 8,724 to 16,663; the annual rate increased from 2.9 to 5.3 cases per 100,000 population. Most of these cases were in men (91.1% of all primary and secondary syphilis cases in 2013) and mostly in men who have sex with men. The rate per 100,000 among men increased from 5.1 in 2005 to 9.8 in 2013.

Treatment

No vaccine exists, but antibiotic therapy (usually penicillin G) is usually highly effective, including treatment of congenital syphilis.

Bejel

Treponema pallidum endemicum

This disease is rare (in the US) and is caused by organisms related to T. pallidum. T. pallidum endemicum is morphologically and serologically indistinguishable from Treponema pallidum.

Bejel, also known as endemic syphilis, is not transmitted sexually but via contact, for example hands to broken skin and mouth to mouth. The disease can also be spread by sharing eating utensils. It is a disease of low income groups with poor hygiene and often begins in childhood.

Depending on the route of transmission, skin or mucous membranes are the first to be infected but the bacterium can spread deeper to the bones. Thus, one sees sores in the mouth, throat and the nasal passages and the infected

lesions can penetrate deep into the tissue causing major malformations of the face and limbs. This results in severe bone pain and there is also swelling of the lymph nodes. The *T. pallidum* organisms can be found in swabs of the sores.

Treatment
Treatment of bejel, which can be completely curative, is similar to syphilis, that is penicillin G or tetracycline.

Epidemiology
Bejel is found in the Middle-East, Africa, Australia and central Asia. It is also known as Sahel disease in West Africa.

Pinta

Treponema carateum

Pinta is another non-venereal, treponematous disease which is caused by *T. carateum*. It occurs in the New World, particularly the Caribbean, central America and northern South America. Pinta is the Spanish for "painted". Again, it is a disease of poor regions with sub-standard hygiene and is spread by personal contact through cuts in the skin. This results in scaly red lesions (hence the name) which form a lump at the site of the primary infection. Small satellite lesions form around the primary lesion and lymph node swelling is also seen. Some months after the primary infection, the patient experiences more scaly red lesions that are now flat and tend to itch. These are the pintids and occur around or distant from the site of the primary infection. The colour of the pintids changes to blue black with time and then can lose pigmentation. Unlike bejel, the disease does not spread deep into the tissues and bones. Detection is via serology or direct examination of lesion specimens under the light microscope.

Treatment
Treatment of pinta is again curative and can be accomplished by a single injection of penicillin G.

Yaws
Treponema pertenue

Yaws (figure 2.2.30) is another chronic treponematous disease of poor hygiene. It can be very disfiguring. It strikes mainly children in Africa, south Asia and northern South America.

FIGURE 3.2.30A: YAWS IS A CRIPPLING AND DISFIGURING DISEASE AFFECTING SOME 50 MILLION PEOPLE IN THE WORLD

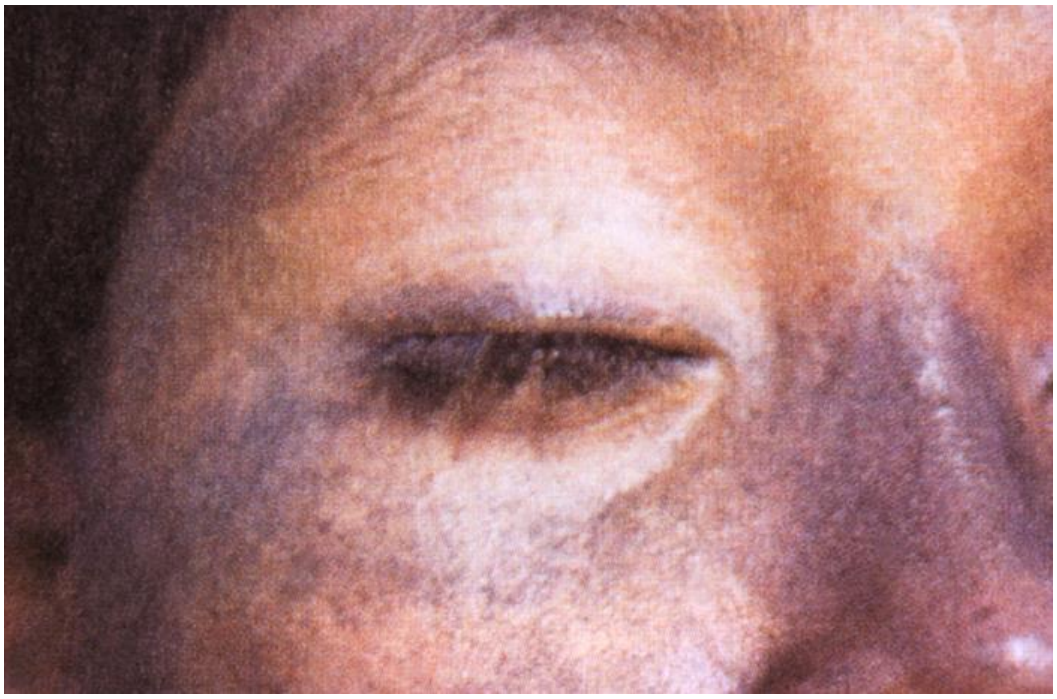


FIGURE 2.2.30B: DISCOLORED AREAS INDICATIVE OF PINTA. PATHOLOGIC CHANGES ACCOMPANYING THIS DISCOLORATION INCLUDE THICKENING OF THE EPIDERMIS, FOLLOWED BY SCALINESS AND DRYING OF THE SKIN, KNOWN AS ACANTHOSIS.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

The causative agent is *T. pertenue*. As with pinta and bejel, spread is via direct contact through skin lesions. About a month after the infection, a papule forms at the infection site which transforms into a crusted ulcer that takes months to heal. Painful swelling of the lymph nodes occurs. Later, soft growths appear on the face, buttocks and limbs. They can also occur in the bottoms of the feet causing the infected person to have a very characteristic walk which gives rise to the name of "crab yaws". Further formation of tumours and ulcers on the

face can cause bone malformation and can be disfiguring. Microscopy (of samples from the lymph nodes) is diagnostic and there are various serological tests.

Treatment of yaws is also a single penicillin G injection which can be completely curative

Lyme disease

Borrelia burgdorferi

Lyme disease is caused by Borrelia burgdorferi (figure 2.2.31a,b and 2.2.36) and is a relatively newly recognized disease. It is found widely in the United States (figure 2.2.32) but is most concentrated in the north east and mid-west. The number of cases peaked in 2009 (figure 2.2.33a).

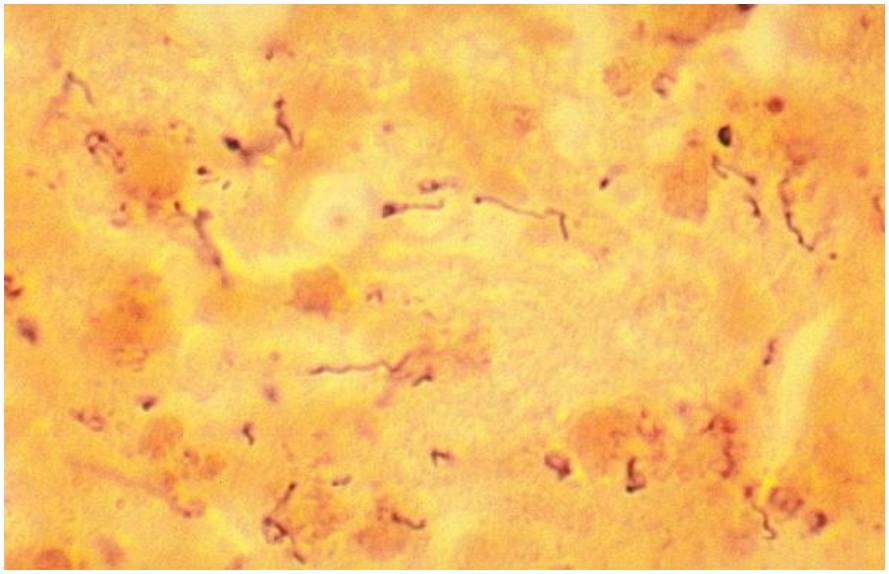


FIGURE 2.2.31A: HISTOPATHOLOGY SHOWING *BORRELIA BURGDORFERI* SPIROCHETES IN LYME DISEASE. DIETERLE SILVER STAIN.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

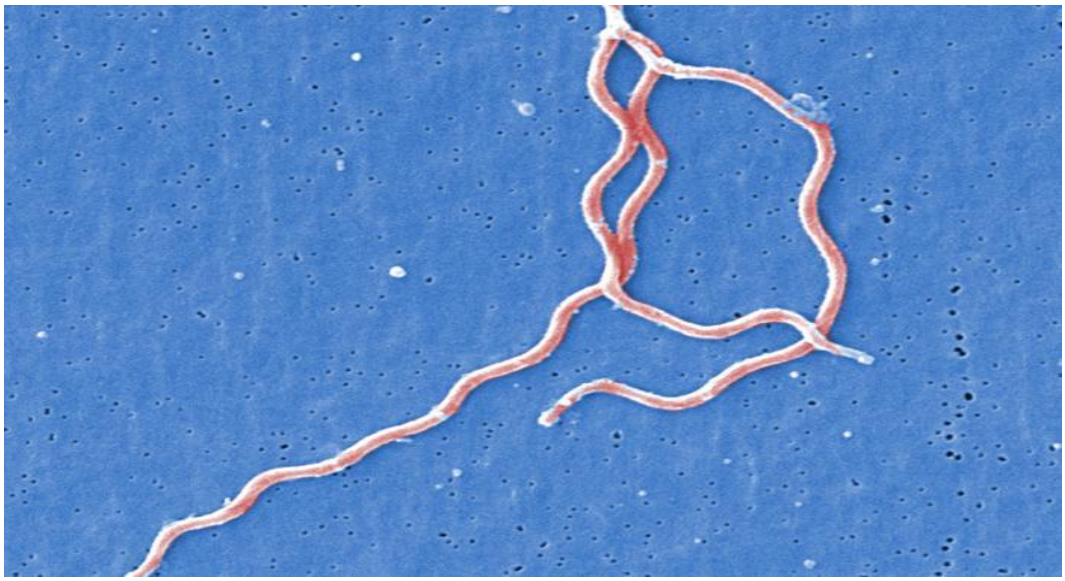


FIGURE 2.2.31B: UNDER A HIGH MAGNIFICATION, THIS DIGITALLY-COLORIZED SCANNING ELECTRON MICROGRAPH DEPICTS THREE GRAM-NEGATIVE, ANAEROBIC, *BORRELIA BURGDORFERI* BACTERIA, WHICH HAD BEEN DERIVED FROM A PURE CULTURE.

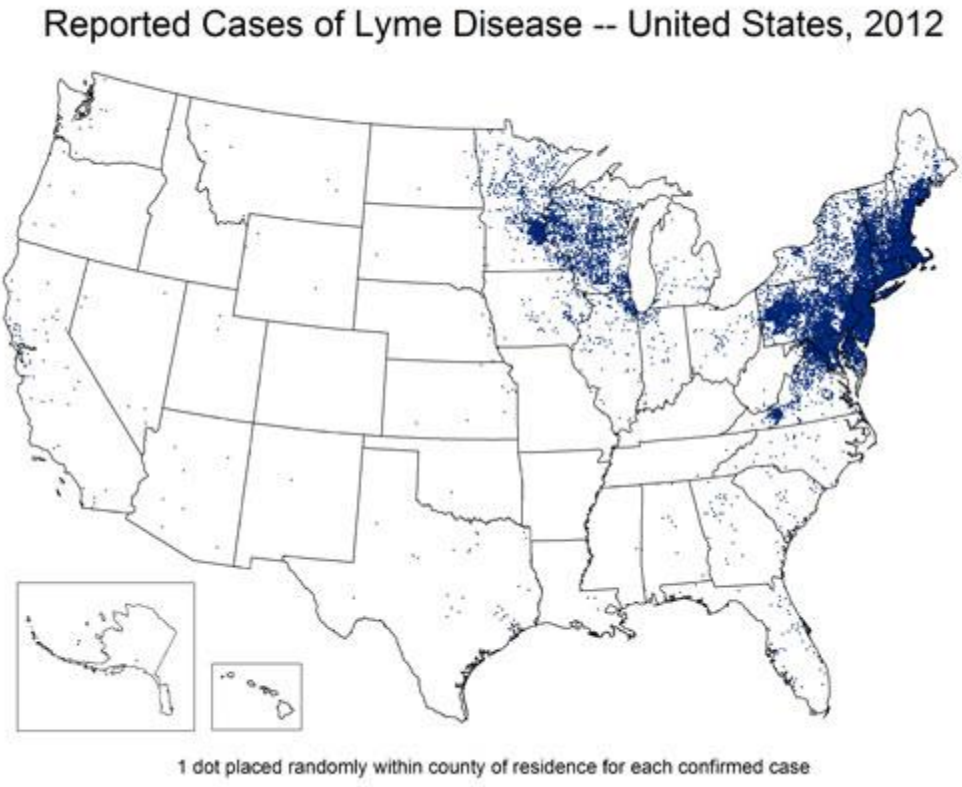


FIGURE 2.2.32 INCIDENCE OF LYME DISEASE BY COUNTY IN THE UNITED STATES 2012.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

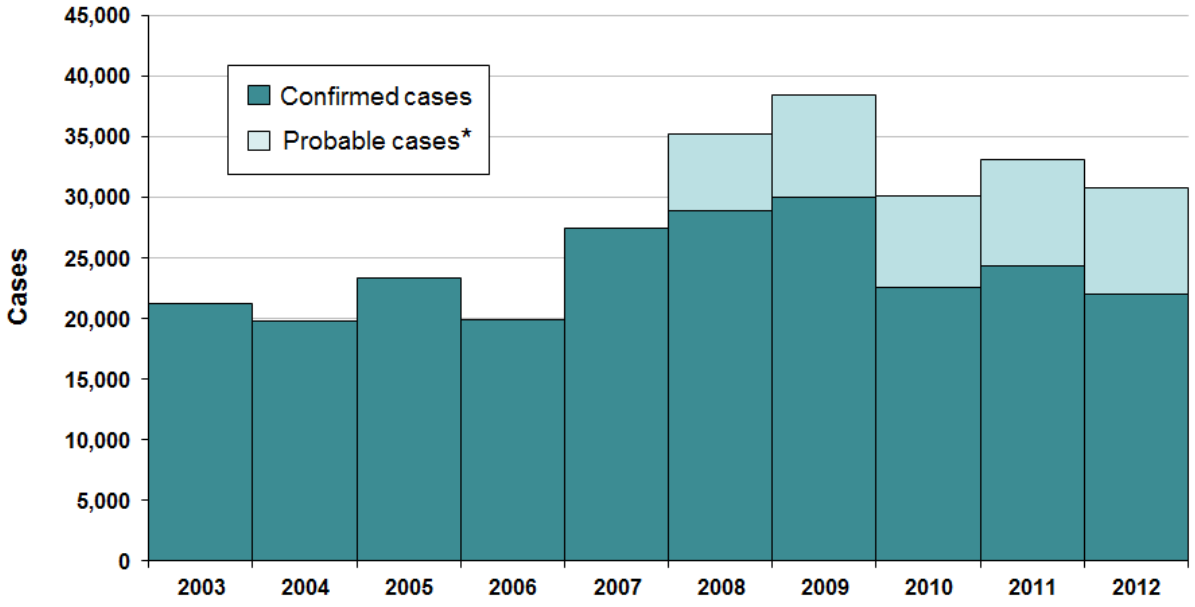


FIGURE 2.2.33A: THE NUMBER OF REPORTED CASES OF LYME DISEASE FROM 2003 THROUGH 2012. THE NUMBER OF CONFIRMED CASES RANGED FROM A LOW OF 19,804 IN 2004 TO HIGH OF 29,959 IN 2009.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

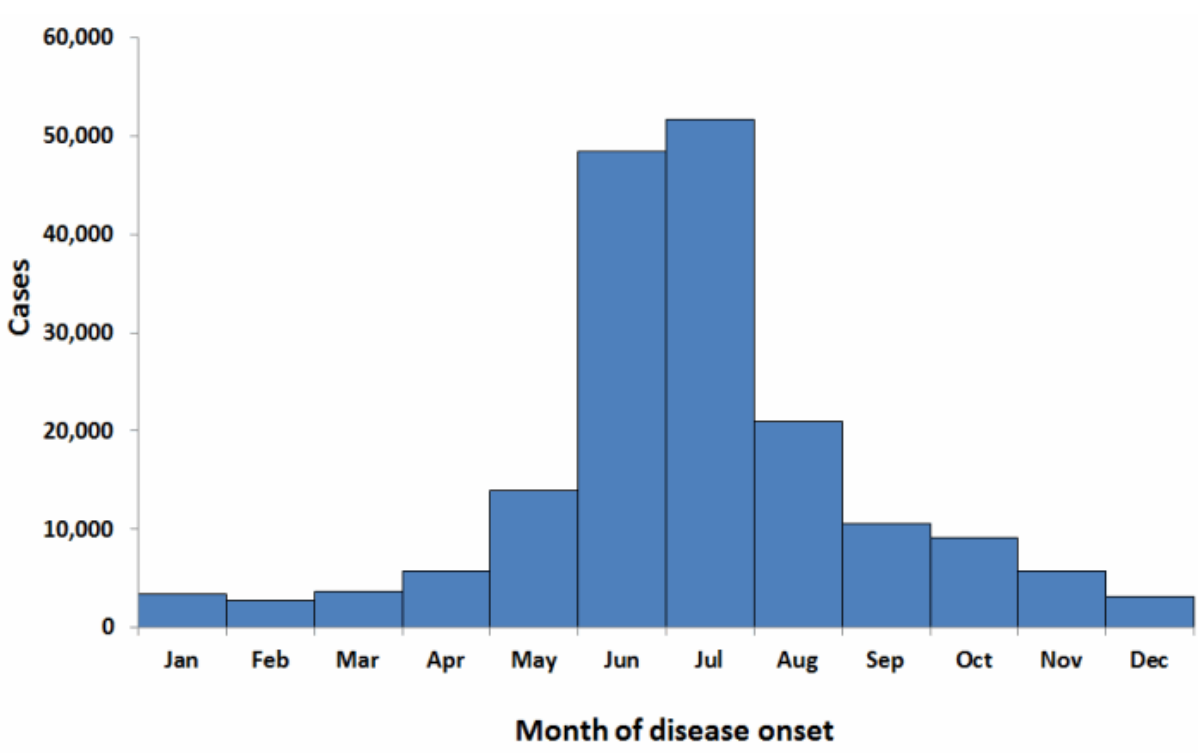


FIGURE 2.2.33B: LYME DISEASE PATIENTS ARE MOST LIKELY TO HAVE ILLNESS ONSET IN JUNE, JULY, OR AUGUST AND LESS LIKELY TO HAVE ILLNESS ONSET FROM DECEMBER THROUGH MARCH.

COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

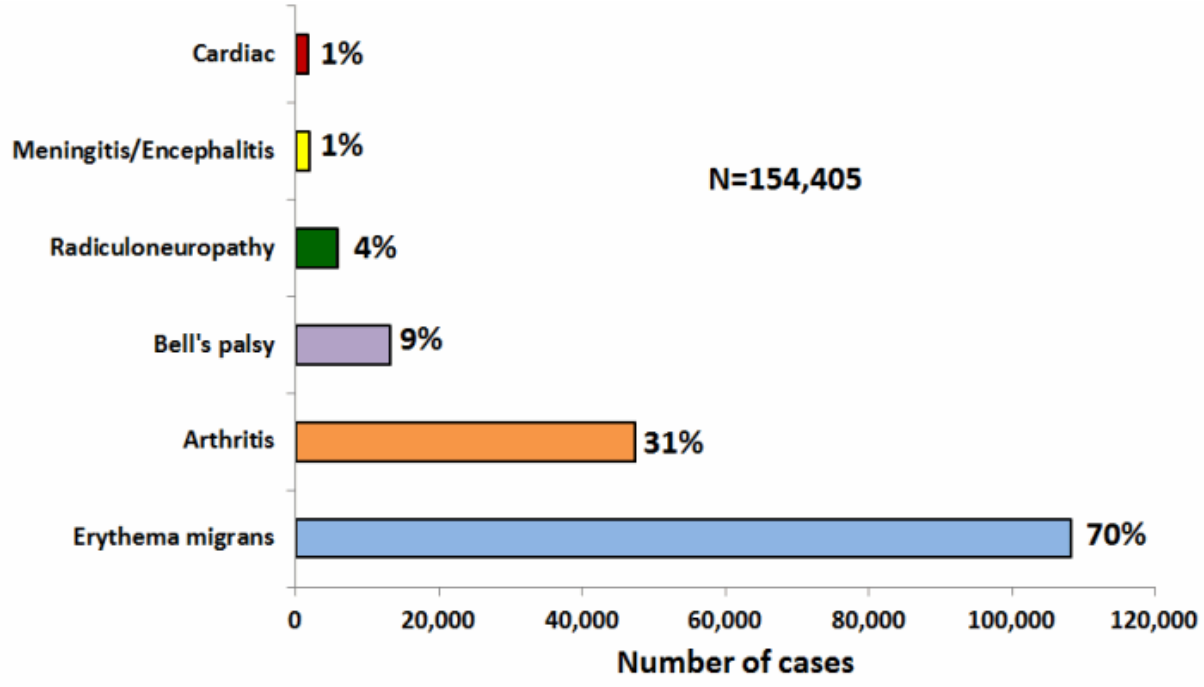


FIGURE 2.2.33C: BREAKDOWN OF REPORTED LYME DISEASE CASES FROM 2001 TO 2010 BY DISEASE MANIFESTATION. THE MAJORITY OF CASES ARE THE EM RASH. OTHER MANIFESTATIONS ARE LESS COMMON, SOME PATIENTS HAVE MORE THAN ONE PRESENTATION.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Although clinically first described in 1975, the role of a tick-borne spirochete was not proven until 1983. These ticks (figure 2.2.35) infect a large array of wild life. A tick bite leads to transmission of B. burgdorferi causing an erythematous skin rash (figure 2.2.34) in a few days along with a transient bacteremia leading to (weeks or months later) severe neurologic symptoms or polyarthritis. Cardiac problems may occur in a minority of cases (figure 2.2.33c). Cases of Lyme disease occur primarily in the summer months in the United States because of increased outdoor activities leading to increased likelihood of picking up a tick.

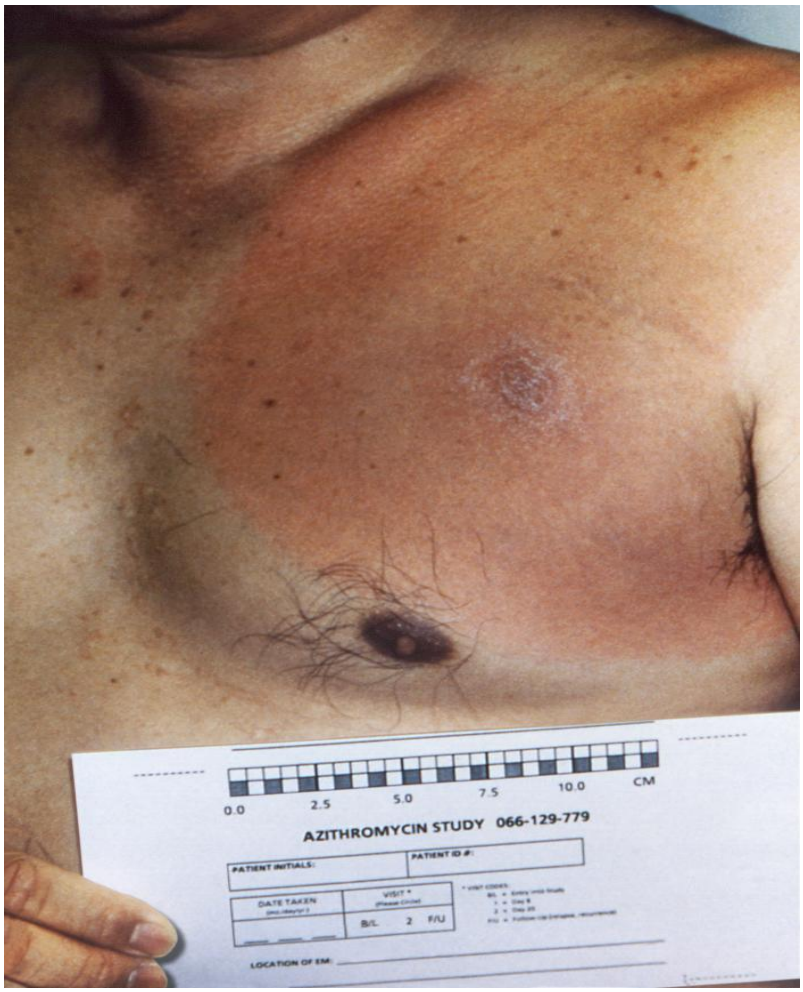


FIGURE 2.2.34A: LEFT ANTERIOR CHEST AND SHOULDER REGION OF A PATIENT WHO'D PRESENTED WITH THE ERYTHEMA MIGRANS (EM) RASH CHARACTERISTIC OF WHAT WAS DIAGNOSED AS LYME DISEASE, CAUSED BY THE BACTERIUM, *BORRELIA BURGDORFERI*.

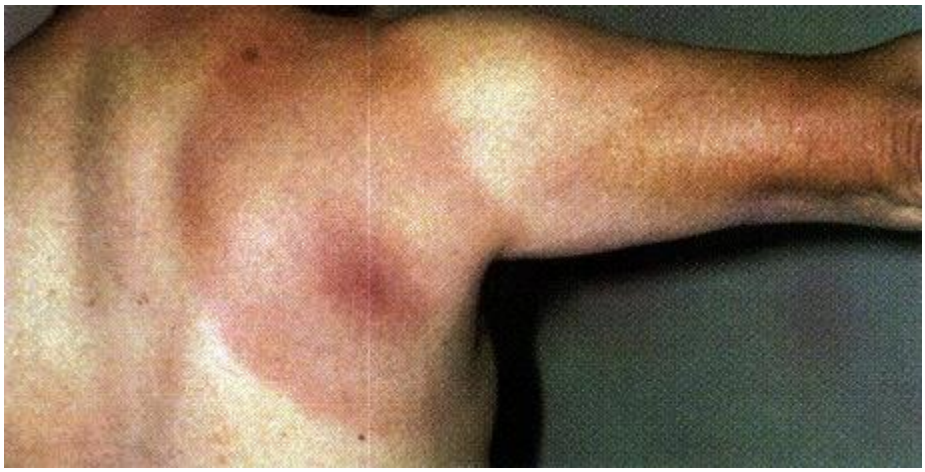


FIGURE 3.2.34B: LYME DISEASE RASH COURTESY:

COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

FIGURE 2.2.35: *IXODES SCAPULARIS* (DEER TICK), TICK VECTOR FOR LYME DISEASE. ITS ABDOMEN IS ENGORGED WITH A HOST BLOOD MEAL, THIS IMAGE SHOWS A LATERAL VIEW OF A FEMALE.

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

If antibiotic therapy is initiated early, a cure is usually achieved. However, late antibiotic administration (penicillin or tetracycline) is often ineffective.

The life cycle of Lyme disease ticks is shown in figure 2.2.36a.

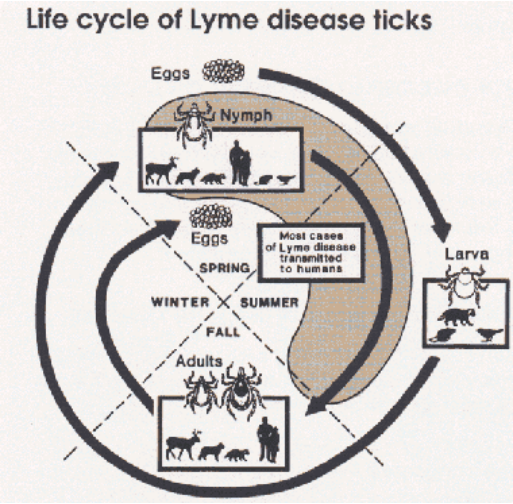


FIGURE 2.2.36

COURTESY: BAILEY AND SCOTT 'S TEXT BOOK OF MEDICAL MICROBIOLOGY TWELFTH EDITION 2016

Diagnosis

B. burgdorferi is highly fastidious, growing extremely slowly in tissue culture (not bacteriological) media. The vast majority of body fluid or tissue samples from patients with Lyme disease do not yield spirochetes on culture. Lyme disease is thus usually diagnosed by detection of serum antibodies to *B. burgdorferi*. However, acutely antibodies may not occur in detectable titer, making early diagnosis difficult. However, late diagnosis may lead to ineffective treatment. Many patients are unaware of having had a tick bite or a rash.

Etiology

The chronic arthritis clinically resembles rheumatoid arthritis. Live agent is almost never cultivated from the joint (in common with other forms of reactive arthritis such as Reiter's syndrome and rheumatic fever). However, small numbers of persistent spirochetes and borrelial antigens have been detected histologically in human tissues. Whether the organism persists in a viable form or not remains to be determined. Thus, there is no clear explanation for the immunopathologic stimulus for chronic tissue injury in Lyme arthritis.

RELAPSING FEVER

Borrelia hermsii and Borrelia recurrentis

There are two types of relapsing fever:

- Tick-borne relapsing fever (TBRF)
- Louse-borne relapsing fever (LBRF)

Tick-borne relapsing fever occurs in the western United States and is usually linked to sleeping in rustic, rodent-infested cabins in mountainous areas. Louse-borne relapsing fever is transmitted by the human body louse and is generally restricted to refugee settings in developing regions of the world.

There are fewer than 100 cases of relapsing fever per year in US. During the years 1990-2011, 483 cases of TBRF were reported in the western United States.

Relapsing fever (with associated bacteremia) is caused by species of Borrelia that are transmitted by tick (Borrelia hermsii, rodent host) and lice (*B. recurrentis*, human host) bites. The term relapsing fever is derived from the following repeating cycle. As an immune response develops the disease relapses. However, the antigens expressed change and the disease reappears. The organism is extremely difficult to culture and there is no serological test. The organism is generally detected by blood smear.

Leptospirosis

There are fewer than 100 cases of leptospirosis per year in US. This flu-like or severe systemic disease is a zoonotic infection. Leptospira are transmitted in water contaminated with infected urine from wild animals (including rodents) and farm animals and can be taken in through broken skin (e.g. bathing). Leptospira particularly infect the kidney, brain and eye. They are the most readily culturable of the pathogenic spirochetes; but this is not routine and diagnosis is usually by serology.

Treatment

Leptospirosis is treated with antibiotics, such as doxycycline or penicillin, which should be given early in the course of the disease. Intravenous antibiotics may be required for persons with more severe symptoms. Persons with symptoms suggestive of leptospirosis should contact a health care provider.

NEISSERIA

Neisseria are Gram negative diplococci (pairs of cocci). These bacteria grow best on chocolate agar (so-called because it contains heated blood, brown in colour); a modified (selective) chocolate agar commonly used is Thayer Martin. The colonies are oxidase positive (i.e. produce cytochrome oxidase) which is demonstrated by flooding the plate with a dye which on oxidation changes colour.

FIGURE 2.2.37

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

N. gonorrhoeae (figure 2.2.37), found only in man, is the causative agent of *gonorrhoea*, the second most common venereal disease. *Gonorrhoea* has recently declined after a peak in 1976. The disease particularly occurs in younger adults and is found equally in males and females. Highest rates in the United States are in the southeast.

N. gonorrhoeae often causes an effusion of polymorphonuclear cells. A smear may show the presence of Gram negative cocci present in cells. However, culture is essential for definitive diagnosis. There is a fluorescent antibody test.

A common feature of disseminated gonoccocal disease is arthritis. Although commonly considered a form of septic arthritis, in many cases gonococci cannot be isolated from the joint (i.e. they are "reactive" in nature). Dermatitis is also common.

Penicillin therapy is still usually effective. However, resistant strains producing beta lactamases are sufficiently common that alternatives are recommended for all gonococcal infections; this includes ceftriaxone (a beta lactamase-resistant cephalosporin).

Because of increasing antibiotic resistance, new therapies to treat *gonorrhoea* have been sought. Two new antibiotic regimens using existing drugs – injectable gentamicin in combination with oral azithromycin and oral gemifloxacin in combination with oral azithromycin – successfully treated *gonorrhoea* infections in a clinical trial. The injectable gentamicin/oral azithromycin combination appears to be 100% effective in curing genital *gonorrhoea* infections, and while the oral gemifloxacin/oral azithromycin combination was 99.5% effective.

There is no vaccine since strains are highly variable in their external antigens (both outer membrane and pili). Both are involved in the initial adhesion of the organism to genital epithelium.

IgA proteases (also produced by *N. meningitidis*) are involved in successful colonization. As for many other bacterial infections, a role for both the lipopolysaccharide and peptidoglycan in tissue injury have been suggested. Exotoxins are not believed to be of importance in pathogenesis.

Outbreaks occur usually among adults living in confined and crowded conditions (e.g. university dorms, army barracks, prisons). Initial infection of the upper respiratory tract (involving binding by pili) leads to invasion into the bloodstream and from there to the brain. Indeed, it is the second most common cause of meningitis (pneumococcus is the most common). Neisseria meningitis is usually fatal if untreated but responds well to antibiotic therapy. Thus, rapid diagnosis is important. The organism is often detectable in spinal fluid (Gram negative diplococci within polymorphonuclear cells) or antigenically. Culture on Thayer Martin (or similar) agar is essential for definitive diagnosis. Penicillin is the drug of choice.

Meningococci vary antigenically and can be serogrouped with anti-capsular antibodies. The capsule is an important pathogenesis factor allowing inhibition of phagocytosis.

There are effective meningococcal vaccines that protect against most types of meningococcal disease, although they do not prevent all cases. There are two vaccines against Neisseria meningitidis available in the United States: meningococcal polysaccharide vaccine (Menomune) and meningococcal conjugate vaccine (Menactra, Menveo and MenHibrix). In the United States, vaccines are approved and routinely used against serogroups C and Y (in addition to A and W, which circulate globally), but not B. A serogroup B meningococcal vaccine that is licensed for use in Europe, Canada, and Australia has been used in the United States to help control 2 outbreaks of this disease in universities.

Non-pathogenic species morphologically resembling Neisseria are found in the normal flora of the oropharynx but can be differentiated from the pathogenic Neisseria readily. These occasionally cause opportunistic human disease (including pneumonia).

3.7 ANAEROBES AND PSEUDOMONAS IN OPPORTUNISTIC

ANAEROBES

Obligate anaerobes are bacteria that cannot survive in the presence of a high oxidation-reduction potential (redox potential) / high oxygen content. During metabolism, bacteria can produce toxic bi-products from oxygen (including superoxide radicals and hydrogen peroxide). Strict anaerobes lack certain enzymes (including superoxide dismutase and catalase) that detoxify these products.

Polymicrobic anaerobic infection

Strict anaerobes cannot grow in healthy tissues due to their oxygen content. When tissue injury occurs with limitation of the blood (and oxygen) supply, conditions are created for opportunistic growth of obligate anaerobes. Often more than one species will infect the same site. Simultaneous infection with a facultative anaerobe (which uses up the already diminished oxygen supply) also encourages growth of obligate anaerobes.

Endogenous versus exogenous infection

Most anaerobes in the normal flora are non-spore formers and anaerobic infections often occur from this source. However, contamination of wounds can also occur with anaerobic spore-formers (e.g. clostridia) which are common in the environment (e.g. soil). Non-spore-formers rarely produce exotoxins in contrast to spore-formers.

Sites of anaerobes in normal flora

Strict anaerobes are present in large numbers in the intestine (95 to 99% of total bacterial mass), but also in the mouth and genitourinary tract. The most common infections resulting from abdominal surgery or other gut injury are Enterobacteriaceae (facultative anaerobes) and Bacteroides fragilis (see below). These are minor components of the gut flora and demonstrate the important point that certain organisms more readily produce opportunistic infections than others.

Problems in identification of anaerobic infections

•They are often derived from the normal flora. One must be confident that one has not isolated a contaminant.

•If air gets into the sample during sampling or transportation to the clinical laboratory, then the organism may not be isolatable.

•Slow growth of the organism (due to inefficiency of fermentation) means isolation takes several days or longer.

Identification in the clinical laboratory after isolation;

Two systems are commonly used: Biochemical systems and/or gas chromatographic identification of volatile fermentation products (short chain fatty acids / alcohols)

ANAEROBIC NON-SPORE-FORMERS OF CLINICAL IMPORTANCE

•Gram-negative rods (Bacteroides [e.g. B. fragilis] and Fusobacterium)

•Gram-positive rods (*Actinomyces, Arachnia, Eubacterium, Bifidobacterium*, *Lactobacillus, Propionibacterium*)

•Gram-positive cocci (*Peptostreptococcus and Peptococcus*)

•Gram-negative cocci (*Veillonella, Acidominococcus*)

Bacteroides fragilis

B. fragilis is the most important strict anaerobic non-spore-former causing clinical disease. It has a prominent capsule that is involved in pathogenesis since it is:

•Anti-phagocytic

•Directly involved in abscess formation

This bacterium also produces an endotoxin which differs in composition from typical endotoxin and is of low toxicity.

•Case report: Multidrug-Resistant Bacteroides fragilis - Seattle, Washington, 2013

ANAEROBIC SPORE-FORMERS (CLOSTRIDIA)

These are Gram-positive rods. They are found in the environment (particularly soil) but also intestine of man and animals.

Tetanus

Clostridium tetani

Clostridium tetani, a gram-positive rod that forms a terminal spore is commonly found in the soil, dust and animal faeces. Contamination of wounds, which provide anaerobic conditions, can lead to spore germination and tetanus, a relatively rare (in western countries) but frequently fatal disease. Death occurs in about 11% of cases with most of these in the more elderly patients (over 60 years of age). Tetanus is also known as lockjaw because of the patient's inability to open the mouth as a result of muscle paralysis. The rarity of the disease results from an excellent vaccine and most cases that are now seen in the United States are in adults who never received the vaccine. Thus, currently some 60% of cases are in adults over 50. In the period 1947 to 2008, tetanus cases in the US dropped by over 95% and deaths by over 99%. From 2000 through 2009 an average of 29 cases were reported per year in the United States. Vaccination has reduced neonatal tetanus in developed countries so that in the United States there have been just two cases since 1989. Both patients were born to unvaccinated mothers.

In third world countries, many (about half) of tetanus cases are in neonates where the unhealed umbilical stump becomes infected, often as a result of cutting the umbilical cord with a contaminated knife. Many neonatal deaths result (about 270,000 in 1998). This occurs when the mother has no protective immunity to pass on to the infant.

Infection usually occurs when spores (in dirt, faeces or saliva) enter wounds and scratches where they germinate and produce tetanus toxin. Puncture wounds, such as by a needle or nail, other wounds and scratches and burns can all lead to *C. tetani* infections. More rarely, surgical procedures and dental extractions can lead to tetanus. Tetanus can also be contracted from the use of intravenous drugs.

The organism is non-invasive and thus remains in the local wound. The exotoxin (tetanospasmin) binds to gangliosides receptors on inhibitory neurones in central nervous system in which glycine is commonly the neurotransmitter. This stops nerve impulse transmission to muscle leading to spastic paralysis. The toxin can act at peripheral motor nerve end plates, the brain, spinal cord and also in the sympathetic nervous system. Because inhibitory neurons are involved, the result is unopposed muscle contraction.

In generalized tetanus, the most common form, the patient typically experiences lockjaw (trismus). This is a stiffness of the jaw muscles that results in inability to open the mouth or swallow leading to the appearance of a sardonic smile (*risus sardonicus*). Speech as a result of spasm of the vocal cords may be affected. Continued severe muscle contractions, which can even cause broken bones, and resulting spasms, often lasting for minutes over a period of weeks, can be fatal. The patient often experiences headaches and/or a fever (a rise of 2 to 4 degrees) with sweating, elevated heart rate and blood pressure. Tetanus patients in hospital often experience nosocomial infections. Aspiration pneumonia is often a late complication.

On average, about eight days after infection symptoms of tetanus appear (though the incubation period can be a short as three days and as long as three weeks). The incubation period seems to depend on the distance of the infection site from the central nervous system. In neonates the average latent period is about a week.

Other forms of tetanus

Cephalic tetanus is a rare infection involving the middle ear. It can affect cranial nerves. Local tetanus is also rare and manifests itself as localized muscle contractions in the area of infection. Few cases of local tetanus are fatal.

Vaccination

Vaccination of infants with tetanus toxoid have almost eliminated this disease in the United States. The toxoid consists of tetanus toxin that has been inactivated using formalin that stimulates anti-toxin antibodies. It comes in two forms: precipitated and fluid. The precipitated form yields more rapid seroconversion and higher anti-toxin titters. If infants receive the complete vaccination regimen, virtually 100% protection is achieved. Boosters should be given every ten years.

Diagnosis

Diagnosis is clinical and bacteria are only derived from wounds in a minority of cases.

Treatment

Tetanus is an emergency situation and requires hospitalization. The patient is immediately treated with human tetanus immune globulin (or equine antitoxin). Drugs can control muscle spasms. The wound requires aggressive washing and treatment with antibiotics.

Gas Gangrene

Clostridium perfringens

Clostridium perfringens, a gram positive rod causes wound colonization (gas gangrene) after soil, and to a lesser extent intestinal tract, contamination. It is primarily seen in time of war as a result on non-sterile field hospitals and projectile wounds. The term gas gangrene refers to swelling of tissues due to release of gas, as fermentation products, of clostridia. Progression to toxaemia and shock is frequently very rapid.

Gas gangrene can easily be identified by the large, blackened sores and loud and distinctive sound (crepitus) caused by gas escaping from necrotic tissue. It is a moist gangrene, in contrast to dry gangrene which is not caused by a

bacterial infection.The organism produces several tissue degrading enzymes (including lecithinase [alpha toxin], proteolytic and saccharolytic enzymes). Necrosis and destruction of blood vessels and the surrounding tissue, especially muscle, result (myonecrosis is a condition of necrotic damage, specific to muscle tissue). This creates an anaerobic environment in adjacent tissue and the organism spreads systemically. Death can occur within two days. Nowadays, treatment (including anti-toxin, antibiotic therapy, debridement) is extremely effective and amputation and death is rare. The determination of production of lecithinase is important in laboratory identification of the organism.

This bacterium is also a significant cause of food poisoning by enterotoxin producing strains.

Botulism

Clostridium botulinum

Botulism (a rare but fatal form of food poisoning) is caused by a potent nerve exotoxin (botulinum toxin). It is a serious paralytic illness caused by Clostridium botulinum and, more rarely, by strains of *Clostridium butyricum* and *Clostridium baratii.*
The toxin (of which there are seven types, designated as A through G but only types A, B, E and F cause illness in humans) binds to receptors on peripheral nerves, where acetylcholine is the neurotransmitter and inhibits nerve impulses. Flaccid paralysis and often death (from respiratory and/or cardiac failure) ensue. The organism does not grow in the gut, but pre-formed exotoxin from prior germination of spores may be present in inadequately autoclaved canned food (usually at home).

- Besides food poising, *C. botulinum* can cause:

- Wound botulism but is even rarer than botulism food poisoning.

- In addition, iatrogenic botulism can occur from accidental overdose of botulinum toxin.

C. botulinum does not readily grow in the adult intestine due to competition with the normal flora and their requirement for an anaerobic, low acidity environment. In infants, where the flora is not established, colonization with C. botulinum can occur. Infant botulism, although uncommon, is now the predominant form of botulism. In the United States, there are about 150 cases of botulism per year of which three quarters are infant botulism and 15% come from contaminated food (usually home-reserved food). The remainder are wound botulism (mostly associated with black-tar heroin injection). The spores can remain viable for many years.

Symptoms

After eating contaminated food, the symptoms of botulism occur usually with a day or two but sometimes there may be a period of up to a week before they appear. Vision and swallowing are affected and the patient may become nauseated and constipated. Muscle paralysis ensues, usually starting at the head and, when the respiratory muscles are affected, death can result. While the bacterium does not grow in the adult large intestine, it can in infants who ingest spores that are ubiquitous in the environment - Eating honey contaminated by spores is one source. Again, an early symptom is constipation and general malaise. With muscle paralysis, swallowing becomes difficult and paralysis of the head muscles leads to the characteristic floppy baby symptoms. Impaired respiratory muscles lead to breathing difficulties and possibly to death. When severe wounds are infected, the conditions are right for the growth of clostridia leading to similar symptoms to food-borne botulism except that the gastro-intestinal tract is not involved.

Treatment

Treatment for adults includes an enema to clear the gastro-intestinal tract of the toxin and injection of anti-toxin (antibodies produced in horses). It is important that the anti-toxin is given early to neutralize the toxin and protect nerve endings from damage. The horse-derived anti-toxin is not used in infants who receive, instead, human botulism immune globulin. Antibiotics are not used to treat botulism, although they may be used in secondary infections, because of the possibility of more toxin being released as bacteria are lyzed. Supportive treatment of infants is based on helping them breath and on tube feeding. Adults may also require a respirator and possibly a tracheotomy and intensive medical and nursing care for several months.

Death from botulism has become much rarer in the past 50 years. The proportion of patients with botulism who die has fallen from about 50% to 3 to 5%. Some patients die from infections or other problems as a result of being paralyzed for weeks or months. Patients who survive an episode of botulism poisoning may have fatigue and shortness of breath for years and long-term therapy may be needed to aid recovery. However, complete recovery from botulism usually occurs over a period of months as the damaged nerve endings are replaced.

Clostridium difficile

C. difficile is frequently a nosocomial infection. The organism, a gram positive rod, can cause a variety of diseases including:

- pseudomembranous colitis, a form of gastroenteritis.
- toxic mega colon
- perforations of the colon
- sepsis
- Patients at elevated risk include those that have received:
- antibiotics
- proton pump inhibitors
- gastrointestinal surgery/manipulation
- long length of stay in healthcare settings
- a serious underlying illness

Those with immunocompromising conditions and advanced age are also at high risk. However, *C. difficile* infection is rarely fatal.

Symptoms

When the normal flora of the intestine is altered by antibiotic therapy, this organism - which is present in the gastro-intestinal tract of many babies - can grow and colonize. *C. difficile* produces an enterotoxin and pseudomembranous colitis can result. Symptoms, which include abdominal cramps and watery diarrhoea, start some days (4 to 8) after initiation of antibiotic therapy. In mild cases, there is no blood in the diarrhoea but, in severe cases, bloody diarrhoea, a distended tender abdomen and fever can occur.

Treatment
Therapy includes discontinuation of the implicated antibiotic (e.g. ampicillin). Severe cases require specific antibiotic therapy (e.g. with vancomycin).

AEROBES: Pseudomonas *aeruginosa* spp*.* are typical examples*.*

Pseudomonads are aerobic, gram-negative rods with polar flagella. They are oxidase positive, in contrast to Enterobacteriaceae. These organisms are found in most environments including in water and soil and air. Among the genus Pseudomonas, the majority of human infections are caused by P. aeruginosa, although other related organisms also cause disease. Normally, individuals with compromised immune systems such as those infected with HIV, organ transplant recipients and burns patients are particularly prone to pseudomonad infections and mortality can be high (e.g. as much as 90% in heart infections). In burns and wounds, there is destruction of blood vessels which limits access of phagocytes that would normally clear the region of the pathogen. Cystic fibrosis patients are also at risk for infection since alteration of the respiratory epithelium commonly allows colonization and development of pneumonia. This is often seen in children who may suffer recurrent bouts of pseudomonad pneumonia resulting in fever, a wheezing productive cough, distended abdomen, breathing difficulties and cyanosis. This is often accompanied by weight loss.

Pseudomonads are opportunistic pathogens. Nosocomial infections by *P. aeruginosa* are particularly common in intensive care units and can lead to fatal pneumonia in which the patient has a productive cough, chills, breathing difficulties and cyanosis. The problem is compounded by the often encountered resistance of pseudomonads to common antibiotics. Moreover, the slime layer that is produced over the surface of these organisms has an anti-phagocytic effect making their control by the immune system phagocytes difficult; yet, they stick readily to other cells. They produce tissue-damaging toxins.

Infections by *P. aeruginosa* are a common cause of bacteremia, that is bacterial blood infections. Heart valves, particularly of intravenous drug users, can also become infected. Symptoms include general malaise with fever with joint and muscle pain.

Pseudomads can infect the skin as a result of bathing in infected waters, resulting in a itching rash in otherwise healthy individuals. This is the so-called "hot tub folliculitis". Sometimes these skin infections can be severe and result in headache, sore eyes, stomach and breast pain and earache. Injury can lead to infections of soft tissues and of bone and joints and the bacteria can also spread to these sites from a bacteremia. Bone involvement is sometimes seen in diabetics as well as persons who are undergoing surgery. Infection of wounds can result in the characteristic fruity smell and blue-green secretions (pyocyanin).

Among other pseudomonad-caused infections are those of the urinary tract, often as a result of catheter use or surgery, the brain which can develop abscesses and meningitis, and the eyes and ears. Swimmer's itch is an innocuous infection of the ear canal by these bacteria but older patients can experience life-threatening infections of the ear which sometimes cause paralysis of facial muscles. Abrasion of the cornea can lead to infection and resultant corneal ulcers which, if left untreated, can cause severe damage and loss of sight. Some eye medications and prolonged use of soft contact lenses can exacerbate the infection.

Identification of a pseudomonad infection includes pigment production: pyocyanin (blue-green) and fluorescein (green-yellow, fluorescent) and biochemical reactions (oxidase test). Cultures have fruity smell. Since hospitals are so commonly infected with pseudomonads, the presence of the organism is not sufficient to prove it as a source of the infection. Techniques such as X-rays can be used to assess deep tissue and bone infections.

Resistance of pseudomonads to various antibiotics is a problem. Two such drugs simultaneously are often employed for up to 6 weeks, either by mouth or intravenously. Eye infections are treated with antibiotic drops. In the case of infections of deep tissues such as in the brain, joints or bone, surgery to remove damaged tissue may be required. Moreover, amputation may be necessary in infections of the limbs of burns patients or those with infected wounds.

The toxicity of pseudomonads results from production of Toxin A which ADP ribosylates elongation factor-2 (EF2 - used in protein synthesis). In this, pseudomonad toxin is similar to diphtheria toxin

3.8 *MYCOBACTERIA AND CORYNEBACTERIA*

MYCOBACTERIA

In the 1980's, many experts felt that the days of tuberculosis as a threat to the US population had passed and the incidence of new cases (around 20,000 a year) was slowly decreasing, even though it was still the leading infectious cause of death world-wide. The situation in the 1990's has changed dramatically. The incidence of tuberculosis has slightly increased and the disease is certainly not going away (This is primarily due to the AIDS epidemic). At the same time multiple drug-resistant strains of M. tuberculosis are appearing regularly. The M. avium - M. intracellulare complex, long considered a group of organisms that only rarely infects man, is now recognized as one of the leading opportunists associated with AIDS. M. leprae is the causative agent of leprosy which remains a major disease in the third world. Due to eradication of infected cattle and pasteurization of milk M. bovis (a zoonotic cause of tuberculosis) is rarely seen in the United States. Mycobacteria are obligate aerobic, acid-fast rods.

Mycobacterium tuberculosis

Tuberculosis is extremely common in third world countries and WHO estimates that about one third of the world's population is infected, although the rate of mortality due to tuberculosis around the world has fallen by 45% between 1990 and 2012. It is estimated that around the world in 2012:

•1.3 million people died of the disease, including 320,000 HIV-infected patients.

•About 8.6 million people showed signs of infection and sickness, including 1.1 million HIV-infected people.

•About half of deaths among HIV-infected patients were in women.

•Among children, in 2012, 530,000 became sick with tuberculosis. 74,000 HIV-negative children died.

In the United States there were 9,945 cases of tuberculosis in 2012 (3.2 cases peer 100,000 population). There was a decline of 5.4% in the number of cases over those reported in 2011. In 1953, there were 84,304 cases of tuberculosis (a case rate of 52.6 per 100,000 population). Rates were higher in the older population and an increasing proportion of cases is seen in foreign-born

persons. In the United States in 2013, 64% of tuberculosis cases and 91% of multidrug–resistant cases occurred in people born in other countries. Three quarters of these cases came from 15 countries.

Pathogenesis of tuberculosis

Mycobacterium tuberculosis is spread via aerosols when an infected person coughs or sneezes; however, many people who become infected do not show any symptoms of the disease. This is a latent tuberculosis infection and these people are not infectious. The bacteria are kept under control by the immune system. In contrast, other people's immune system cannot control the bacteria and they show overt tuberculosis disease. Some, about 5 to 10%, people with latent tuberculosis may develop active disease many years after infection when their immune system weakens for a variety of reasons. Especially prone to activation of overt disease are people whose immune system has been weakened by HIV infection.

CDC lists the following categories of people who are at high risk for developing disease

•Close contacts of a person with infectious tuberculosis

•People who have immigrated from areas of the world with high rates of tuberculosis

•Children less than 5 years of age who have a positive tuberculosis test

•People with high rates of tuberculosis transmission including:

homeless people

intravenous drug users

HIV-infected people

•People who work or reside with people who are at high risk for tuberculosis in facilities or institutions such as hospitals, homeless shelters, correctional facilities, nursing homes, and residential homes for those with HIV

Medical conditions that lead to a weakened immune system and therefore predispose people to overt tuberculosis disease include:

•HIV infection

•Drug abuse

•Silicosis

•Diabetes mellitus

•Severe kidney disease

•Low body weight

•Organ transplants

•Head and neck cancer

•Corticosteroids treatment

•Treatment for rheumatoid arthritis or Crohn's disease

M. tuberculosis bacteria infect the lungs (pulmonary tuberculosis) and are distributed systemically within macrophages where they survive intracellularly. Inhibition of phagosome-lysosome fusion and resistance to lysosomal enzymes have both been suggested to play a role. Cell-mediated immunity develops which causes infiltration of macrophages and lymphocytes with development of granulomas (tubercles). The disease can be diagnosed (figure 6) by skin testing for delayed hypersensitivity with tuberculin (also know as protein purified purified from Mycobacterium tuberculosis, PPD). A positive test does not indicate active disease; merely exposure to the organism.

Other pathogenesis factors (of considerably less importance than delayed hypersensitivity) include mycobactin (a siderophore) and cord factor which damages mitochondria.

Symptoms of tuberculosis

These depend on the site of infection. In pulmonary tuberculosis, symptoms include:

•a cough that lasts 3 weeks or longer

•chest pain

•blood or sputum (phlegm from deep inside the lungs)

•weakness/fatigue

•weight loss

•appetite loss

•chills

•fever

•night sweats

Diagnosis and identification

A positive skin (Mantoux) test shows whether a person has been infected by the bacteria but people who have been inoculated against tuberculosis using the BCG vaccine can also give a positive test. X-ray imaging is also often used. The presence of acid fast bacteria in sputum is a rapid presumptive test for tuberculosis. Subsequently, when cultured, M. tuberculosis will grow very slowly producing distinct non-pigmented colonies after several weeks. M. tuberculosis can be differentiated from most other mycobacteria by the production of niacin. A rapid alternative to culture is polymerase chain amplification (PCR).

There are also blood tests called interferon-gamma release assays (IGRAs) which are done on blood samples. These are not affected by prior BCG vaccination.

Treatment

Tuberculosis is usually treated for extensive time periods (9 months or longer) since the organism grows slowly and may become dormant. By using two or more antibiotics (including rifampin, rifapentine and isoniazid), the possibility of resistance developing during this extended time is minimized.

Recommended treatment for overt tuberculosis includes some of ten approved drugs taken over a period of six to nine months. These drugs include, as a first line of attack:

•isoniazid

•rifampin

•ethambutol

•pyrazinamide

A new anti-tuberculosis drug

In 2013, a new anti-tuberculosis drug, bedaquiline, was approved by the Federal Drug Administration for use in patients infected with multi-drug resistant Mycobacterium tuberculosis. This is the first new drug to treat tuberculosis in over 40 years. The drug inhibits part of the F0 subunit of ATP synthase in the bacterial membrane and probably inhibits the proton pump leading to altered pH homeostasis and ATP depletion. Because this is a unique target, not inhibited by other anti-bacterial drugs, cross resistance with other anti-tuberculosis drugs does not occur. Nevertheless, since the bacterium can develop resistance to bedaquiline, it is only approved for use in combination therapy with other drugs

Vaccination

The BCG vaccine (Bacillus de Calmette et Guerin, an attenuated strain of M. bovis) has not been shown to be effective in many studies, yet in others a protective effect has been seen. In the United Kingdom, a protective effect of 60 to 80% has been reported. It is not known why the efficacy of BCG is so different in different studies. In most cases, however, efficacy seem to wane over a number of years. In the United Kingdom all school children received BCG vaccination until 2005. This was abandoned because it was not found to be cost effective since 94 children would have to be immunized in 1953 to prevent one case of tuberculosis but the fall in the incidence of the disease meant that by 1988 12,000 children would need vaccination to prevent one case.

In the United States, where the incidence of tuberculosis is low, widespread vaccination is not practiced. Indeed, immunization (resulting in a positive tuberculin test) is felt to interfere with diagnosis.

In other countries, BCG vaccination has been carried out in school children and mass vaccination campaigns.

Atypicals

The "atypicals" generally infect the immunocompromised host and are thus not transmitted man-man. With the AIDS epidemic, the atypical mycobacteria have taken on new importance with the recognition that the M. avium complex (MAC) results in the most commonly associated systemic bacterial infection. Atypical mycobacteria can cause tuberculosis-like or leprosy-like, diseases, and are not susceptible to certain common anti-tuberculous antibiotics.

. Mycobacterium avium complex and AIDS

M. avium generally infects AIDS patients when their CD4+ cell count decreases greatly (below 100/mm3). M. tuberculosis infects AIDS patients much earlier in the disease. This clearly demonstrates the much greater virulence of M. tuberculosis. The incidence of systemic disease (versus primarily pulmonary) is much greater in tuberculosis associated with AIDS than in its absence. Furthermore, histologically lesions often appear lepromatous (non-granulomatous, with many organisms). It is rare to find a case of M. avium infection that is not AIDS associated. However, M. tuberculosis is a much more virulent organism. Approximately 20% of the total tuberculosis cases in the US are caused by AIDS. This helps explain why TB is no longer on the decline. Increased homelessness is also suggested to be a factor in the rise of tuberculosis.

Treatment of M. avium also involves a long-term regimen of multiple drug combinations. However, this organism does not always respond to the drug regimens used to treat M. tuberculosis. Appropriate drug combinations are still under investigation in clinical trials. Since M. tuberculosis is the more virulent organism, the drug regimen selected is primarily against M. tuberculosis. If M. avium is suspected other agents effective against this organism are included.

Other atypicals

Presence or absence of pigmentation (and its dependence on growth in the light) and slow or fast growth rates of atypical mycobacteria allow some differentiation - the "Runyon Groups". Modern techniques allow ready speciation of mycobacteria based on their cellular fatty acid and/or mycolic acid profiles. This is only performed in reference laboratories. Mycolic acids are components of a variety of lipids found only in mycobacteria, nocardia and corynebacteria. The chain length of these mycolic acids is longest in

mycobacteria, intermediate in nocardia and shortest in corynebacteria. This explains why mycobacteria are generally acid fast; nocardia less acid fast; and corynebacteria are non-acid fast.

Mycobacterium leprae

M. leprae is the causative agent of leprosy (Hansen's Disease), a chronic disease often leading to disfigurement. It is rarely seen in the U.S. but common in the third world. The organism infects the skin, because of its growth at low temperature. It also has a strong affinity for nerves. In "tuberculoid" leprosy, there are few organisms due to control by active cell-mediated immunity. In "lepromatous" leprosy, due to immunosuppression by the organism, the opposite is found. Although uncommon in the U.S., millions of cases occur worldwide. Treatment with antibiotics (initially dapsone and now multi-drug) is effective and the overall disease incidence worldwide is down. The organism does not grow in culture media. However, it grows well in the armadillo (which has a low body temperature), allowing production of M. leprae antigens and pathogenesis studies. M. leprae has traditionally been identified on the basis of acid-fast stains of skin biopsies and clinical picture. Lepromin is used in skin testing.

CORYNEBACTERIA

Corynebacterium diphtheriae

C. diphtheriae grows best under strict aerobic conditions It is Gram positive and pleomorphic.

Colonization of the upper respiratory tract (pharynx and nose) and less commonly skin with C. diphtheriae can lead to diphtheria. The organism does not produce a systemic infection. However, in addition to a pseudo membrane being formed locally (which can cause choking), systemic and fatal injury results primarily from circulation of the potent exotoxin (diphtheria toxin). The latter begins over a period of a week. Thus treatment involves rapid therapy with anti-toxin. The gene for toxin synthesis is encoded on a bacteriophage (the tox gene). Corynebacteria that are not infected with phage, thus do not generally cause diphtheria. Diphtheria is now a disease of almost historic importance in the U.S. due to effective immunization of infants (in conjunction

with pertussis and tetanus, DPT) with a toxoid (inactive toxin) which causes production of neutralizing antibodies. However, colonization is not inhibited and thus C. diphtheriae is still found in the normal flora (i.e. a carrier state exists). Immunity can be monitored with the Schick skin test. Treatment in non-immune individuals primarily involves injection of anti-toxin. Antibiotics are also administered at this time.

The toxin consists of two types of polypeptide. One binds to host cells; the other then becomes internalized and inhibits protein synthesis. The exotoxin catalyses the covalent attachment of the ADP-ribose moiety of NADH to a rare amino acid, diphthamide, present in EF2 (elongation factor 2). The toxin is not synthesized in the presence of iron as an iron-repressor complex forms which inhibits expression of the tox gene.

C. diphtheriae are identified by growth on Loeffler's medium followed by staining for metachromatic bodies (polyphosphate granules, Babes-Ernst bodies). The term "metachromatic" refers to the colour difference of the intracellular polyphosphate granules (pink) compared to the rest of the cell (blue). Characteristic black colonies are seen on tellurite agar from precipitation of tellurium on reduction by the bacteria. Production of exotoxin can be determined by in vivo or in vitro tests.

Other organisms which morphologically resemble C. diphtheriae ("diphtheroids" which include other corynebacteria and also Propionibacterium) are found in the normal flora. Isolates should not be confused with these organisms.

4.0 CONCLUSION

Streptococci are facultatively anaerobic, Gram-positive organisms that often occur as chains or pairs and are catalase-negative (in contrast, staphylococci are catalase positive). Streptococci are subdivided into groups by antibodies that recognize surface antigens. These groups may include one or more species. The most important groupable streptococci are A, B and D. Streptococcus pneumoniae is a leading cause of pneumonia in all ages (particularly the young and old), often after "damage" to the upper respiratory tract (e.g. following viral infection). *Staphylococcus aureus* is one of the commoner causes of opportunistic nosocomial and community infections. *S. aureus* has gained notoriety because of the increased incidence of Methicillin-

resistant. *Staphylococcus aureus* (MRSA) Infections. Mycobacteria; 'otherwise referred to as acid fast bacilli' is the cause of multidrug resistant strains of *M. tuberculosis* regularly occurring now and also the leading opportunists associated with AIDS. M. leprae is the causative agent of leprosy which remains a major disease in the third world. *Spirochetes* and *Neisseria* are the most important genera of spirochetes are Treponema, Borrelia and Leptospira. These are Gram negative bacteria that are long, thin, helical and motile. While Neisseria are Gram negative diplococci (pairs of cocci).

Anaerobes Obligate anaerobes are bacteria that cannot survive in the presence of a high oxidation-reduction potential (redox potential) / high oxygen content.

Aerobes such as Pseudomonads are aerobic, gram-negative rods with polar flagella. They are oxidase positive, in contrast to Enterobacteriaceae. These organisms are found in most environments including water, soil and air.

5.0 SUMMARY

At the end of this unit we have considered the detailed microbiological features of the following bacteria with respect to disease manifestations and public /environmental health relevance and significance.

- Groups A, B and D streptococcus, viridans streptococci
- Enterococcus faecalis (formerly group D).
- Streptococcus pneumonia
- Staphylococcus infections; food poisoning, toxic shock, MRSA
- Neisseria and Spirochetes
- Anaerobes and Pseudomonas in Opportunistic
- Mycobacteria and Corynebacteria

6.0 TUTOR-MARKED ASSIGNMENT

a. What are the Problems in identification and isolation of pathogens associated with anaerobic infections?
b. Distinguish clearly between Anaerobes and Aerobes

c. Succinctly discuss the enterococcus group of bacteria

Write brief notes on the following

d. Mycobacteria
e. Corynebacteria
a. Staphylococcus

7.0 REFERENCES/FURTHER READING

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Unit 3 ZOONOSES ;

1.0 INTRODUCTION

Zoonosis refers to a disease primarily of animals which can be transmitted to humans as a result of direct or indirect contact with infected animal populations.

Bordetella pertussis is the organism of major clinical significance within this genus for now; it causes whooping cough in infants and young children. However, a closely related organism, *B. parapertussis* can also cause a milder form of bronchitis. *B. bronchosepticus*, another member of the genus *Bordetella*, is the causative agent of respiratory diseases in cats and swine, but can cause broncho-pulmonary symptoms in severely immunosupressed individuals.

Mycoplasmas are the smallest free-living bacteria. They range from 0.2 - 0.8 micrometers and thus can pass through some filters used to remove bacteria. They have the smallest genome size and, as a result, lack many metabolic pathways and require complex media for their isolation. The mycoplasmas are facultative anaerobes, except for M. pneumoniae, which is a strict aerobe. A characteristic feature that distinguishes the mycoplasmas from other bacteria is the lack of a cell wall. Thus, they can assume multiple shapes including round, pear shaped and even filamentous. The family *Mycoplasmataceae* contains two genera that infect humans: *Mycoplasma* and *Ureaplasma*, which are usually referred

to collectively as mycoplasmas. Although there are many species of mycoplasmas, only four are recognized as human pathogens; *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum*. Although there are other species that have been isolated from humans, their role in disease is not well established. The diseases caused by *M. pneumoniae, M. hominis*, *M. genitalium* and *U. urealyticum*

2.0 OBJECTIVES

At the end of this unit you should be able to know, describe and microbiologically characterise the following group of pathogens;

- *Brucellosis* as a typical zoonosis
- *Bordetella, Haemophilus, and Legionella*
- *Mycoplasma and Uroplasma*

3.1 ZOONOSIS

Morphology and physiology Brucella are Gram-negative, non-motile, coccobacilli. They are strict aerobes and grow very slowly (fastidious) on blood agar. In the host, they live as facultative intracellular pathogens.

Epidemiology

Brucellosis is primarily a disease of animals and it affects organs rich in the sugar erythritol (breast, uterus, epididymis, etc.). The organisms localize in these animal organs and cause infertility, sterility, mastitis, abortion. They may also be carried asymptomatically. Humans in close contact with infected animals (slaughterhouse workers, veterinarians, farmers, dairy workers) are at risk of developing undulant fever. There are 100 to 200 cases of brucellosis seen in the United States annually, although the worldwide incidence is estimated at 500,000. Species of Brucella that are known to infect humans include:

•*B. abortus* (cattle)

•*B. suis* (swine)

•*B. melitensis* (goats/sheep)

•*B. canis* (dogs)

•*B. ovis* (goats/sheep)

There are other species of *Brucella* that infect many animals from whales to rats. Although brucellosis has largely been eradicated in most developed countries through animal vaccination, it persists in many underdeveloped and developing countries. Areas where there is a higher risk of Brucella infection include:

•Areas around the Mediterranean

•Eastern Europe

•Asia

•Africa

•Caribbean

•Middle East

Transmission

Infection may occur by:

•Direct contact with infected animals or animal products

•Ingestion of animal products such as unpasteurized milk, milk products (including cheeses), undercooked meat. This is probably the most frequent cause of human Brucella infections and are of particularly important for tourists

•Breathing in bacteria/ this may occur in people in contact with infected animals or in laboratories where Brucella is handled.

•Entry through wounds or mucous membranes. In addition, to those mentioned above, this may occur in hunters. Infected game animals may include (CDC): Bison, elk, caribou, moose, feral hogs.

•Breast feeding. Person to person transmission is very rare but has been reported

•Sexual activity. Again this is very rare

•Possibly in tissue transplantation or organ transplant. Again, this is very rare.

CDC notes that it is especially important that expectant mothers who have been exposed to Brucella should consult a physician as post-exposure prophylaxis may be required. The bacteria are engulfed by neutrophils and monocytes and localize in the regional lymph nodes, where they proliferate intracellularly. If the Brucella organisms are not destroyed or contained in the lymph nodes, the bacteria are released from the lymph nodes resulting in septicaemia. The organisms migrate to other lympho-reticular organs (spleen, bone marrow, liver, testes) producing granulomas and/or micro abscesses.

Symptoms

B. abortus and *B. canis* cause a mild suppurative febrile infection whereas *B. suis* causes a more severe suppurative infection which can lead to destruction of the lymphoreticular organs and kidney. *B. melitensis* is the cause of most severe prolonged recurring disease. The bacteria enter the human host through the mucous membranes of the oropharynx (ingestion/inhalation routes), through abraded skin, or through the conjunctiva.

Symptoms include:

•Recurrent fever

•Chills

•Sweats

•Fatigue

•Myalgia,

•Profound muscle weakness

•Anorexia.

•Joint involvement occurs often

Brucellosis may be either acute or chronic. Mortality due to Brucellosis is rare (less than 3%) and is generally due to endocarditis.

Pathogenesis

The symptoms of brucellosis are due to the presence of the organism and appear 2 to 4 weeks (sometimes up to 2 months) after exposure. While in the phago-lysosome, *B. abortus* releases 5'-guanosine and adenine which are capable of inhibiting the degranulation of peroxidase-containing granules and thus inhibit the myeloperoxidase-peroxide-halide system of bacterial killing. The intracellular persistence of bacteria results in granuloma formation in the reticuloendothelial system organs and tissue damage due to hypersensitivity reactions, mostly type-IV.

Diagnosis

Diagnosis is based on prolonged (at least a week) presence of undulating fever, myalgia, arthralgia and the history of exposure (contact with animals or consumption of unprocessed material from infected animals). Definitive diagnosis can be made by culturing blood samples on blood enriched media. The (fastidious) organisms grow very slowly (4 to 6 weeks in blood culture). *B. abortus* but not other *Brucella* grows better in 5% CO2 atmosphere. On blood agar, they produce white glistening colonies. Serology can be used to further confirm the diagnosis.

Prevention and treatment

Prolonged (6 to 8 weeks) treatment with rifampin or doxycycline along with streptomycin or tetracycline is used to treat human Brucella infections. Control measures include animal vaccination and avoidance of infected material.

PLAGUE

Plague is caused by Yersinia pestis and is the disease known in the middle ages as the black death. This is because it frequently leads to gangrene and blackening of various parts of the body. Capillary fragility results in haemorrhages in the skin which also result in black patches.

Morphology and physiology

Yersinia pestis is a pleomorphic, Gram-negative, bipolar staining, facultatively aerobic, non-motile bacillus. Optimal temperature for growth is 28 degrees C. It is a facultative intracellular parasite.

Epidemiology, transmission and symptoms

The three documented pandemics of plague (Black Death) have been responsible for the death of hundreds of millions of people. Today, sporadic infections still occur. In the U.S., animal (sylvatic) plague occurs in a number of western states, usually in small rodents and in carnivores which feed on these rodents. The last urban outbreak of plague occurred in the United States in 1924-25. There was an outbreak of plague in India in 1994. In both cases, rats were the vector.

Sporadic plague still occurs in the rural United States. Infected animals include:

•squirrels (rock and ground)

•wood rates

•prairie dogs

•mice

•chipmunks

•voles

•rabbits

•also carnivores may be infected by eating infected prey. In the United States, pneumonic plague has occurred in recent years from contact with infected cats that have been infected after eating infected rodents.

Humans are usually infected by carrier rodent fleas. The flea acquires the *Y. pestis* organisms during a blood meal from infected rodents. The bacteria lose their capsule, multiply in the intestinal tract and partially block the proventriculus. When the flea feeds on a human host, it may regurgitate some of the organisms into the wound.

Humans also can be infected in other ways such as by contact with infected animals or handling carcasses of infected animals. This usually results in bubonic or septicaemia plague.

Plague can also be spread in aerosols in a cough or sneeze from a person (or pet cat) with pneumonic plague.

The bulk of non-capsular organisms are phagocytized and destroyed by neutrophils. However, a few organisms are taken up by histiocytes which are unable to kill them and allow them to resynthesize their capsule and multiply.

The encapsulated organisms, when they are released from histiocytes, are resistant to phagocytosis and killing by neutrophils. The resulting infection spreads to the draining lymph nodes which become hot, swollen, tender and haemorrhagic giving rise to the characteristic black buboes whence the name of the disease, bubonic plague, is derived. Within hours the organism spreads into the spleen, liver and lungs resulting in pneumonia. While in the circulation, the organism causes diffuse intra-vascular coagulation resulting in intra-vascular thrombi and purpuric lesions all over the body. If untreated, the infection has a very high (up to 90%) mortality rate. The organisms in exhaled in cough droplets, infect other humans in close proximity and cause pneumonic plague, which is more difficult to control and has 100% mortality.

PATHOGENESIS

Many factors play direct and indirect roles in *Y. pestis* pathogenesis.

Low calcium response (lcr)
This is a plasmid-coded gene that enables the organism to grow in a low Ca++ (intracellular) environment. It also coordinates the production of several other virulence factors, such as V, W and yops (Yersinia outer proteins).

V and W proteins
These plasmid-coded proteins are associated rapid proliferation and septicaemia.

Yops
A group of 11 proteins, which are coded by plasmids, are essential for rodent pathogenesis and are responsible for cytotoxicity, inhibition of phagocyte migration and engulfment and platelet aggregation.

Envelope (F-1) antigen
This is a protein-polysaccharide complex which is highly expressed at 37 degrees in the mammalian host but not in the flea and is anti-phagocytic.

Coagulase and Plasminogen activator
Both of these are plasmid-coded proteins. Coagulase is responsible for micro thrombi formation and plasminogen activator promotes the dissemination of

the organism. It also destroys C3b on the bacterial surface, thus attenuating phagocytosis.

Diagnosis

Diagnosis is based on appearance of buboes (swollen lymph glands). The diagnosis is confirmed by culture of a lymph node aspirate. Extreme caution is warranted in handling of the specimen, as it is highly infectious. In cases of pneumonic and septicaemia plague there may be no obvious signs.

Prevention and Treatment

Hospitalization and strict isolation are the rule for this serious but easily treatable disease. Streptomycin and gentamycin are highly effective but other effective antibiotics include tetracycline, fluoroquinolones and chloramphenicol. For most recent CDC recommendations go here.
An effective formalin-killed vaccine is available but is recommended only for people at a high risk. The disease is internationally quarantinable and reporting of cases is mandatory. Control of urban plague is based upon flea and rodent control.

Summary of types and symptoms of plague

Bubonic plague

This usually occurs as a result of a bite by an infected flea. The lymph nodes closest to the bite become swollen as the bacteria proliferate. They can then spread to other parts of the body.

Symptoms of bubonic plague

Sudden onset of

- Fever
- Headache
- Weakness
- Formation of buboes (swollen, tender lymph nodes). See below

Pneumonic plague

Anteroposterior chest x-ray of a plague patient revealing bilateral infection, greater on the patient's left side, which was diagnosed as a case of pneumonic plague, caused by the bacterium, Yersinia pestis.

This is usually the result of inhaling aerosols from an infected patient or animal. It can also develop as the result of spread of bacteria to the lungs in other forms of plague that remain untreated. It is the most serious form of plague as it can easily be spread.

Symptoms of pneumonic plague

•Fever

•Headache

•Weakness

•Pneumonia (chest pain, cough with sometimes watery or bloody mucous, shortness of breath)

Septicaemia plague

A 59 year-old man's hands who had been infected by the plague bacterium, Yersinia pestis, after having come into contact with both an infected cat, and a dead mouse in his neighbourhood. The gangrenous condition of the fingers had turned the dead digits black, and mummified.

Two days after the exposure the patient developed fever and myalgias, and by the following day he had developed a left axillary bubo. Seven days after the initial exposure he became critically ill and was admitted to the hospital with multiple organ failure. Initial blood cultures were positive for double-curved, Gram-negative *Y. pestis* rods.

The patient was treated with gentamicin and survived, but necrosis of the hands and feet developed during hospitalization. He subsequently required amputation of the hands and feet.

This comes from flea bits or contact with an infected animal. It may the initial disease or the consequence of untreated bubonic plague.

Symptoms of septicaemic plague

•Fever
•Chills
•Weakness

•Abdominal pain

•Shock

•Bleeding in skin and organs

•Skin, particularly at extremities, becomes black and necrotic

ANTHRAX

Morphology and physiology

Bacillus anthracis is the causative agent of anthrax. It is a Gram-positive, aerobic, spore-forming large bacillus. Spores are formed in culture, in the soil, and in the tissues and exudates of dead animals, but not in the blood or tissues of living animals. Spores remain viable in soil for decades.

Epidemiology, transmission and symptoms

Anthrax is a major disease threat to herbivorous animals (cattle, sheep, and to a lesser extent horses, hogs, and goats). People become infected by the cutaneous route (direct contact with diseased animals, industrial work with hides, wool, brushes, or bone meal), by inhalation (Woolsorter's disease), or by ingestion (meat from diseased animals). It is not contagious.

•Cutaneous anthrax accounts for more than 95% of human cases. Spores enter through small break in skin, germinate into vegetative cells which rapidly proliferate at the portal of entry. Within a few days, a small papule emerges that becomes vesicular. The latter is filled with blue-black oedema fluid. Rupture of this lesion will reveal a black eschar at the base surrounded by a zone of induration. This lesion is called a malignant pustule; however, no pus or pain are manifested. The lesion is classically found on the hands, forearms or head. The invasion of the bloodstream will lead to systemic dissemination of bacteria.

•Pulmonary anthrax results from inhalation of B. anthracis spores which are phagocytized by the alveolar macrophages where they germinate and

replicate. The injured host cell and organisms infect the hilar lymph node where marked haemorrhagic necrosis may occur. The patient may manifest fever, malaise, myalgia, and a non-productive cough. Once in the hilar lymph node, infection may spread into the blood stream. Respiratory distress and cyanosis are manifestations of toxaemia. Death results within 24 hours. This form of anthrax is of significance in biological warfare.

•Gastrointestinal anthrax results from ingestion of meat-derived from an infected animal and leads to bacterial proliferation within the gastrointestinal tract, invasion of the epithelium and ulceration of the mucosa. The invasion spreads to the mesenteric lymph nodes and then to the bloodstream. Initially, there is vomiting and diarrhoea followed by blood in the faeces. Invasion of the bloodstream is associated with profound prostration, shock and death. Because of strict control measures, this form of anthrax is not seen in the U.S. Without treatment of gastrointestinal anthrax, the majority of patients die but with antibiotic treatment, 60% or more survive.

•Injection anthrax is are and has not been seen in North America but has occurred in heroin users in northern Europe. Symptoms are similar to cutaneous anthrax but the infection may be deeper at the site of needle entry. The bacteria can spread more rapidly from site of infection to other parts of the body than is the case with cutaneous anthrax.

•Meningeal Anthrax. All forms of anthrax above can progress to meningeal encephalitis with deep brain haemorrhagic lesions and infection of the cerebro-spinal fluid. It is almost always fatal.

Summary of types and symptoms of anthrax

Cutaneous anthrax
Symptoms

•Blisters near site of infection

•After the blister, a painless ulcer appears with a black center with swelling. This is most often on the face, neck, arms or hands

Pulmonary anthrax
Symptoms

•Cough
•Shortness of breath
•Nausea and stomach cramps
•Headache
•Confusion
•Severe sweats
•Fever and chills
•Malaise

Gastrointestinal anthrax

Symptoms

•Fever and chills
•Headache
•Neck swelling
•Red face and eyes
•Sore throat, hoarseness and pain swallowing
•Nausea and bloody vomit
•Stomach cramps and stomach swelling
•Diarrhoea, sometimes bloody
•Faintness
•Malaise

Injection anthrax

Symptoms

•Fever and chills

•Small blisters at site of drug injection

•A painless ulcer with a black center with swelling at site of injection after blisters appear

•Deep abscess at injection site

Pathogenesis

The virulence factors of B. anthracis include a number of exotoxins and the capsule.

Exotoxin

A plasmid-encoded, heat-labile, heterogeneous protein complex made up of 3 components:

- Edema Factor (EF)
- Lethal Factor (LF)
- Protective Antigen (PA).

In vivo, these three factors act synergistically (for toxic effects). The protective antigen binds to surface receptors on eucaryotic cells and is subsequently cleaved by a cellular protease. The larger C-terminal piece of PA remains bound to the receptor and then binds either EF or LF, which enters the cell by endocytosis. Edema Factor, when inside the cells binds calmodulin-dependent and acts as adenylate cyclase. Lethal factor's mechanism of action involves activation of macrophages and production of cytokines which cause necrosis, fever, shock and death. Individually, the three proteins have no known toxic activity. Antibodies to protective antigens prevent PA binding to cells stop EF and LF entry.

Capsule

The capsule consists of a polypeptide of D-glutamic acid which is encoded by a plasmid and is anti-phagocytic. It is not a good immunogen and, even if any antibodies produced, they are not protective against the disease.

Diagnosis

Clinical diagnosis of anthrax can be confirmed by direct examination or culture. Fresh smears of vesicular fluid, fluid from under the eschar, blood, or spleen or lymph node aspirates are stained with polychrome methylene blue and examined for the characteristic blunt ended, blue-black rods with a pink capsule. In case of a negative finding, the specimen can be cultured on blood agar plates. Cultured organisms stain as Gram-positive long thin rods.

Prevention and Treatment

Antibiotics

Penicillin and the 4-quinolone, Ciprofloxacin (Cipro), are the antibiotics of choice.

Anti-toxin

Antibody to the toxin complex is neutralizing and protective. This may be used in combination with antibiotics. There are two vaccines available. One is for use for immunizing cattle and other herbivorous animals and the other for at-risk humans (certain laboratory workers, people who handle animals (veterinarians) and some military personnel.

LISTERIOSIS

Listeriosis is serious disease which is almost always caused by eating contaminated food. It most affects newborn children, older and immunocompromised people and pregnant women. There are about 1,600 cases of Listeriosis annually in the United States and about 260 deaths. In 2013, the incidence of Listeriosis was 2.6 cases per million.

Morphology and Physiology

L. monocytogenes is a facultative intracellular, Gram-positive coccobacillus which often grows in short chains. It is different from other Gram-positive organisms in that it contains a molecule chemically and biologically similar to the classical lipopolysaccharide, the listerial LPS. The organism forms beta haemolytic colonies on blood agar plates and blue-green translucent colonies on colourless solid media. Upon infecting a cell (macrophages and parenchymal cells), the organism escapes from the host vacuole (or phagosome) and undergoes rapid division in the cytoplasm of the host cell before becoming encapsulated by short actin filaments. These filaments reorganize into a long tail extending from only one end of the bacterium. The tail mediates movement of the organism through the cytoplasm to the surface of the host cell. At the cell periphery, protrusions are formed that can then penetrate neighbouring cells and allow the bacterium to enter. Due to this mode of cell-cell transmission, the organisms are never extracellular and exposed to humoral antibacterial agents (e.g., complement, antibody). *L. monocytogenes* is readily killed by activated macrophage.

Epidemiology and symptoms

Listeria monocytogenes is a ubiquitous organism found in the soil, vegetation, water, and in the gastrointestinal tract of animals. Exposure to the organism can lead to asymptomatic miscarriage or disease in humans. At greatest risk for the disease are the foetus, neonates, cancer patients and immuno-compromised persons. In the United States, a number of recent outbreaks have been traced to cheese, cole slaw (cabbage), milk, and meat. The organisms can grow at 4^{o}C which means that organism replication continues in refrigerated foods. Laboratory isolation can employ a cold enrichment technique.

Listeriosis has been categorized in two forms:

a. Neonatal disease and
b. Adult disease.

Neonatal Disease

Neonatal disease can occur in two forms:

•Early onset disease, acquired transplacetally in utero
In utero acquired infection (granulomatosus infantiseptica) causes abscesses and granulomas in multiple organs and very frequently results in abortion.
•Late onset disease acquired at birth or soon after birth.
Exposure on vaginal delivery results in the late onset disease resulting in meningitis or meningo-encephalitis with sepsis within 2 to 3 weeks.

Adult Disease

Infection in normal adults results in self-resolving flu-like symptoms and/or mild gastrointestinal disturbance. Chills and fever are due to bacteremia. In pregnant women, infection can lead to miscarriage, still birth or premature birth with life-threatening.
In immunosuppressed individuals listeriosis can produce serious illness, leading to meningitis. It is one of the leading causes of bacterial meningitis in patients with cancer and in renal transplant recipients. In the elderly, the early symptoms may go unnoticed and the infection may lead to acute

manifestations of sepsis (high fever, hypo-tension). A complication of the bacteremia is endocarditis.

Pathogenesis

Listeriolysin O, a β-haemolysin, is related to streptolysin, and pneumolysin and is produced by virulent strains. It disrupts the phagocytic vacuole and is instrumental in cell-cell transmission of the organism. The toxin is oxygen labile and immunogenic.

Diagnosis

Listeriosis is indicated when blood and CSF monocytosis is observed. The organism can be isolated on most laboratory media.

Treatment and control

Penicillin (ampicillin) alone or in combination with gentamycin have been effective. Immunity is cell-mediated.

TULAREMIA

Morphology and physiology

Francisella tularensis is a small, Gram-negative, non-motile, encapsulated, pleomorphic coccobacillus (short rod). It is a facultative intracellular parasite which grows poorly or not at all on most laboratory media and requires a special glucose cysteine blood agar for isolation. Care must be taken in handling the sample because of the low infectious dose.

Epidemiology and symptoms

Francisella tularensis is the causative agent of tularaemia (a reportable disease in the U.S.). Unlike plague, tularaemia occurs routinely in all 50 of the United States. Its primary reservoirs are rabbits, hares, rodents and ticks. People most commonly acquire tularaemia via insect bites (ticks primarily, but also deer flies, mites, blackflies, or mosquitoes) or by handling infected animal tissues. Human disease (rabbit or deer fly fever) is characterized by a focal ulcer at the site of entry of the organisms and enlargement of the regional lymph nodes.

In 2013, there were 203 cases of tularaemia in the United States (incidence: 0.6 cases per million populations). Because the disease is spread by ticks and flies, it is most common in the summer months.

As few as10 - 50 bacilli will cause disease in humans if inhaled or introduced intradermally, whereas a very large inoculum (~108 organisms) is required for the oral route of infection. Incubation period is 3 - 10 days. A small skin papule usually develops at the site of entry. Ulceration occurs together with fever, chills, malaise, fatigue, and usually lymphadenopathy. Bacteremia usually occurs and the bacilli then grow intracellularly in the reticuloendothelial system. Dissemination of the organisms through the bloodstream permits focal lesions to develop in numerous organs. The patient will normally exhibit one of several clinical syndromes and the infection can be life-threatening, although most cases are mild.

Forms of tularaemia

Ulceroglandular tularaemia

This form is most common (70 - 85% of cases) in which a painful ulcerating papule, which has a necrotic center and raised periphery, develops at the site of infection (usually from a tick or fly bite). The patient experiences a fever (which can be as high as 104 degrees F) together with lymph node swelling particularly in the arm pit and groin.

Glandular tularaemia

This is acquired in the same way as Ulceroglandular tularaemia; however, there is lymphadenopathy but no ulcer.

Oculoglandular tularaemia

This is often acquired when a person handling infected meat rubs the bacteria into the eye. There is inflammation of the eye and swelling of regional lymph glands.

Pneumonic tularaemia

This is a very serious disease that comes from breathing in the bacteria in dust or aerosols. It results in difficulty breathing, chest pain and cough. It can also

result from not treating other forms of tularaemia resulting is dissemination to the lungs.

Oropharyngeal tularaemia

In this form, the disease is acquired by eating or drinking contaminated food or drink. Patients experience pharyngotoncillitis with lymphadenopathy accompanied by mouth ulcers. This is a rare form of tularaemia.

Pathogenesis

The capsule of the organism renders it resistant to phagocytosis. Intracellularly, the organisms resist killing by phagocytes and multiply. Most of the symptoms are due to cell-mediate hypersensitivity.

Diagnosis

F. tularensis is difficult to visualize in direct smears. The organism can be isolated from specimens of sputum, or lymph node aspirates inoculated on chocolate blood agar. Blood cultures are often negative. The organism grows very slowly and hence must be incubated for several days. The identity of the organism is confirmed with specific antisera.

Prevention and treatment

Streptomycin is the drug of choice for all forms of tularemia. Untreated, cases have a fatality rate of 5 - 15%. A live attenuated organism vaccine is available but its use is restricted to those persons who are at risk. Immunity appears to be cell mediated. One must avoid handling infected animals, watch out for ticks and utilize clean water supplies.

ERY SIPELOID

This is an occupational disease of butchers, meat processors, farmers, poultry workers, fish handlers: swine and fish handlers are particularly at risk. The causative agent, Erysipelothrix rhusiopathiae, is a Gram-positive anaerobic rod

which infects through skin abrasion while handling contaminated animal products or soil. Generally, the organism produces an inflammatory skin lesion, on fingers or hand, which is violaceus and has a raised edge. It spreads peripherally, as the discoloration in the central area fades. The painful lesion is pruritic and causes a burning or throbbing sensation. It lacks suppuration and thus is distinguishable from staphylococcal erysipelas. Diffuse cutaneous infection and septicaemia are rare. The organism can be cultured easily on most laboratory media. It is easily treatable with penicillin.

3.2 *BORDETELLA, HAEMOPILUS, AND LEGIONELLA*

Bordetella pertussis is the only organism of major clinical significance within this genus; it causes whooping cough in infants and young children. However, a closely related organism, *B. parapertussis* can also cause a milder form of bronchitis. *B. bronchosepticus*, another member of the genus Bordetella, is the causative agent of respiratory diseases in cats and swine, but can cause broncho-pulmonary symptoms in severely immunosupressed individuals.

Bordetella pertussis

Morphology and physiology

B. pertussis is an extremely small, strictly aerobic, Gram negative, non-motile *coccobacillus* (short rod). Compared to other *Bortdetella* species, *B. pertussis* does not grow on common laboratory media and can be distinguished from *B. parapertussis* in that *B. pertussis* is oxidase positive but urease negative, while *B. parapertussis* is oxidase negative and urease positive. *B. bronchosepticus* is positive for both enzymes.

Epidemiology and symptoms

Most of the patients with whooping cough are less than a year old although older children may also get the disease. The severity of disease is also age-related. The organism, contained in aerosol droplets, gains access via inhalation and colonizes the bronchial ciliary epithelial cells. After a week to 10 days of incubation period, mild symptoms of rhinitis, mild cough and sneezing occur (catarrhal stage) which last 1-2 weeks. Further proliferation of the organism compromise ciliary function and is accompanied by increased frequency and intensity of symptoms. This leads to the paroxysmal stage,

characterized by paroxysms of cough followed by a prolonged and distressing inspiratory gasp (whoop). The cough, which recurs at variable intervals and often every few minutes, may last for 2-3 weeks. The cough interferes with oral intake, and the swallowed mucus may induce vomiting, resulting in severe dehydration and weight loss. Hypoxia during prolonged attacks may lead to seizure, hypoxic encephalopathy or coma. The cough episodes slowly decrease and there is gradual recovery over 3-16 weeks (convalescent stage). Pneumonia (due to B. pertussis or other bacterial pathogens), otitis media, rectal prolapse and meningo-encephalitis are among the secondary complications.

Pathogenesis

The symptoms following the infection are due to many factors. In addition to the attachment to and growth on ciliated cells, the organism produces a number of exotoxins which contribute to these symptoms.

Pertussis toxin (pertussigen)

Pertussis toxin is an oligopeptide AB-type exotoxin that is the major cause of pertussis (abnormal cough). It causes T cell lymphocytosis and has adjuvant properties. It also causes hypoglycemia, increased IgE synthesis, and increased histamine and endotoxin sensitivity. The organism inhibits many leukocyte functions, including chemotaxis, phagocytosis and respiratory burst and impairs NK cell killing. It also contributes to bacterial binding to ciliated epithelial cells. It exerts many of its effects by covalent addition of ADP-ribose to the GTP binding Gi protein and thereby preventing the deactivation of adenylate cyclase. This results in the accumulation of large amounts of cAMP which leads to increased mucus secretion and interferes with many cellular functions.

Adenylate cyclase toxin

This exotoxin penetrates the host cells, is activated by calmodulin and catalyzes the conversion of ATP to cAMP. Like pertussigen, it also inhibits phagocyte and NK cell functions. However, in contrast with pertussigen, the cAMP increase caused by this toxin is short-lived.

Tracheal cytotoxin

This is a peptidoglycan-like molecule (monomer) which binds to ciliated epithelial cells, thus interfering with ciliary movement. In higher concentrations, it causes ciliated epithelial cell extrusion and destruction. The destruction of these cells contributes to pertussis.

Dermonecrotic (heat-labile) toxin

Dermonecrotic toxin is a very strong vaso-constrictor and causes ischemia and extravasation of leukocytes and, in association with tracheal cytotoxin, causes necrosis of the tracheal tissue.

Filamentous haemagglutinins (agglutinogens)

These are not exotoxins but are filament-associated lipo-oligo-saccharides which are implicated in the binding of the organism to ciliated epithelial cells. Antibodies against these molecules are protective, probably by preventing bacterial attachment.

Lipopolysaccharide (LPS)

Like LPS of other gram negative bacteria, these endotoxins cause a number of pathophysiological effects. When released in relatively large quantities following bacterial cell lysis, they cause irreversible shock and cardiovascular collapse. In smaller quantities, they activate a variety of inflammatory mediators (TNF, IL1, IL6, prostaglandins, etc.) and generate complement activation products.

Diagnosis

Symptoms are characteristic. Laboratory diagnosis is made by obtaining a nasopharyngeal aspirate and primary culture on Bordet-Gengou medium (potato-glycerol-blood agar). Growth is inhibited by peptones, unsaturated fatty acids, sulphides, etc. found in ordinary media. The organism grows as small transparent haemolytic colonies. It can be serologically distinguished from *B. parapertussis* and *B. bronchosepticus*.

Prevention and treatment

A killed whole bacterial vaccine is normally administered as DPT combination. An acellular vaccine consisting of filamentous haemagglutinins and detoxified pertussigen is also available and is recommended for booster shots. Erythromycin is the current drug of choice.

HAEMOPHILUS

The genus Haemophilus contains many species but H. influenzae is the most common pathogen. Other species of Haemophilus that are of clinical importance to immuno-competent humans are *H. ducreyi* (causes chancroid: an STD), *H. influenzae aegyptius* (associated with conjunctivitis and Brazilian purpuric fever) and H. parainfluenzae (a rare cause of pneumonia and endocarditis). There are several species of Hemophilus that are normal flora, but may be pathogenic in immuno-compromised hosts. The capsulated strain of H. influenzae (type b) is most virulent, although some non-encapsulated (non typable) strains are also pathogenic.

Haemophilus influenzae

Morphology and physiology

H. influenzae is a small Gram negative bacillus which can be grown on chocolate agar (heated blood) and requires hemin (factor X) and nicotinamide adenine dinucleotide (NAD+:factor V) for growth which is enhanced by high CO2 concentration (5%). It does not grow on normal blood agar. The factor V and factor X requirement can be used to distinguish between H. influenzae which requires both, H. parainfluenzae which requires factor V only and H. ducreyi which requires factor X only. H. influenzae are divided into several strains on the basis of capsular polysaccharides (a-f) or the absence of a capsule (non-typable).

Epidemiology and symptoms

H. influenzae causes a variety of clinical symptoms some of which may depend on the presence of the bacterial capsule. Until the availability of the Hib vaccine, the type-b H. influenzae was the main cause of meningitis in children between 6 months and 5 years, although older children, adolescents and adults can also be infected. The infection initially causes a runny nose, low

grade fever and headache (1-3 days). Due to its invasive nature the organism enters the circulation and crosses the blood-brain barrier, resulting in a rapidly progressing meningitis (stiff neck), convulsions, coma and death. Timely treatment may prevent coma and death, but the patient may still suffer from deafness and mental retardation. Type-b H. influenzae may also cause septic arthritis conjunctivitis, cellulitis, and epiglottitis, the latter results in the obstruction of the upper airway and suffocation. H. influenzae of other types may rarely cause some of the symptoms listed above. Non-typable strains of H. influenzae are the second commonest cause of otitis media in young children (second to Streptococcus pneumoniae). In adults, these organisms cause pneumonia, particularly in individuals with other underlying pulmonary infections. These organisms also cause acute or chronic sinusitis in individuals of all ages.

Pathogenesis

The exact mechanism of pathogenesis is not known but the presence of capsule, which is anti-phagocytic, is a major factor in virulence. Type-b H. influenzae are more invasive and pathogenic than other strains. The lipopolysaccharide is responsible for the inflammatory process. The organisms also produce IgA1-specific protease which may aid their mucosal colonization.

Diagnosis

Presumptive diagnosis is based on history, physical examination and symptoms. Blood cultures are positive in more than 50% of symptomatic patients, except those with conjunctivitis. Polyribitol phosphate (PRP), a component of the capsular polysaccharide is present in the serum, cerebrospinal fluid (CSF) and concentrated urine of more than 95% of H. influenzae-b meningitis cases. Gram-negative *cocobacilli* can be found in the CSF in more than 80% of meningitis cases. Some Gram-stained preparations may be useful in rapid diagnosis of septic arthritis and lower respiratory diseases.

Treatment and prevention

Unless prompt treatment is initiated, H. influenzae-b meningitis and epiglotitis are almost 100% fatal. Due to common resistance to ampicillin and some resistance to chloramphenicol, cephalosporin, which penetrates the blood brain barrier, is the antibiotic of choice in these cases. Other diseases caused by this organism can be treated with ampicillin (if susceptible) or choice of trimethoprim-sulphamethoxazol, tetracyclin and cefaclor.

Hib-C vaccine which consists of capsular PRP conjugated to tetanus toxoid has been used successfully to provide protection and is a part of the recommended routine vaccination schedule.

Haemophilus ducreyi

This is a significant cause of genital ulcers (chancroid) in Asia and Africa but, is seen less commonly in the United States. The incidence is approximately 4000-5000 per year with clusters found in California, Florida, Georgia and New York. The infection is asymptomatic in women but about a week following sexual transmission to a man, it causes appearance of a tender papule with erythematous base on the genitalia or the peripheral area. The lesion progresses to become a painful ulcer with inguinal lymphadenopathy. *The H. ducreyi* lesion (chancroid) is distinguished from a syphilitic lesion (chancre) in that it is a comparatively soft lesion. The organism is more fastidious than *H. influenzae* but can be grown on chocolate agar, supplemented with IsovitaleX in 5%-10% CO_2 atmosphere and the growth can be detected in 2-4 days.

Haemophilus influenzae aegyptius

This bacterium, previously known as *H. aegyptius*, causes an opportunistic organism which can result in a fulminant pediatric disease (Brazilian purpuric fever) characterized by an initial conjunctivitis, followed by an acute onset of fever, accompanied by vomiting and abdominal pain. Subsequently, the patient develops petechiae, purpura, shock and may face death. The pathogenesis of this infection is poorly understood. The growth conditions for this organism are the same as those for *H. influenzae*.

Both *H. ducreyi* and *H. influenzae aegyptius* can be treated with erythromycin.

LEGIONELLA

In 1976*, Legionella pneumophila* was recognized as a newly described pathogen after an outbreak of pneumonia among a group of Legionnaires at a convention in Philadelphia. The disease was subsequently referred to as Legionnaires' disease. Another flu-like form of the disease is referred to as Pontiac fever. *L. pneumophila* is now recognized as a ubiquitous aquatic saprophyte which causes epidemics and sporadic infections. The organisms are spread via aerosols and no person to person transmission has been reported.

Legionellae are facultative intracellular pathogens, which stain poorly as Gram negative rods. The causative agent was not recognized previously, since it does not grow on conventional agar such as sheep blood agar. Nowadays *L. pneumophila* is cultured on medium that contains iron and cysteine which are vital for growth (e.g. charcoal yeast extract agar). However, primary isolation is still difficult from clinical specimens.

Organisms of Clinical Importance

After recognition of their unique culture characteristics, a large number of other species of *Legionella* were isolated from environmental and clinical samples. These organisms are only occasional causes of human disease and the vast bulk of legionellosis is caused by *Legionella pneumophila* (most are serogroup 1 and 6).

The second most common cause of pneumonia is *Legionella micdadei*. This organism also stains weakly acid fast on primary isolation, but loses this property in vitro. This does not mean that it is anyway related to the *Mycobacteria*.

Microbiology

Legionellae are poorly staining Gram negative rods which are identified by growth on buffered charcoal yeast extract (BCYE), and require L-cysteine and iron for growth. The organisms are fairly slow growing requiring 3 to 7 days at 35 degrees. Colonies are small with a ground glass appearance.

The Center for Disease Control (CDC) lists four tests for the identification of Legionnaires' disease:

•Culture

•Urine antigen

•Paired serology

•Direct fluorescent antibody stain.

PCR tests for *L. pneumophila* in clinical specimens are available; however the CDC does not recommend the routine use of genetic probes or PCR for detection in clinical samples.

Public Health

Legionella pneumophila is an organism that resides in the environment in pools of stagnant water worldwide. It is found as an intra-cellular agent within protozoa and a component of biofilms. Legionnaires' disease is recognized as a sporadic infection, often associated with travel, an epidemic disease of community-acquired pneumonia and a nosocomial infection. It often infects hot water towers and air conditioning systems. When found in buildings, anti-bacterial treatment of the water supply is recommended. One recently identified source of Legionella infections is the water used in car windscreen washers, the reservoirs being warmed by the car engine. The use of windscreen washer fluid (which contains methanol) solves this problem.

The organism is transmitted in contaminated air but not spread person-person. Legionellosis is listed as one of the Nationally Notifiable Diseases by the Centers for Disease Control.

Clinical Presentation

Legionellae present as two distinct clinical diseases. The first is Legionnaires' disease, a typical pneumonia with an incubation period of 2 to 10 days. The mortality rate is as low as 20 % for healthy individuals and as high as 75% for the immune compromised persons. Legionnaires' disease is treated with erythromycin. The second form of disease presentation is Pontiac Fever. This illness has an incubation period of 1 to 2 days and is self-limiting with flu-like symptoms and no reported mortality.

Pathogenesis

Pathogenesis of Legionellae species requires the organism be phagocytosed into monocytes via complement receptors. Once inside the monocytes, the bacteria prevent phagosome-lysosome fusion and proceed to replicate until they lyse the phagosome which leads to apoptosis of the monocyte and release of the bacteria. Humoral immunity has little effect and the sensitized T helper (TH1) cells are required to activate the infected cells. Interferon- gamma is also critical to the elimination of Legionellae.

3.3 MYCOPLASMA AND UROPLASMA

The family Mycoplasmataceae contains two genera that infect humans: Mycoplasma and Ureaplasma, which are usually referred to collectively as mycoplasmas. Although there are many species of mycoplasmas, only four are recognized as human pathogens; Mycoplasma pneumoniae, Mycoplasma hominis, Mycoplasma genitalium, and Ureaplasma urealyticum. Although there are other species that have been isolated from humans, their role in disease is not well established. The diseases caused by *M. pneumoniae, M. hominis, M. genitalium* and *U. urealyticum* are presented in the table below:

Organism	**Disease**
M. pneumoniae	Upper respiratory tract disease, tracheobronchitis, atypical pneumonia
M. hominis	Pyelonephritis, pelvic inflammatory disease, postpartum fever
M. genitalium	Non-gonococcal urethritis
u. urealyticum	Non-gonococcal urethritis

Morphology and Physiology

The mycoplasmas are the smallest free-living bacteria. They range from 0.2 - 0.8 micrometers and thus can pass through some filters used to remove bacteria. They have the smallest genome size and, as a result, lack many metabolic pathways and require complex media for their isolation. The

mycoplasmas are facultative anaerobes, except for *M. pneumoniae*, which is a strict aerobe. A characteristic feature that distinguishes the mycoplasmas from other bacteria is the lack of a cell wall. Thus, they can assume multiple shapes including round, pear shaped and even filamentous.

The mycoplasmas grow slowly by binary fission and produce "fried egg" colonies on agar plates; the colonies of *M. pneumoniae* have a granular appearance. Due to the slow growth of mycoplasmas, the colonies may take up to 3 weeks to develop and are usually very small. The colonies of Ureaplasma are extremely small and thus Ureaplasma are also called T-strains (tiny strains).

Members of the genus *Mycoplasma* lack a cell wall, and are therefore, difficult to treat with many antibiotics, which have a negative effect on bacterial cell-wall synthesis such as penicillin. CDC

The mycoplasma all require sterols for growth and for membrane synthesis. The three species can be differentiated by their ability to metabolize glucose (*M. pneumoniae*), arginine (*M. hominis*) or urea (*U. urealyticum*). The fourth species *M. genitalium* is extremely difficult to culture.

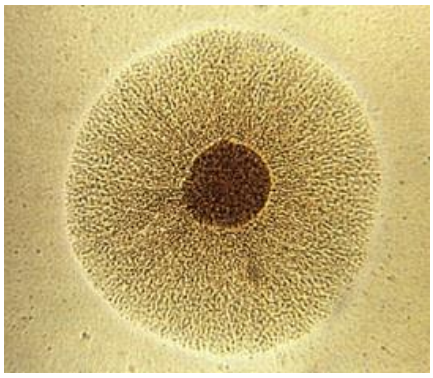

FIGURE 2.3.1: ***MYCOPLASMA HOMINIS***

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

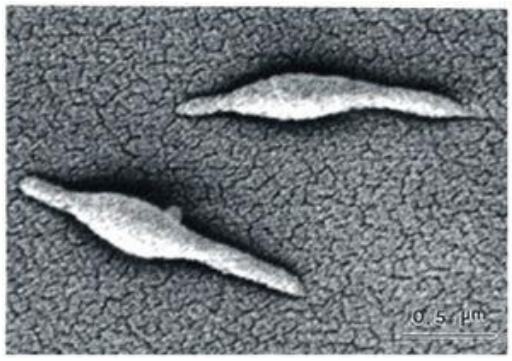

FIGURE 2.3.2: *MYCOPLASMA PNEUMONIAE*

COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

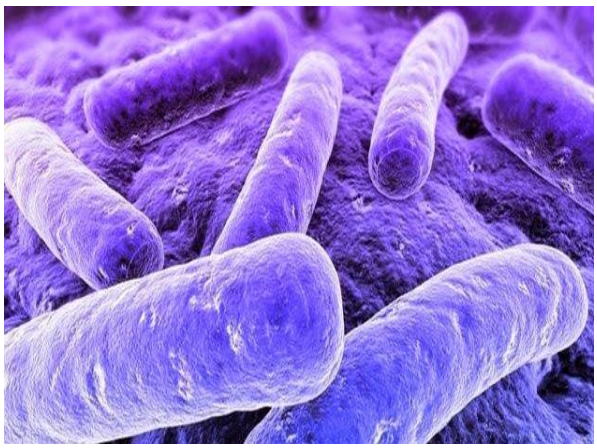

FIGURE 2.3.3; MYCOPLASMA GENITALIUM

COURTESY: DR. ALVIN FOX EMERITUS PROFESSOR OF MICROBIOLOGY UNIVERSITY OF SOUTH CAROLINA

Pathogenesis

Adherence factors

The mycoplasmas are extracellular pathogens that adhere to epithelial cell surfaces. Thus, adherence proteins are one of the major virulence factors. The adherence protein in M. pneumoniae has been identified as a 168kD protein called P1. The P1 Adhesin localizes at tips of the bacterial cells and binds to sialic acid residues on host epithelial cells.

The nature of the adhesins in the other species has not been established. Colonization of the respiratory tract by *M. pneumoniae* results in the cessation of ciliary movement. The normal clearance mechanisms of the respiratory tract do not function, resulting in contamination of the respiratory tract and the development of a dry cough.

Toxic Metabolic Products

The intimate association of the mycoplasma and the host cells provides an environment in which toxic metabolic products accumulate and damage host tissues. Both hydrogen peroxide and superoxide, which are products of mycoplasma metabolism, have been implicated in pathogenesis since oxidized host lipids have been found in infected tissues. Furthermore, the mycoplasmas have been shown to inhibit host cell catalase, thereby increasing the peroxide concentrations.

Immunopathogenesis

Mycoplasmas can activate macrophages and stimulate cytokine production and lymphocyte activation (*M. pneumoniae* is a superantigen). Thus, it is has been suggested that host factors also contribute to pathogenesis. Experimental evidence in animals supports this suggestion. Ablation of thymus function before infection with *M. pneumoniae* prevents the development of pneumonia and animals in which thymic function is restored develop pneumonia at an exacerbated rate. Epidemiologic data in humans suggest that repeated infections are required before clinical disease is observed, again suggesting a role for host related factors in pathogenesis; most children are infected from 2 - 5 years of age but disease is most common in children 5-15 years of age.

M. pneumoniae

Epidemiology

Pneumonia caused by *M. pneumoniae* occurs worldwide and no increased seasonal activity is seen. However, epidemics occur every 4 - 8 years. The disease is spread by close contact via aerosolized droplets and thus is most easily spread in confined populations (e.g., families, schools, army barracks). The disease is primarily one of the young (5 - 15 years of age).

Clinical syndrome

The most common clinical syndrome following infection with *M. pneumoniae* is tracheobronchitis, which is seen in 70-80% of the infections. Approximately one third of infected persons will develop pneumonia which is usually mild but of long duration. Pneumonia caused by this agent has been referred to a 'primary atypical pneumonia' and 'walking pneumonia'.

The incubation time following infection is approximately 2 - 3 weeks at which time fever, headache and malaise are gradually observed. These symptoms may be accompanied by a persistent non-productive hacking cough. Respiratory symptoms appear somewhat later and persist for several weeks. Interestingly, in *M. pneumoniae* pneumonia X-ray examination will show signs of pneumonia even before respiratory symptoms appear. Organisms can be cultured from sputum before symptoms occur and throughout the course of the disease. Resolution of the disease is slow but it is rarely fatal. The disease must be differentiated from other 'atypical' pneumonias.

Immunity

Complement activation via the alternative pathway and phagocytic cells both play a role in resistance to infection. As the infection proceeds, antibodies play a role in controlling infection, particularly IgA. The development of delayed type hypersensitivity, however, is associated with the severity of the disease, which supports the suggestion that pathogenesis is at least, in part, Immunopathogenesis.

Laboratory Diagnosis

In the early stages of infection diagnosis must be made on clinical grounds. However, as the infection progresses several laboratory tests are available.

•Microscopy
This is not particularly useful because of the absence of a cell wall but it can be helpful in eliminating other possible pathogens.

•Culture

Sputum (usually scant) or throat washings must be sent to the laboratory in special transport medium. It may take 2 -3 weeks to get a positive identification. Culture is essential for a definitive diagnosis.

•Serology

•Complement fixation test - There is a good complement fixation test that has good sensitivity and specificity. However, the titers do not peak until 4 - 6 weeks after infection. A fourfold rise in titer is indicative of a recent infection. Since antibodies may persist for up to 1 year, a sustained high titer does not necessarily indicate a current infection.

•Cold agglutinins - Approximately 34% - 68% of patients with M. pneumoniae infection develop cold agglutinins. Cold agglutinins are antibodies that agglutinate human erythrocytes at 4 ^{O}C but not at 37 ^{O}C. The antigen to which the antibodies are directed is the I antigen. These antibodies arise before the complement fixing antibodies and they decline faster. Cold agglutinins are not specific for *M. pneumoniae* infections, they can also appear in other infections and in other diseases (e.g. Infectious mononucleosis, influenza infections, cold agglutinin disease, leukaemia). However, if present in a patient with clinical signs of *M. pneumoniae* infection, a presumptive diagnosis can be made.

•ELISA - There is a new ELISA for IgM that has been used for diagnosis of acute infection. It is sensitive and specific. However, it is not yet commercially available.

Treatment and Prevention

Since mycoplasmas lack a cell wall, the penicillins and cephalosporins are ineffective. The antibiotics of choice are tetracycline (adults only) and erythromycin. Prevention is a problem due to the long duration of the disease. It is problematic to isolate patients to avoid close contact for a long period of time. No vaccines are currently available.

M. hominis and *U. urealyticum*

Clinical syndromes

M. hominis is associated with pyelonephritis, pelvic inflammatory disease and post-partum fevers. *U. urealyticum* is associated with non-gonococcal urethritis.

Epidemiology

Colonization with *M. hominis* and *U. urealyticum* can occur during birth but in most cases the infection will be cleared. Only in a small number of cases does colonization persist. However, when individuals become sexually active, colonization rates increase. Approximately 15% are colonized with *M. hominis* and 45% - 75% with U. urealyticum. The carriers are asymptomatic but the organisms can be opportunistic pathogens.

Laboratory Diagnosis; Laboratory diagnosis is by culture.

Treatment and Prevention; Since mycoplasmas lack a cell wall, the penicillins and cephalosporins are ineffective. The antibiotics of choice are tetracycline (adults only) and erythromycin. Abstinence or proper barrier protection are means of prevention.

4.0 CONCLUSION

Zoonosis refers to a disease primarily of animals which can be transmitted to humans as a result of direct or indirect contact with infected animal populations. Typical examples of zoonotic diseases Anthrax, Brucellosis e.t.c. Bordetella pertussis is the only organism of major clinical significance within this genus; it causes whooping cough in infants and young children. However, a closely related organism, *B. parapertussis* can also cause a milder form of bronchitis. *B. bronchosepticus*, another member of the genus Bordetella, is the causative agent of respiratory diseases in cats and swine, but can cause broncho-pulmonary symptoms in severely immunosupressed individuals. Mycoplasmas are the smallest free-living bacteria. They range from 0.2 - 0.8 micrometers and thus can pass through some filters used to remove bacteria. They have the smallest genome size and, as a result, lack many metabolic pathways and require complex media for their isolation. The mycoplasmas are facultative anaerobes, except for M. pneumoniae, which is a strict aerobe. A

characteristic feature that distinguishes the mycoplasmas from other bacteria is the lack of a cell wall. The family Mycoplasmataceae contains two genera that infect humans: Mycoplasma and Ureaplasma, which are usually referred to collectively as mycoplasmas.

5.0 SUMMARY

At the end of this unit, you have learnt about the basic microbiological principles, Key members of the group, and diagnostic features of the following;

- Zoonotic microbes
- Bordetella, Haemophilus, and Legionella
- Mycoplasma and Uroplasma

6.0 TUTOR MARKED ASSINGMENT

a. Discuss the Immunopathogenesis of the *Mycoplasma*.

Write brief notes on the following

b. Bordetella
c. Uroplasma
d. Zoonotic microbes

e. Comment on the Public Health relevance of *Legionella pneumophila*

7.0 REFERENCES /SUGGESTED FURTHER READING

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Unit 4; CHLAMYDIA and RICKETTSIA:

1.0 INTRODUCTION:

Chlamydiaceae; Members of the Chlamydiaceae are small obligate intracellular parasites and were formerly considered to be viruses. However, they contain DNA, RNA and ribosomes and make their own proteins and nucleic acids and are now considered to be true bacteria. They possess an inner and outer membrane similar to gram-negative

bacteria and a lipopolysaccharide but do not have a peptidoglycan layer. Although they synthesize most of their metabolic intermediates, they are unable to make their own ATP and thus are energy parasites. The family Chlamydiaceae consists of two genera. One species of Chlamydia and two of Chlamydophila are important in causing disease in humans. Chlamydia trachomatis can cause urogenital infections, trachoma, conjunctivitis, pneumonia and lymphogranuloma venereum (LGV). Chlamydophila pneumoniae can cause bronchitis, sinusitis, pneumonia and possibly atherosclerosis. Chlamydophila *psittaci* can cause pneumonia (psittacosis). Members of the Chlamydiaceae are small obligate intracellular parasites and were formerly considered to be viruses. However, they contain DNA, RNA and ribosomes and make their own proteins and nucleic acids and are now considered to be true bacteria. They possess an inner and outer membrane similar to gram-negative bacteria and a lipopolysaccharide but do not have a peptidoglycan layer. Although they synthesize most of their metabolic intermediates, they are unable to make their own ATP and thus are energy parasites.

Rickettsia, Ehrlichia, Anaplasma and Coxiella; The Rickettsia, Ehrlichia, Anaplasma and Coxiella are all small obligate intracellular parasites which were once thought to be part of the same family. Now, however, they are considered to be distinct unrelated bacteria. Like the Chlamydia these bacteria were once thought to be viruses because of their small size and intracellular life cycle. However, they are true bacteria, structurally similar to Gram negative bacteria. They are small Gram negative coccobacilli that are normally stained with Giemsa since they stain poorly with the Gram stain. Although these bacteria are able to make all the metabolites necessary for growth, they have an ATP transport system that allows them to use host ATP. Thus, they are energy parasites as long as ATP is available from the host. All of these organisms are maintained in animal and arthropod reservoirs and, with the exception of *Coxiella*, are transmitted by arthropod vectors (e.g., ticks, mites, lice or fleas). Humans are accidentally infected with these organisms. The reservoirs, vectors and major diseases caused by these organisms are summarized in a table in this unit.

2.0 OBJECTIVE

At the end of this unit, you should be able to discuss in details the microbiology of the: -

- Physiology and Structure of the Chlamydiaceae.
- Rickettsiae
- Methods for the treatment and control of these infections.

3.0 CONTENTS

3.1 Physiology and Structure of the Chlamydiaceae

Elementary bodies (EB)

EBs are the small (0.3 - 0.4 µm) infectious form of the chlamydia. They possess a rigid outer membrane that is extensively cross-linked by disulfide bonds. Because of their rigid outer membrane, the elementary bodies are resistant to harsh environmental conditions encountered when the chlamydia are outside of their eukaryotic host cells. The elementary bodies bind to receptors on host cells and initiate infection. Most chlamydia infects columnar epithelial cells but some can also infect macrophages.

Reticulate bodies (RB)

RBs are the non-infectious intracellular forms of the chlamydia. They are the metabolically active replicating form of the chlamydia. They possess a fragile membrane lacking the extensive disulfide bonds characteristic of the EB.

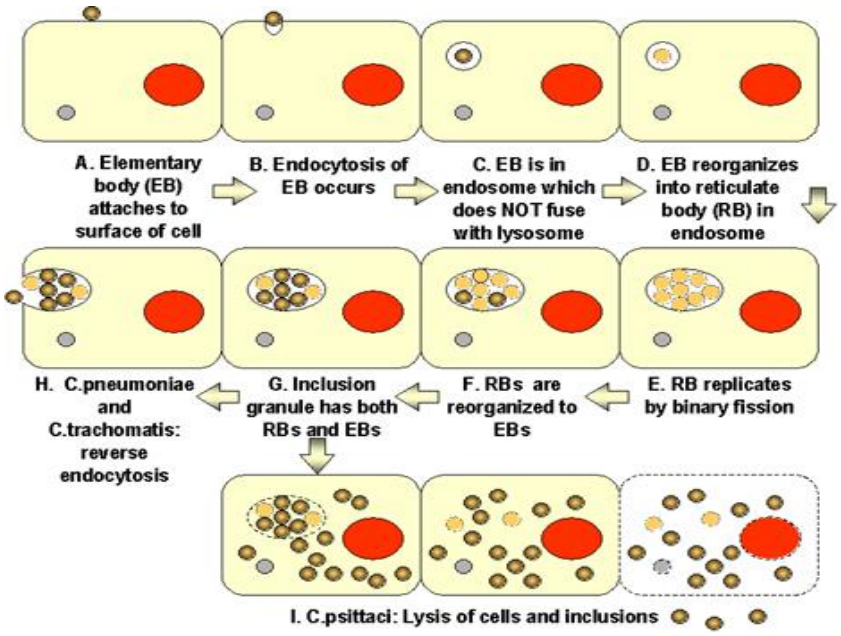
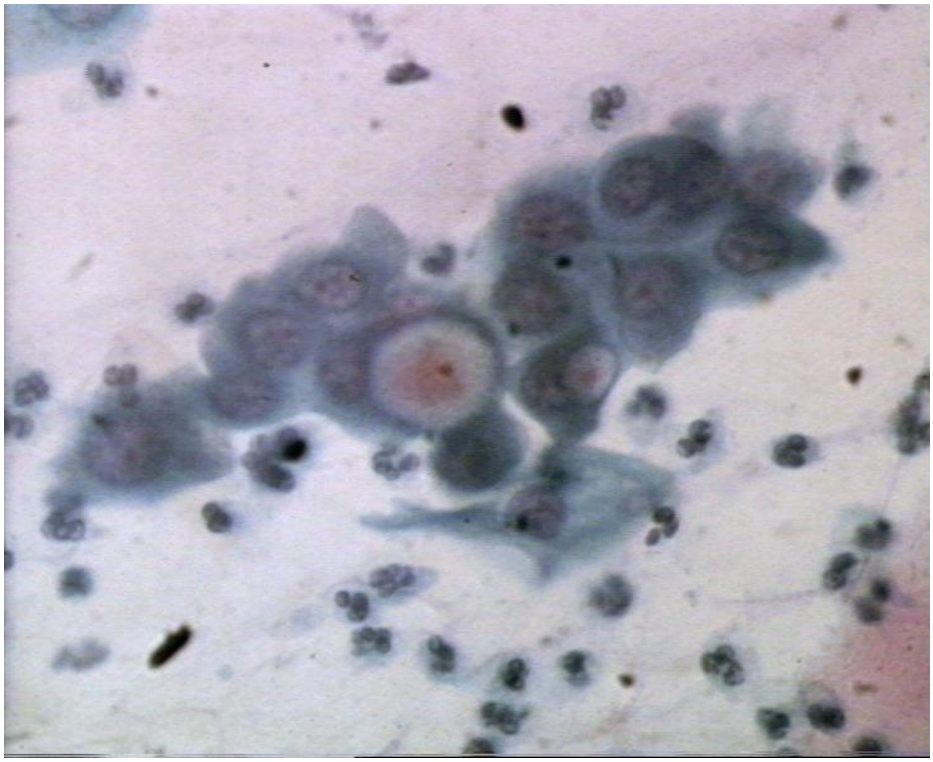


Figure 2.4.1: The developmental cycle of chlamydia

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Developmental cycle (Figure 3.4.1) - The EBs bind to receptors on susceptible cells and are internalized by endocytosis and/or by phagocytosis. Within the host cell endosome, the EBs reorganize and become RBs. The chlamydia inhibits the fusion of the endosome with the lysosomes and thus resist intracellular killing. The entire intracellular life cycle of the chlamydia occurs within the endosome. RBs replicate by binary fission and reorganize into EBs. The resulting inclusions may contain 100 - 500 progeny (Figure 3.4.2). Eventually, the cells and inclusions lyse (*C. psittaci*) or the inclusion is extruded by reverse endocytosis (*C. trachomatis* and *C. pneumoniae*) (Figure 3.4.1).



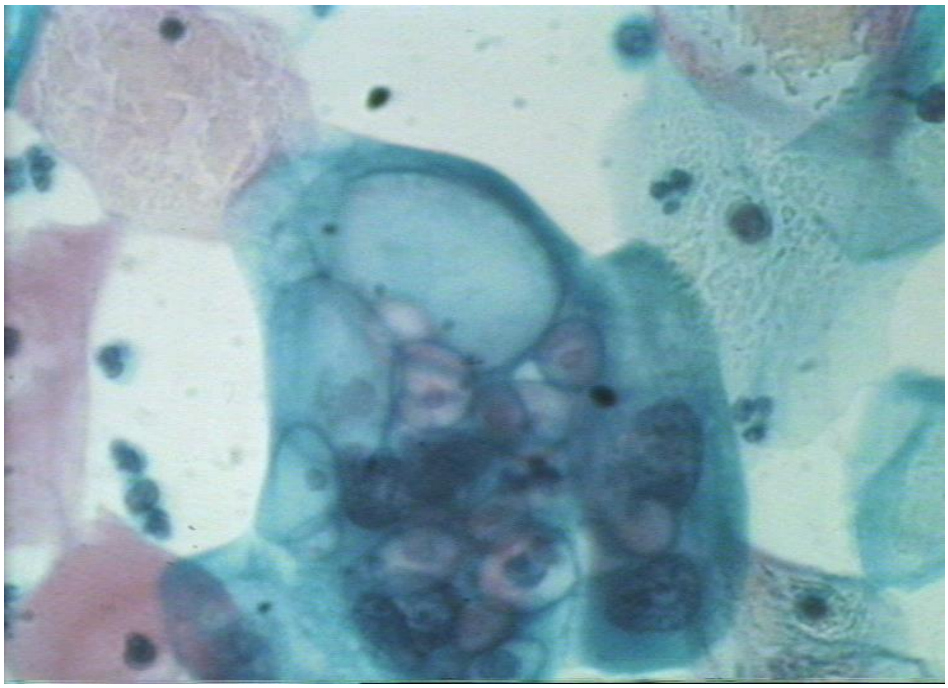


Figure 2.4.2 Chlamydial inclusions

Courtesy; Microbiology Department, Bingham University New Karu Nasarawa State of Nigeria

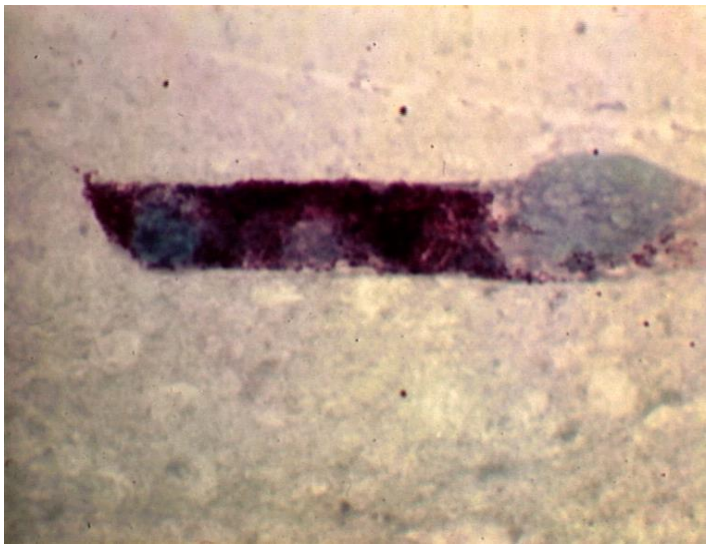


Figure 2.4.3: Chlamydial inclusions in an endothelial cell

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

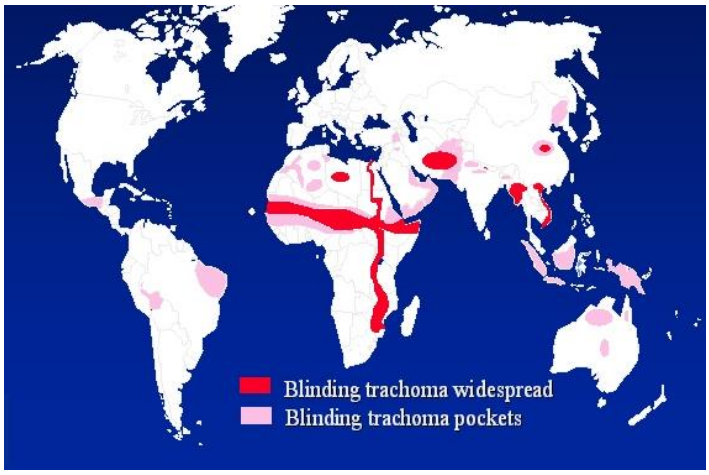


Figure 2.4.4: Distribution of trachoma

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Chlamydia trachomatis

C. trachomatis is the causative agent of trachoma, urogenital disease, infant pneumonia and lymphogranuloma venereum.

Biovar

C. trachomatis has a limited host range and only infects human epithelial cells (one strain can infect mice). The species is divided into three biovar (biological variants): trachoma, lymphogranuloma venereum and mouse pneumonitis.

Serovars

The human biovar have been further subdivided in to several serovars (serological variants; equivalent to serotypes) that differ in their major outer membrane proteins and which are associated with different diseases (see table below)

Serovars	Disease	Distribution
ABBa C	Trachoma	Asia and Africa
D-K	Disease of eye and genitals Conjuctitis Urethritis Cervicitis Respiratory System: Infant pneumonia	Worldwide
LV1 LGV2 LGV3	Lymph granuloma venereum	Worldwide

Pathogenesis and Immunity

C. trachomatis infects non-ciliated columnar epithelial cells. The organisms stimulate the infiltration of polymorphonuclear cells and lymphocytes which leads to lymphoid follicle formation and fibrotic changes. The clinical manifestations result from destruction of the cells and the host inflammatory response. Infection does not stimulate long lasting immunity and reinfection results in a inflammatory response and subsequent tissue damage.

Epidemiology

•Ocular infections

a. *C. trachomatis* (biovar: trachoma) is found worldwide primarily in areas of poverty and overcrowding (Figure 2.4.4). It is estimated that 500 million people are infected worldwide and 7 - 9 million people are blind as a consequence. *C. trachomatis* biovar: trachoma is endemic in Africa, the Middle East, India and Southeast Asia. In the United States, Native Americans are most commonly infected. Infections occur most commonly in children. The organism can be transmitted by droplets, hands, contaminated clothing, flies, and by passage through an infected birth canal.

•Genital tract infections

a. C. trachomatis (biovar: trachoma) is the most common sexually transmitted bacterial disease in the United States (4 million new cases each year) and 50 million new cases occur yearly worldwide. In the United States, the highest infection rates occur in Native and African Americans with a peak incidence in the late teens/early twenties.

b. C. trachomatis (biovar: LGV) is a sexually transmitted disease that occurs sporadically in the United States but is more prevalent in Africa, Asia and South America. Humans are the only natural host. Incidence is 300 - 500 cases per year in the United States with male homosexuals being the major reservoir of the disease.

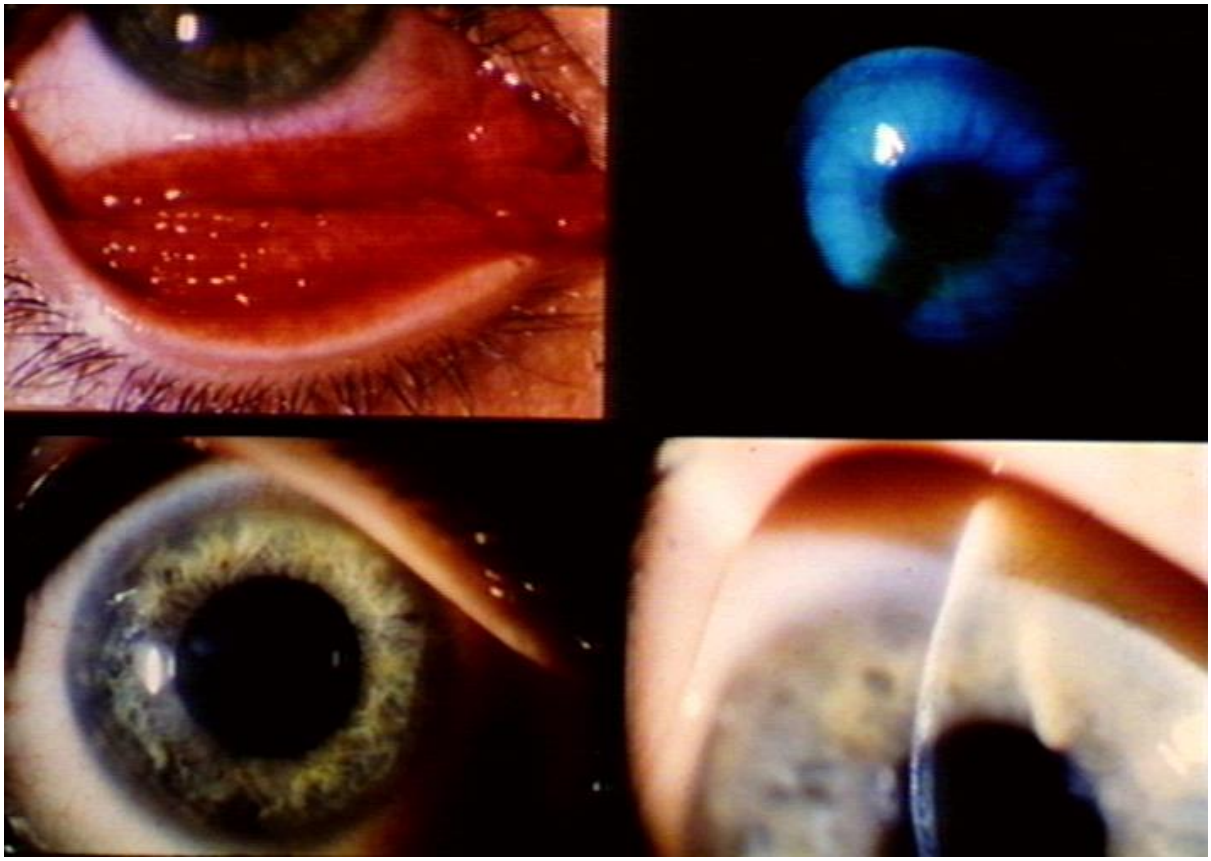


Figure 2.4.5 Chlamydial kerato-conjunctivitis

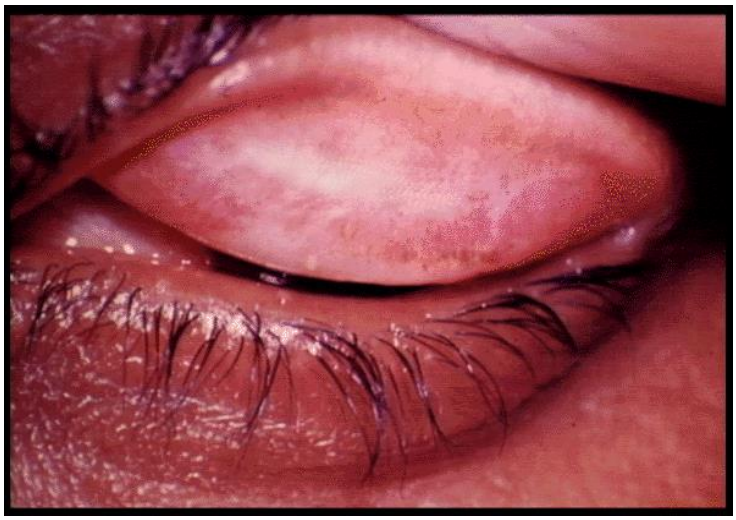


Figure 2.4.6: Trachomatous SCARRING: the presence of scarring in the tarsal conjunctiva. Scars are easily visible as white lines, bands, or sheets in the tarsal conjunctiva. They are glistening and fibrous in appearance. Scarring, especially diffuse fibrosis, may obscure the tarsal blood vessels

Figures 3.4.5-6

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Clinical Syndromes

•Trachoma

Chronic infection or repeated reinfection with C. trachomatis (biovar: trachoma) results in inflammation and follicle formation involving the entire conjunctiva (Figure 2.4.5 and 2.4.6). Scarring of the conjunctiva causes turning in of the eyelids and eventual scarring, ulceration and blood vessel formation in the cornea, resulting in blindness. The name trachoma comes from 'trakhus' meaning rough which characterizes the appearance of the conjunctiva. Inflammation in the tissue also interferes with the flow of tears which is an important antibacterial defence mechanism. Thus, secondary bacterial infections occur.

•Inclusion conjunctivitis

Inclusion conjunctivitis is caused by C. trachomatis (biovar: trachoma) associated with genital infections (serovars D - K). The infection is characterized by a mucopurulent discharge, corneal infiltrates and occasional corneal vascularization. In chronic cases corneal scarring may occur. In neonate’s infection results from passage through an infected birth canal and becomes apparent after 5 - 12 days. Ear infection and rhinitis can accompany the ocular disease.

•Infant pneumonia

Infants infected with C. trachomatis (biovar: trachoma; serovars: D - K) at birth can develop pneumonia. The children develop symptoms of wheezing and cough but not fever. The disease is often preceded by neonatal conjunctivitis.

•Ocular lymphogranuloma venereum
Infection with the LGV serovars of C. trachomatis (biovar: LGV) can lead to oculoglandular conjunctivitis. In addition to the conjunctivitis, patients also have an associated lymphadenopathy.

•Urogenital infections

In females, the infection is usually (80%) asymptomatic but symptoms can include cervicitis, urethritis, and salpingitis. Postpartum fever in infected mothers is common. Premature delivery and an increased rate of ectopic pregnancy due to salpingitis can occur. In the United States, tubal pregnancy is the leading cause of first-trimester, pregnancy-related deaths. In males, the infection is usually (75%) symptomatic

After a 3-week incubation period patients may develop urethral discharge, dysuria and pyuria. Approximately 35 - 50% of non-gonococcal urethritis is due to C. trachomatis (biovar: trachoma). Post-gonococcal urethritis also occurs in men infected with both Neisseria gonorrhoeae and C. trachomatis. The symptoms of chlamydial infection oc**cur** after treatment for gonorrhoea because the incubation time is longer.

Up to 40% of women with untreated (undiagnosed) chlamydia will develop pelvic inflammatory diseases and about 20% of these women will become infertile. Many untreated cases (18%) result in chronic pelvic pain.

Women infected with chlamydia have a 3 - 5 fold increased risk of acquiring HIV.

•Reiter's syndrome

Reiter's syndrome is a triad of symptoms that include conjunctivitis, polyarthritis and genital inflammation. The disease is associated with HLA-B27. Approximately 50 - 65% of patients have an acute C. trachomatis infection at the onset of arthritis and greater than 80% have serological evidence for C.

trachomatis infection. Other infections (shigellosis or Yersinia enterocolitica) have also been associated with Reiter's syndrome.

•Lymphogranuloma venereum (C. trachomatis biovar: LGV)

The primary lesion of LGV is a small painless and inconspicuous vesicular lesion that appears at the site of infection, often the penis or vagina. The patient may also experience fever, headache and myalgia. The second stage of the disease presents as a marked inflammation of the draining lymph nodes. The enlarged nodes become painful 'buboes' that can eventually rupture and drain. Fever, headache and myalgia can accompany the inflammation of the lymph nodes. Proctitis is common in females; lymphatic drainage from the vagina is perianal. Proctitis in males results from anal intercourse or from lymphatic spread from the urethra. The course of the disease is variable but it can lead to genital ulcers or elephantiasis due to obstruction of the lymphatics.

Laboratory diagnosis

There are several laboratory tests for diagnosis of *C. trachomatis* but the sensitivity of the tests will depend on the nature of the disease, the site of specimen collection and the quality of the specimen. Since chlamydia are intracellular parasites, swabs of the involved sites rather than exudate must be submitted for analysis. It is estimated that as many as 30% of the specimens submitted for analysis are inappropriate.

•Cytology

Examination of stained cell scrapings for the presence of inclusion bodies (Figures 2.4.2 and 2.4.3) has been used for diagnosis but this method is not as sensitive as other methods.

•Culture

Culture is the most specific method for diagnosis of *C. trachomatis* infections. Specimens are added to cultures of susceptible cells and the infected cells are examined for the presence of iodine-staining inclusion bodies. Iodine stains glycogen in the inclusion bodies. The presence of iodine-staining inclusion bodies is specific for C. trachomatis since the inclusion bodies of the other species of chlamydia do not contain glycogen and stain with iodine.

•Antigen detection

Direct immunofluorescence and ELISA kits that detect the group specific LPS or strain-specific outer membrane proteins are available for diagnosis. Neither is as good as culture, particularly with samples containing few organisms (e.g. asymptomatic patients).

•Serology

Serological tests for diagnosis are of limited value in adults, since the tests do not distinguish between current and past infections. Detection of high titer IgM antibodies is indicative of a recent infection. Detection of IgM antibodies in neonatal infection is useful.

•Nucleic acid probes

Three tests based on nucleic acid probes are available. These tests are sensitive and specific and may replace culture as the method of choice.

Treatment and prevention

Tetracyclines, erythromycin and Sulfonamides are used for treatment but they are of limited value in endemic areas where reinfection is common. Vaccines are of little value and are not used. Treatment coupled with improved sanitation to prevent reinfection is the best way to control infection. Safe sexual practices and prompt treatment of symptomatic patients and their sexual partners can prevent genital infections.

Chlamydophila psittaci

C. psittaci is the causative agent of psittacosis (parrot fever). Although the disease was first transmitted by parrots, the natural reservoir for C. psittaci can be any species of bird. Thus, the disease has also been called ornithosis from the Greek word for 'bird'.

Pathogenesis

The respiratory tract is the main portal of entry. Infection is by inhalation of organisms from infected birds or their droppings. Person-to-person transmission is rare. From the lungs the organisms enter the blood stream and are transported to the liver and spleen. The bacteria replicate at these sites where they produce focal areas of necrosis. Haematogenous seeding of the

lungs and other organs then occurs. A lymphocytic inflammatory response in the alveoli and interstitial spaces leads to oedema, infiltration of macrophages, necrosis and sometimes haemorrhage. Mucus plugs may develop in the alveoli causing cyanosis and anoxia.

Epidemiology

Approximately 50 - 100 cases of psittacosis occur annually in the United States with most infections occurring in adults. The organism is present in tissues, faeces and feathers of infected birds that are symptomatic or asymptomatic. There may also be reservoirs in other animals such as cats and cattle. Veterinarians, zoo keepers, pet shop workers and poultry processing workers are at increased risk for developing the disease.

Clinical Syndromes

The illness develops after an incubation time of 7 - 15 days. Symptoms include fever, chills, headache, a non-productive cough and a mild pneumonitis. In uncomplicated cases the disease subsides by 5-6 weeks after infection. Asymptomatic infections are common. In complicated cases convulsions, coma and death (5% mortality rate) can occur. Other complications include carditis, hepatomegaly and splenomegaly.

Laboratory diagnosis

Laboratory diagnosis is based on a serological test. A four-fold rise in titter in paired samples in a complement fixation test is indicative of infection.

Treatment and prevention

Tetracycline or erythromycin are the antibiotics of choice. Control of infection in birds by feeding of antibiotic supplemented food is employed. No vaccine is available.

Chlamydophila pneumoniae

Chlamydophila pneumoniae is the causative agent of an atypical pneumonia (walking pneumonia) similar to those caused by Mycoplasma pneumoniae and Legionella pneumoniae. In addition, it can cause a pharyngitis, bronchitis,

sinusitis and possibly atherosclerosis. The organism was originally called the TWAR strain from the names of the two original isolates - Taiwan (TW-183) and an acute respiratory isolate designated AR-39. It is now considered a separate species of chlamydia.

Pathogenesis

The organism is transmitted person- to-person by respiratory droplets and causes bronchitis, sinusitis and pneumonia.

Epidemiology

The infection is common with 200,000 - 300,000 new cases reported annually, mostly in young adults. Although 50% of people have serological evidence of infection most infections are asymptomatic or mild. The disease is most common in military bases and college campuses (crowding). No animal reservoir has been identified.

Potential link to atherosclerosis: A report in the Journal of the American College of Cardiology documented a high incidence of C. pneumoniae in the arteries of patients with atherosclerosis (79% compared with 4% in the control group). It is still unproven that the link is causal. However, previous reports show a high association between presence of antibodies to C. pneumoniae in serum of patients with atherosclerosis as well as the presence of the organisms in the coronary and carotid arteries.

Clinical Syndrome

Symptoms include a pharyngitis, bronchitis, a persistent cough and malaise. More severe infections can result in pneumonia, usually of a single lobe.

Laboratory diagnosis

Culture is difficult so serological test is most common. A four-fold rise in titre in paired samples is diagnostic.

Treatment and prevention

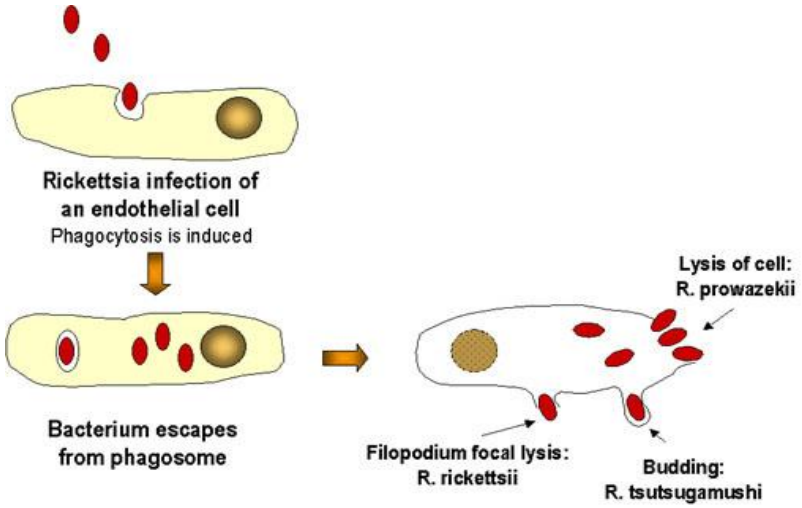
Tetracycline and erythromycin are the antibiotics of choice. No vaccine is available. Rickettsial infections have played a significant role in the history of Western civilization. Epidemic typhus has been known since the 16th century and it has long been associated with famine and war. The outcome of several wars was influenced by epidemic typhus. Typhus killed or caused great suffering to over 100,000 people in the two World Wars. In spite of its long history, it was not until the early part of the 20th century that the causative agent was determined. Howard Ricketts described the causative agent of Rocky Mountain Spotted Fever and was able to culture it in laboratory animals. Others then realized that the causative agent of epidemic typhus was related to the organism that Ricketts described. After the discovery of the importance of arthropod vectors in the spread of typhus, vector control measures were instituted to control the disease. However, as Hans Zinsser has pointed out, typhus is not dead.

3.2 Rickettsia, Ehrlichia, Anaplasma and Coxiella

The Rickettsia, Ehrlichia, Anaplasma and Coxiella are all small obligate intracellular parasites which were once thought to be part of the same family. Now, however, they are considered to be distinct unrelated bacteria. Like the Chlamydia these bacteria were once thought to be viruses because of their small size and intracellular life cycle. However, they are true bacteria, structurally similar to Gram negative bacteria. They are small Gram negative coccobacilli that are normally stained with Giemsa since they stain poorly with the Gram stain. Although these bacteria are able to make all the metabolites necessary for growth, they have an ATP transport system that allows them to use host ATP. Thus, they are energy parasites as long as ATP is available from the host. All of these organisms are maintained in animal and arthropod reservoirs and, with the exception of Coxiella, are transmitted by arthropod vectors (e.g., ticks, mites, lice or fleas). Humans are accidentally infected with these organisms. The reservoirs, vectors and major diseases caused by these organisms are summarized in the table below:

Disease	Organism	Vector	Reservoir
Rocky Mountain spotted fever	*R rickettsii*	Tick	Ticks, wild rodents
Ehrlichiosis	*E chaffeensis*		Deer
	E ewingii	Tick	Small mammals
Anaplasmosis			
	A. phagocytophilium	Tick	Deer
			Small mammals
Rickettsial pox	*R. akari*	Mite	Mites, wild rodents
Scrub typhus	*R. tsutsugamushi*	Mite	Mites wild rodents
Epidemic typus	*R. prowazekii*	Louse	Humans, squirrel fleas, flying squirrels
Murine typhus	*R. typhi*	Flea	Wild rodents
Q fever	*C burnetii*	None	Cattle, sheep, goats, cats

The Rickettsia preferentially infect endothelial cells lining the small blood vessels by parasite-induced phagocytosis (figure 2.4.7). Once in the host cell, the bacteria lyse the phagosome membrane with a phospholipase and get into the cytoplasm where they replicate.



Figures 2.4.7

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

The mode of exit from the host cell varies depending upon the species. R. prowazekii exits by cell lysis while R. rickettsii get extruded from the cell through local projections (filopodia). F actin in the host cell associates with *R. rickettsii* and the actin helps to "push" the bacteria through the filopdia (figure 3.4.8). O. tsutsugamushi exits by budding through the cell membrane and remains enveloped in the host cell membrane as it infects other cells.

Antigenic structure

Based on their antigenic composition, the Rickettsia are divided into several groups. The organisms in each group, the diseases caused by the organisms and their geological distribution are summarized in the table below:

Spotted fever group		
Organism	Disease	Distribution
R. rickettsii	Rocky Mountain spotted fever	Western hemisphere
R. akari	Rickettsial pox	USA, former Soviet Union
R. conorii	Boutonneuse fever	Mediterranean countries, Africa, India, Southwest Asia
R. sibirica	Siberian tick typhus	Siberia, Mongolia, northern China
R. australis	Australian tick typhus	Australia
R. japonica	Oriental spotted fever	Japan
Typhus group		
Organism	Disease	Distribution
R. prowazekii		
R. typhi		
Scrub typhus group		
Organism	Disease	Distribution
O. tsutsugamushi	Scrub typhus	Asia, northern Australia, Pacific Islands

Pathogenesis and Immunity

Pathogenesis is primarily due to destruction of the infected cells by the replicating bacteria. Destruction of endothelial cells results in leakage of blood and subsequent organ and tissue damage due to loss of blood into the tissue spaces. No evidence for immunopathological damage has been obtained. Both humoral and cell mediated immunity are important in recovery from infection. Antibody-opsonized Rickettsia are phagocytosed and killed by macrophages and delayed type hypersensitivity develops following rickettsial infections.

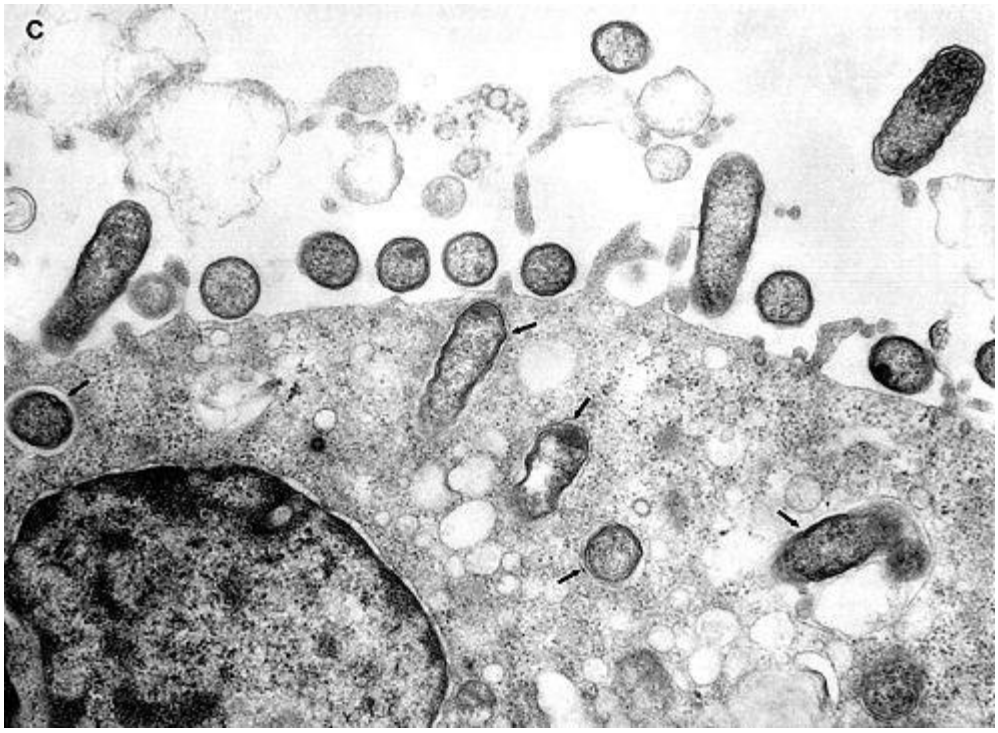


Figure 2.4.8: Attachment of rickettsiae to the surface of an endothelial cell is followed by their entry into the cell via rickettsia- induced phagocytosis. Following phagocytosis, the phagosome membrane (arrow) is lost and the rickettsiae escape into the host cell cytoplasm.

Figures 2.4.8

Courtesy: Dr. Alvin Fox Emeritus Professor of Microbiology University of SOUTH CAROLINA

Rickettsia rickettsii (Rocky Mountain spotted fever)

Epidemiology

Rocky Mountain Spotted Fever is the most common rickettsial disease in the United States with 400 - 700 cases occurring annually.

While the disease was originally described in the Rocky Mountain states, it is now most common in the South Central states, including South Carolina.

The organism is transmitted by the bite of an infected tick with most infections occurring from April through September because of more frequent human contact with ticks at this time of the year. The Rickettsia in the tick are in a dormant state and must be activated by the warm blood meal. They are then released into the saliva of the tick. Thus, prolonged exposure (24 - 48 hrs) to an infected tick must occur before the organisms can infect the human host. The principal reservoir for R. rickettsii is the ixodid (hard) tick in which transovarian passage occurs. Wild rodents can become infected and act as a reservoir for the bacteria but they are not considered to be the main reservoir.

Clinical syndromes

Rocky Mountain spotted fever begins with the abrupt onset of fever, chills headache and myalgia usually 2 - 12 days after the tick bite. Patients may not recall being bitten by a tick. Rash usually (90% of cases) appears 2 - 3 days later. The rash begins on the hands and feet and spreads centripetally towards the trunk. Rash on the palms and soles is common. Initially, the rash is maculopapular but in the later stages may become petechial and haemorrhagic.

Complications from widespread vasculitis can include gastrointestinal symptoms, respiratory failure, seizures, coma and acute renal failure. Complications occur most frequently in cases in which the rash does not develop, since treatment is usually delayed. Mortality rate in untreated patients is 20%.

Laboratory diagnosis

Initial diagnosis should be made on clinical grounds and treatment should not be delayed until laboratory confirmation is obtained. A fluorescent antibody test to detect antigen in skin punch biopsies is the fastest way to confirm a diagnosis. However, this test is available only in reference laboratories. PCR based methods are also available but limited to reference laboratories. The Weil-Felix test, which is an agglutination test to detect antibodies that cross react with Proteus vulgaris, is no longer recommended. The primary laboratory diagnostic tool is serology. Indirect fluorescent antibody tests and latex agglutination tests are available for serological diagnosis of Rocky Mountain spotted fever.

3.3 METHODS FOR TREATMENT, PREVENTION AND CONTROL OF RICKETTSIAL DISEASES

R. rickettsii is susceptible to tetracyclines and chloramphenicol. Prompt treatment is necessary since morbidity and mortality increases if treatment is delayed. No vaccine is available. Prevention of tick bites (protective clothing, insect repellents, etc.) and prompt removal of ticks are the best preventative measures. It is not feasible to attempt to control the tick reservoir.

Epidemiology

R. akari is found in the United States and sporadic infections occur. The vector is a mouse mite and the reservoirs are mites and mice. In mites, the bacteria are maintained by transovarian transmission. Humans are accidentally infected.

Clinical syndromes

Rickettsialpox is typically a mild disease that has two phases. In the first phase a papule develops at the site of the mite bite and quickly ulcerates and forms an eschar. This initial phase occurs approximately 1 week after the bite. After

an incubation time of 7 to 24 days, the second phase of the disease occurs. This phase is characterized by sudden onset of fever, chills headache and myalgia and is followed 2 to 3 days later with a generalized rash. The rash is papulovesicular and crusts over in the later stages. The pox heals within 2 to 3 weeks without scarring. Fatalities are rare.

Laboratory diagnosis

Not available except in certain reference laboratories

Treatment and prevention and control

Tetracycline and chloramphenicol can speed up recovery. Measures aimed at controlling mouse populations help to prevent the disease.

Epidemiology

Epidemic typhus is a disease transmitted by the human body louse. When an infected louse bites a human, it defecates and the bacteria are found in the feces. Irritation caused by the bite causes the person to scratch the bite and thereby to inoculate the bacteria into abraded skin. Unlike the other rickettsial diseases, humans are the primary reservoir for R. prowazekii. Epidemic typhus occurs among people living in crowded, unsanitary conditions such as those found in wars, famine and natural disasters. Transovarian transmission in the louse does not occur since lice die several weeks after being infected. The disease occurs sporadically in the United States, primarily in the Eastern states where the reservoirs are flying squirrels and their fleas. The fleas are the vector that transmit the disease.

Clinical syndromes

a. Epidemic typhus is characterized by sudden onset of fever, chills, headache myalgia and arthralgia, after an average incubation period of 8 days. Approximately 7 days later, a rash develops in most patients. The rash is maculopapular but can be petechial or haemorrhagic. In contrast to the rash seen with Rocky Mountain Spotted Fever, the rash in epidemic typhus develops on the trunk first and spreads to the extremities (centrifugal spread). Complications include: myocarditis, stupor and delirium. The name typhus comes from the Greek for "smoke" underscoring the fact that stupor and delirium often complicate the disease. Recovery may take several months. The mortality rate varies but can be quite high (60 - 70%) in some epidemics.

b. Brill-Zinsser disease is recrudescent epidemic typhus. It occurs decades after the initial infection. In the United States it is most commonly seen in those who were exposed to epidemic typhus in World War II. The clinical course of the disease is similar to epidemic typhus but is milder and recovery is faster. The skin rash is rarely seen. Diagnosis is made on the basis of a fever with unknown origin and a history of previous exposure to epidemic typhus.

Laboratory diagnosis

Diagnosis should be made on clinical findings and treatment should begin before laboratory confirmation. Weil-Felix antibodies are produced but the test is not recommended. Serology is the primary laboratory test used for diagnosis of R. prowazekii. Indirect fluorescent antibody tests and latex agglutination tests are available. Patients with epidemic typhus initially have an IgM response followed by IgG antibodies whereas patients with Brill-Zinsser disease initially have an anamnestic IgG response. Isolation of the organism is possible but dangerous.

Treatment, prevention and control

Tetracyclines and chloramphenicol are highly effective. Louse control measures can prevent infection. A killed typhus vaccine is available and is recommended for use in high-risk populations.

Rickettsia typhi (Murine or endemic typhus)

Epidemiology

Murine typhus occurs worldwide with approximately 40 - 60 cases being reported in the United States annually. Rats are the primary reservoir for the disease which is transmitted by the rat flea vector. The normal cycle is rat to flea to rat and humans are accidentally infected. Since there is no transovarian transfer in the flea, the flea is not a reservoir for the disease. The cat flea can also be a vector for the disease in the United States. The bacteria are in the flea faeces and are inoculated into abraded skin by scratching the area irritated by the bite.

Clinical syndromes

The symptoms of fever, chills headache and myalgia appear abruptly 1 - 2 weeks after infection. A rash develops in many but not all cases. The rash begins on the trunk and spreads to the extremities, unlike the rash seen in Rocky Mountain Spotted Fever. The disease is mild and resolves within 3 weeks' even if untreated.

Laboratory diagnosis

A serological indirect fluorescent antibody test is used to detect antibodies to *R. typhi*.

Treatment, prevention and control

Tetracyclines and chloramphenicol are effective. Controlling the rodent reservoir is useful in preventing infection. A vaccine is not available.

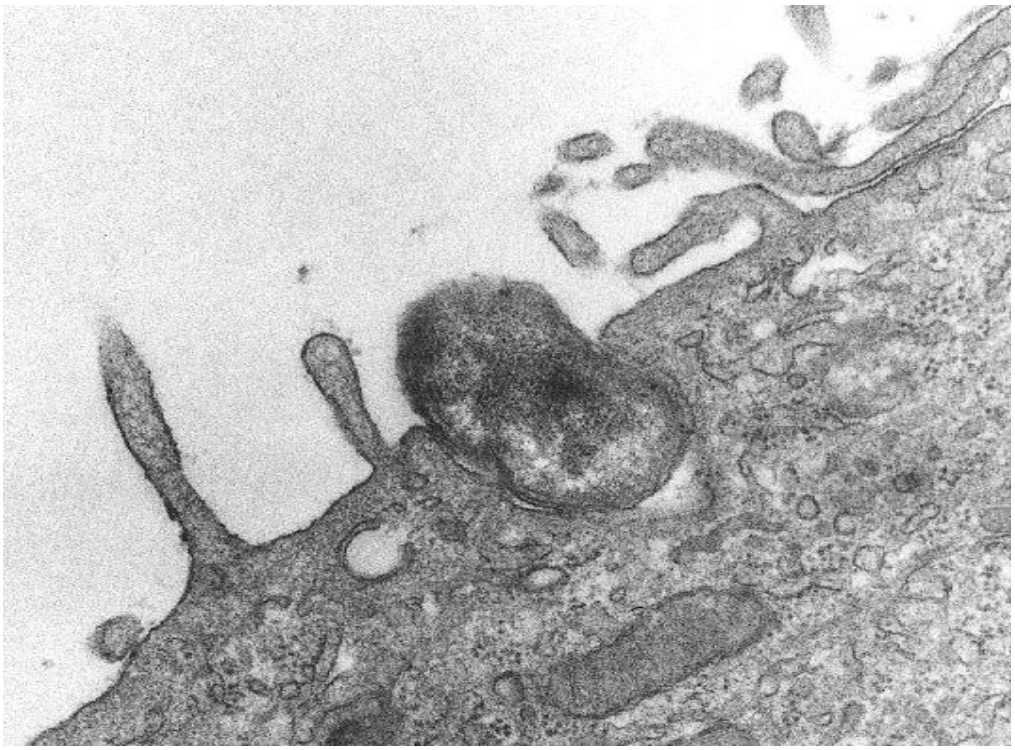


Figure 2.4.9: Phagocytosis of Rickettsia tsutsugamushi by mouse peritoneal mesothelial cell.

Courtesy :Dr Alvin Fox Emeritus Professor University of South Carolina School of Medicine

Orientia (Rickettsia) tsutsugamushi (Scrub typhus)

Epidemiology

Scrub typhus occurs in Asia, Australia and the Pacific Islands. The disease is transmitted to humans by chiggers, the larval form of a mite. The mite is both the reservoir and the vector and passes the bacteria transovarially. Rodents can also act as a reservoir. The normal cycle is mite to rodent to mite; humans are accidentally infected.

Clinical syndromes

The disease is characterized by sudden onset of fever, chills headache and myalgia 1 - 3 weeks after contracting the bacteria. A maculopapular rash develops 2 - 3 days later. The rash appears first on the trunk and spreads to the extremities (centrifugal spread). Mortality rate in outbreaks are variable.

Laboratory diagnosis

Serological tests for antibody are available.

Treatment, prevention and control

Tetracyclines and chloramphenicol are effective. Avoiding exposure to chiggers will prevent the disease.

Ehrlichia and Anaplasma

Replication

The Ehrlichia and Anaplasma preferentially infect leukocytes. They enter the cell by phagocytosis and once in the host cell they inhibit phagolysozomes fusion.

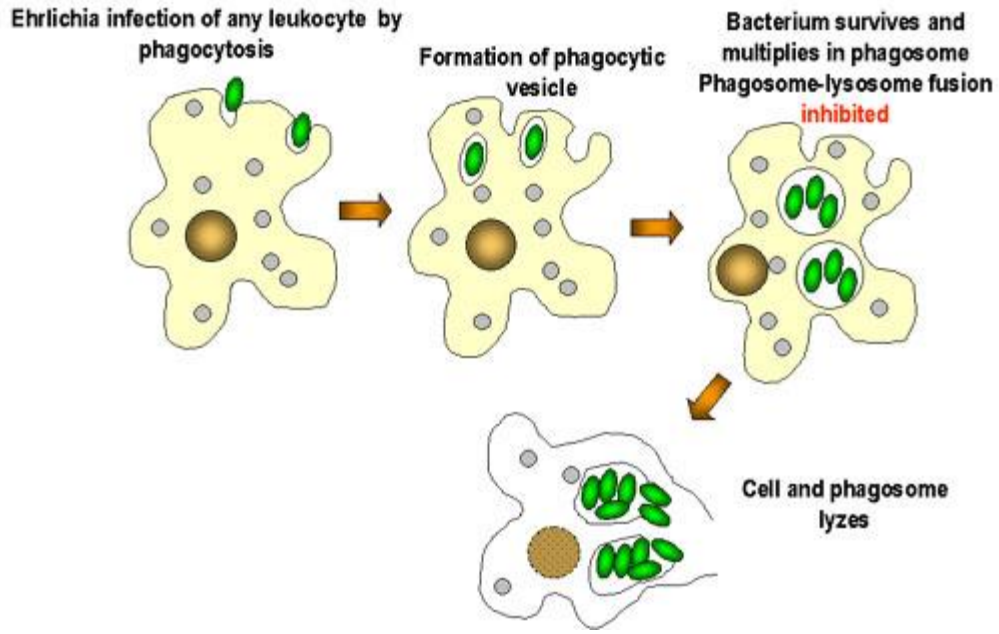


FIGURE 2.4.10 UNDECAPRENOL PHOSPHATE AND THE PEPTIDOGLYCAN SUBUNIT ARE PHOSPHORYLATED

Online resource wikipedia

The organisms grow within the membrane-bound phagosome and are released by lysis of the cell (figure 2.4.10). The inclusion body containing the organisms is called a morula.

Epidemiology

The Ehrlichia are divided into three groups based on genetic homology. Table 3 (Adapted from: Murray, et al., Medical Microbiology) summarizes the human diseases caused by the Ehrlichia and Analplasma, the vectors, reservoirs and the geographic distributions.

Ehrlichia chaffeensis (human monocytic ehrlichiosis)

Clinical syndromes

The disease resembles Rocky Mountain Spotted Fever, except that the rash does not develop in most (80%) patients. In addition, leukopenia is observed due to destruction of the leukocytes. Mortality is low (5%).

Laboratory diagnosis

Microscopic observation of morula in blood smears is rare and although culture is possible, it is rarely attempted. Serological test are available and are the most commonly employed test. DNA probes are available and may replace serological tests.

Treatment, prevention and control

Patients should be treated with doxycycline. Avoidance of tick infected areas and protective measures (clothing and insect repellents) can prevent the disease.

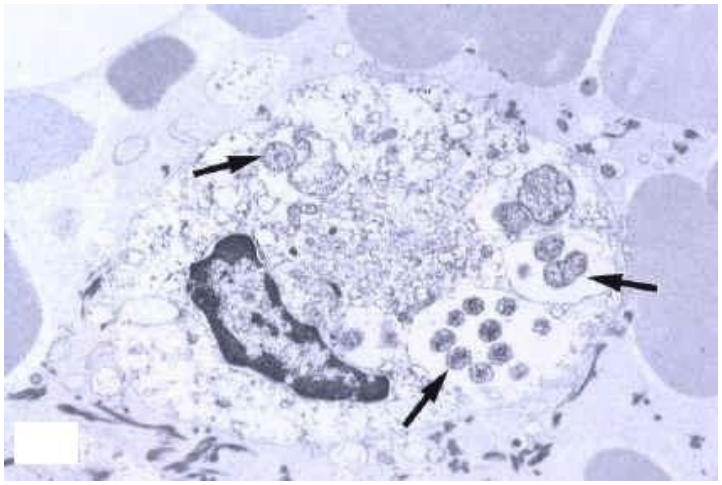


Figure 2.4.11: Electron-photomicrograph of morulae in a bone marrow leukocyte in a patient with ehrlichiosis. Arrows indicate individual ehrlichiae.

2.4.11 Online resource Wikipedia

Ehrlichia ewingii and Anaplasma phagocytophilium (human granulocytic ehrlichiosis)

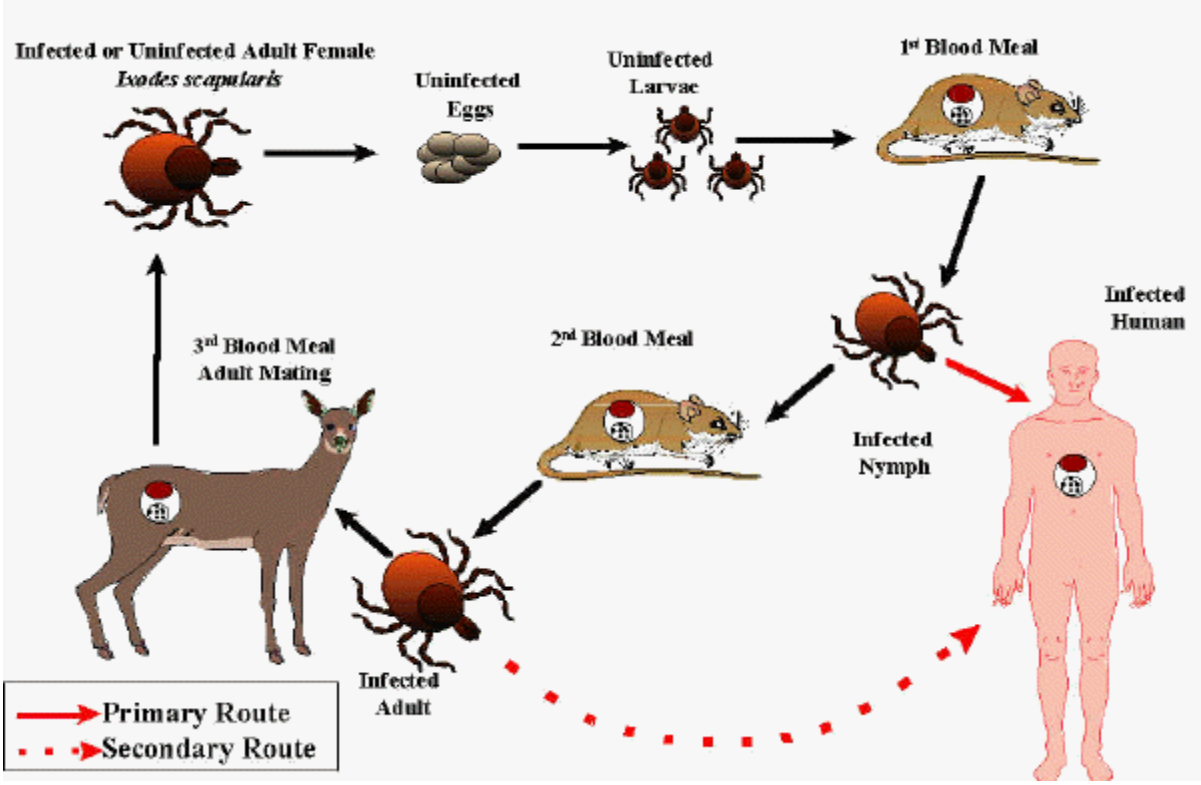


FIGURE 2.4.12: PROPOSED LIFE CYCLE FOR THE AGENT OF HUMAN GRANULOCYTIC EHRLICHIOSIS

Courtesy : Courtesy :Dr Alvin Fox Emeritus Professor University of South Carolina School of Medicine

Clinical syndromes

The disease is similar to human monocytic ehrlichiosis except that mortality rates may be higher (10%)

Laboratory diagnosis

Same as *E. chaffeensis*

Treatment, prevention and control

Same as *E. chaffeensis*

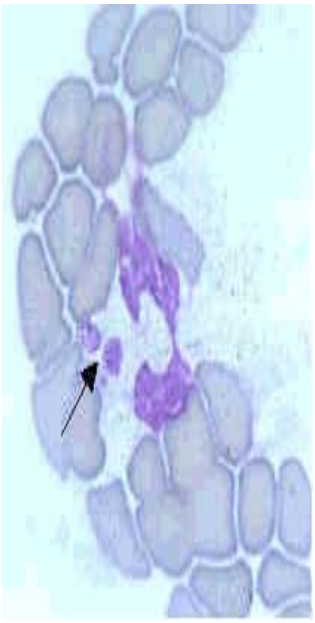


FIGURE 2.4.13; THE PATHOGEN THAT CAUSES HUMAN GRANULOCYTIC EHRLICHIOSIS (HGE) PRIMARILY INFECTS GRANULOCYTES (NEUTROPHILS AND RARELY EOSINOPHILS). THE PATHOGEN IS OFTEN REFERRED TO AS THE AGENT OF HGE OR THE HGE AGENT. THIS SPECIES IS VERY SIMILAR, OR LIKELY IDENTICAL, TO E. PHAGOCYTOPHILA AND E. EQUI.

(MORULAE IN CYTOPLASM OF NEUTROPHIL) CDC ONLINE RESOURCE WIKIPEDIA

Ehrlichia sennetsu (Sennetsu fever)

Clinical syndromes

The disease resembles infectious mononucleosis with fever, lethargy, cervical lymphadenopathy, increased number of peripheral blood mononuclear cells and atypical lymphocytes.

Laboratory diagnosis

Serological tests are available

Treatment

Tetracycline has been used but the disease is benign with no fatalities or serious complications.

COXIELLA

Coxiella burnetii (Q fever) [Q for query]

Replication

C. burnetii infects macrophages and survives in the phagolysozomes where they multiply. The bacteria are released by lysis of the cells and phagolysozomes.

Pathogenesis and immunity

Infection occurs by inhalation of airborne particles. The organism multiplies in the lungs and is disseminated to other organs. Pneumonia and granulomatous hepatitis are observed in patients with severe infections. In chronic disease, immune complexes may play a role in pathogenesis. Phase variation occurs in the LPS of *C. burnetii*. In acute disease, antibodies are produced against the phase II antigen. In chronically infected patients, antibodies to both phase I and phase II antigens are observed. Cellular immunity is important in recovery from the disease.

Epidemiology

C. burnetii is extremely stable in the environment and has "spore-like" characteristics. *C. burnetii* infects a wide range of animals including goats sheep cattle and cats. The organism is found in the placenta and in the feces of infected livestock. The organisms persist in contaminated soil which is a focus for infection. *C. burnetii* is also passed in milk and people who consume non-pasteurized milk can become infected. Arthropods are not common vectors for transmission of *C. burnetii* in humans but ticks are a primary vector for transmission among veterinary species.
C. burnetii is found worldwide and infection is common in ranchers, veterinarians, abattoir workers and others associated with cattle and livestock.

Clinical syndromes

The disease can be mild and asymptomatic and is often undiagnosed. The disease can be acute or chronic. In acute Q fever, the patient presents with headache fever, chills and myalgia. Respiratory symptoms are usually mild ("atypical pneumonia"). Hepatomegaly and splenomegaly may be observed. Granulomas can be seen in histological sections of most patients with Q fever. Chronic Q fever typically presents as endocarditis generally on a damaged heart valve. Prognosis of chronic Q fever is not good.

Laboratory diagnosis

Serology is most commonly used to diagnose Q fever. Antibodies to phase II antigen is used to diagnose acute disease and antibodies to both phase I and phase II antigens to diagnose chronic disease.

Treatment, prevention and control

Tetracycline in used to treat acute Q fever. Chronic disease is treated by a combination of antibiotics. A vaccine is available in some countries, such as Australia, but it has not been approved for use in the United States.

BARTONELLA

Microbiology

The Bartonella are small, Gram-negative aerobic bacilli that are difficult to grow in culture. They are found in many different animals but they cause no apparent disease in animals. Insects are thought to be vectors in human disease. Some species are able to infect erythrocytes while others simply attach to host cells. The table below summarizes the organisms and the diseases they cause.

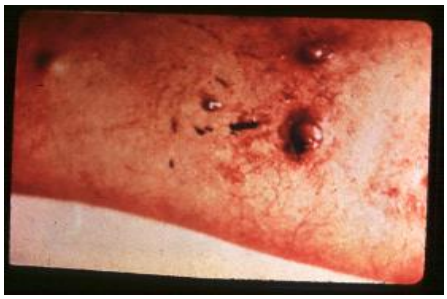


FIGURE 2.4.14: BARTONELLOSIS

COURTESY CDC ONLINE RESOURCES WIKIPEDIA

Bartonellosis- a bacterial onfection caused by Bartonella bacilliformis and found in South America. The infection can occur as either an acute febrile anaemia (Oroya fever) or as a chronic cutaneous eruption (Verruga peruana).

Organism	Disease
B. quintana (formerly Rochalimaea quintana	Trench fever (shin-bone fever, 5 day fever), bacillary angiomatosis, bacillary peliosis, endocarditis
B. henselae	Cat-scratch disease, bacillary angiomatosis, bacillary peliosis endocarditis
B. bacilliformis	Oroya fever (bartonellosis, Carrion's disease)
B. elizabethae	Endocarditis (rare)

Bartonella quintana **(Trench feve**r)

Epidemiology

Trench fever is a disease associated with war. The vector is the human body louse and there is no known reservoir except man. Transovarian transmission in the louse does not occur. The organism is found in the faeces of the louse and is inoculated into humans by scratching. The cycle is human to louse to human.

Clinical syndromes

Infection with B. quintana can result in asymptomatic to severe debilitating illness. Symptoms include fever, chills, headache and severe pain in the tibia. A maculopapular rash may or may not appear on the trunk. The symptoms may reappear at five day intervals and thus the disease is also called five day fever. Mortality rates are very low.

Laboratory diagnosis

Serological tests are available but only in reference laboratories. PCR based tests have been developed.

Treatment, prevention and control

Various antibiotics have been used to treat trench fever. Measures to control the body louse are the best form of prevention.

Bartonella henselae **- (Cat-scratch disease)**

Epidemiology

Cat-scratch disease is acquired after exposure to cats (scratches, bites, and possible cat fleas).

Clinical syndromes

The disease in usually benign, characterized by chronic regional lymphadenopathy.

Laboratory diagnosis

Serological tests are available

Treatment

Cat-scratch disease does not appear to respond to antimicrobial therapy.

4.0 CONCLUSION

Chlamydiaceae are small obligate intracellular parasites formerly considered to be viruses. The family Chlamydiaceae consists of two genera. One species of Chlamydia and two of Chlamydophila are important in causing disease in humans. Rickettsia, Ehrlichia, Anaplasma and Coxiella; The Rickettsia, Ehrlichia, Anaplasma and Coxiella are all small obligate intracellular parasites which were once thought to be part of the same family. Now, however, they are considered to be distinct unrelated bacteria. All of these organisms are maintained in animal and arthropod reservoirs and, with the exception of Coxiella, are transmitted by arthropod vectors (e.g., ticks, mites, lice or fleas). Humans are accidentally infected with these organisms.

5.0 SUMMARY

In this unit we have learnt the following:

- Physiology and Structure of the *Chlamydiaceae*.
- Rickettsiae
- Methods for the treatment and control of these infections

you should be able to discuss in details their microbiological features and details by now with relevance to public health.

6.0 TUTOR MARKED ASSIGNMENT

a) Fully discuss the physiology and structure of the *Chlamydiaceae***.**

b) Copy and complete the table below:

Disease	Organism	Vector	Reservoir
Rocky Mountain spotted fever	?	?	?
Ehrlichiosis	*E chaffeensis*	Tick	?
	E ewingii		?
Anaplasmosis			
	A. phagocytophilium	?	Deer
			Small mammals
?	*R. akari*	Mite	Mites, wild rodents
Scrub typhus	?	Mite	Mites wild rodents
Epidemic *typus*	*R. prowazekii*	?	Humans, squirrel fleas, flying squirrels
Murine typhus	*R. typhi*	Flea	?
Q fever	*C burnetii*	None	?

7.0 **REFERENCES AND SUGGESTED READINGS**

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MODULE 3

SECTION C ; UNIT 1 ENTOMOLOGY.

1.0 INTRODUCTION;

Entomology is simply the study of insects and the diseases they proliferate public health wise .Arthropods belong to the phylum Arthropoda. They are animals with jointed legs, segmented bodies and chitinous exoskeletons. There are over one million species in the phylum Arthropoda (insects and their allies). *Arthron* in Greek means jointed and *poda* means feet. Arthropods are one of the most important sources other than man himself, of human pathogens. Scientific study of insects is known as *entomology*. *Entom* in Greek means insect and *logas* means scientific study. *Medical entomology* is the branch of this science that deals with the arthropods, which are involved with the spread of disease in human beings. Medical significance of arthropods is attributed mainly due to their blood sucking habits and their role as vectors of the agents of bacterial, viral or parasitic infection. They can transmit infection in the following ways:

1. **Mechanical transmission,** in which the arthroipod vector merely transports the organisms from one host to another or from the environment to a host and is not a part of the life cycle of the organism.
2. **Biological transmission,** requiring a period of incubation or development in this host. For example, most species of trypanosomes undergo a period of metacyclic multiplication in the arthropod before becoming infective for man.

There are five main classes in the phylum Arthropoda. Insects and arachnids live in close association with man. Other classes include primarily parasities of the animals. Class Insecta includes mosquitoes, flies, fleas, bugs and lice; and class Arachnida includes ticks, mites, spiders and scorpions.

Arthropods of medical interest ingest the pathogenic organisms and convey these to man by following methods:

1. Non-bloodsucking flies may deposit a vomit-drop containing the pathogens on human food or drink (enteric infections of man).

2.0 OBJECTIVES

At the end of this unit you should be able to discuss the following

- Classification of Arthropods of public health relevance
- Types of insects of medical importance
- Life Cycle of Anopheles Mosquito
- Life Cycle of Simulium damnosum (Black Fly)

3.0 CONTENT

3.1 Classification of Arthropods of public health relevance

Class	Order	Common names
1. *Insecta*	*Diptera*	Mosquitoes, sandflies, tsetse flies, blackflies, deerflies, botflies, warble flies, house flies, flesh flies, blowflies and kads
	Hemiptera	Bed bugs and assassin bug
	Coleoptera	Beetles
	Siphonaptera	Fleas
	Anoplura	Sucking lice
	Maliophaga	Biting lice
	Hymenoptera	Wasps, honeybees and ants

	Dictyoptera	Cockroaches
2. *Arachnida*	*Acari*	Hard ticks, soft ticks, chiggers, itch mites and follicle mites
	Araneae	Spider
	Scorpiones	Scorpions
3. *Pentastomida*		Tongue worm
4. *Myriapoda*	*Diplopoda*	Milipedes
	Chilopoda	Centipedes
5. *Crustacea*	*Copepoda*	Water fleas
	Decapoda	Crabs and crayfish

2. Arthropods may obtain the parasitic organisms in a blood meal form an infected person and deposit them in a vomit-drop in the puncture wound (plague) or in faecal pellets near the puncture wound (epidemic and endemic typhus, trench fever and Chagas' disease) made in the skin of an uninfected person.
3. Contamination from infected haemolymph may occur when the arthropod is crushed on the skin (epidemic typhus and epidemic relapsing fever).
4. Some arthropods discharge the pathogens (malaria sporozoites and virus particles) through the hypopharynx in minute droplets of salivary secretion at the time they produce a blood meal. Filarial larvae from the proboscis (labium and mouth area) of the mosquito are deposited on the skin near the site of the puncture. They then enter through puncture wound or penetrate through the skin on their own.

In many arthropod-associated diseases, the etiologic agents were undoubtedly parasites of their invertebrate hosts long before man came into the life cycle. In some cases, the parasite has been so long and so well adjusted to the arthropod that it produces no obvious injury, namely, *Rickettsia rickettsii* and *Borrelia duttoni in ticks* and *R. tsutsugamushi* in trombiculid mites. In the tick and in the mite the respective parasites are even transmitted vertically (congenitally) and require no vertebrate host for at least several generations. On the other hand, *R. prowazekii* in the body louse can cause extensive and often fatal damage to this ectoparasite, suggesting that the vector-pathogen relationship is an imperfect one. Vector-borne parasitic human infections and

biting characteristics of medically important arthropods are given in the table below respectively.

3.2 TYPES OF INSECTS OF MEDICAL IMPORTANCE

Mosquitoes are readily recognised by a long needle-like proboscis. Adult males and females both feed on plant juices, but the female needs blood for the development of her eggs and is also a voracious predator on a wide variety of vertebrate animals throughout the world. Mosquitoes of importance in human medicine are divided into two broad types:

1. **Anopheline mosquitoes**, numerous species of which transmit malaria.
2. **Culicine mosquitoes,** which are the vectors of many arbovirus infections.

Both anopheline and culicine mosquitoes also act as the intermediate hosts of certain filarial worms. Female mosquitoes lay their eggs on water; larvae and pupae are both aquatic. Most anopheline mosquitoes prefer relatively large expanses of water that do not dry up but many culicine mosquitoes, particularly *Aedes* spp. Will breed in small pockets of water, such as tree holes, water butts, etc. Adults have wide flight range and may be found several kilometres from their breeding ground.

SANDFLIES

Sandflies are tiny flies that are well able to penetrate ordinary mosquito net. They have a restricted flight range, so that the diseases they transmit-notably kalaazar and other forms of leishmaniasis, bartonellosis and sandfly fever-tend to be localized in distribution.

FIGURE 3.1.1 SANDFLIES

COURTESY; MONICA CHEESBROUGH TEXT BOOK OF MEDICAL MICROBIOLOGY

Female flies suck blood, usually at night and breed in dark, moist areas, often in or around human dwellings. Species associated with disease transmission in Africa, the Middle East, Asia and the Mediterranean littoral belong to the genus *Phlebotomous*. In Central and South America, *Lutzomyia* spp. act as vectors of leishmaniasis and Oroya fever.

BEETLES

Some beetles act as intermediate host of dwarf tapeworm *Hymenolepis diminuta,* an uncommon human parasite of minor importance.

FIGURE 3.1.2 REDUVIID BUGS

COURTESY; MONICA CHEESBROUGH TEXT BOOK OF MEDICAL MICROBIOLOGY

Reduviid bugs transmit *Trypanosoma cruzi*, causative agent of Chagas' disease, in South America. They are about 2.5 cm in length – much larger than bed bugs –and unlike them, they have wings. They are usually active at night, settling on the face of an unsuspecting sleeper to take a blood meal and to defecate.

FIGURE 3.1.3 FLEAS

COURTESY; MONICA CHEESBROUGH TEXT BOOK OF MEDICAL MICROBIOLOGY

The infective trypanosomes are in the hindgut and the bitten person becomes infected by rubbing the bug's faeces into the irritating bite wound.

FLEAS

Fleas are small blood-sucking parasites. They have laterally flattened bodies and lack wings. Well-developed hind legs enable them jump from host to host. Many fleas feed on man if given the opportunity. However, the species that is adapted for life on man is the human flea. *Pulex irritans.* It is common throughout the world. Female fleas of another species, *Tunga penetrans*, attack man once they have been fertilized. The fleas burrow into the skin or under the toe-nails, of human host and are known as *jiggers*.

FIGURE 3.1.4 XENOPSYLLA CHEOPSIS

COURTESY; MONICA CHEESBROUGH TEXT BOOK OF MEDICAL MICROBIOLOGY

The abdomen of the gravid female becomes grossly distended *with eggs*, causing pain, irritation and sometimes secondary infection. Jigger fleas are common in dry, sandy soil, mainly in Africa and parts of Central and South America. Human fleas are seldom implicated in the transmission of disease but some other species are important disease vectors. Most notorious is the rat flea, *Xenopsylla cheopsis*, which is the most important but the sole vector of plague. Some forms of typhus are also transmitted by *X. cheopis* and other fleas.

LICE

Lice are wingless insects that undergo incomplete metamorphosis during their development. The ones that parasitize man are blood-sucking species with flattened bodies and short legs that are adapted to cling to hair.

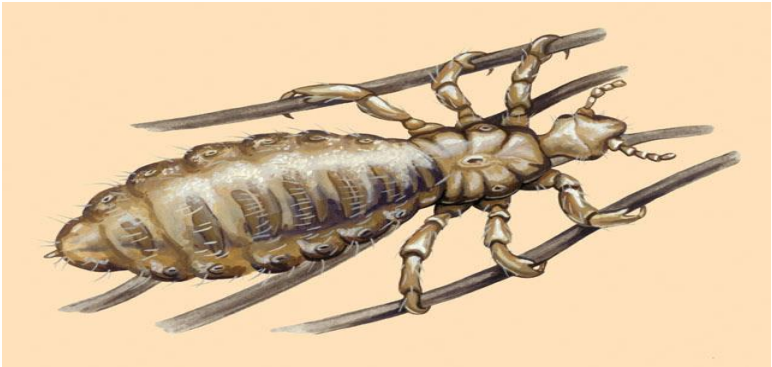

FIGURE 3.1.5; *P. HUMANUS CORPIOUS*

COURTESY; MONICA CHEESBROUGH TEXT BOOK OF MEDICAL MICROBIOLOGY

Body lice and head lice are considered to be variants of the same species, *Pediculus humanus*. Body louse, *P. humanus corpious* is somewhat larger than the head louse, *P. humanus capitis* and there are other minor differences. A third species *Phthirus pubis* is quite distinct morphologically and is known as 'crab' louse. Head lice are usually confined to the hair of the scalp, but body lice live in clothing covering the body rather than on the skin. *Phthirus pubis* is usually found in

Crab lice are known to be involved in disease transmission but body and head lice are classic vectors of epidemic typhus and relapsing fever. Treatment with insecticides such as permethrin, malathion and carbaryl may be effective but resistance occurs.

OTHER BITING FLIES

The tsetse flies, *Glossina* spp., are found in the so called 'fly belts' of sub-Saharan Africa, where they are responsible for transmission of trypanosomiasis in man as well as in cattle and other animals. Usually both male and female feed on blood. Other biting flies responsible for transmission of disease in Africa include species of *Chrysops* (deerflies or mango flies) which act as vectors of *Loa loa* and *Simulium* (blackflies) which act as vectors on onchocerciasis.

MIDGES

Biting midges are tiny flies. The females attack in swarms usually in the evening and may give rise to painful reactions.

FIGURE 3.1.6: MIDGES

Like mosquitoes, they are mostly aquatic. One genus *Culicoides* spp. Transmits filarial worms of *Mansonella* spp.

ARACHNIDS

This group includes spiders, scorpions, ticks and mites. They have four pairs of legs. They do not have wings nor do they have antennae.

TICKS

Ticks are important vectors of human disease. They are of two types: **hard (ixodid) ticks**, which have a chitinous shield on the back and **soft (argasid) ticks**, which lack this feature.

FIGURE 3.1.7; Ticks

COURTESY; MONICA CHEESBROUGH TEXT BOOK OF MEDICAL MICROBIOLOGY

Ticks are obligate blood- feeders. They parasitize a wide variety of animals. Ixodid ticks transmit many *rickettsiae* of the spotted fever group as well as agents of Q fever, Lyme disease, *tularaemia, babesioisis* and some arboviruses.

OTHER ARTHROPODS

Crustaceans (crabs and crayfish) are of interest in human medicine mainly as intermediate hosts of *Paragoniums westermani,* the lung fluke. Copepods (water fleas) are similarly important only as hosts of guinea worm, *Dracunculus medinensis* and the fish tapeworm, *Diphyllobothrium latum.*

3.3 Life Cycle of Anopheles Mosquito

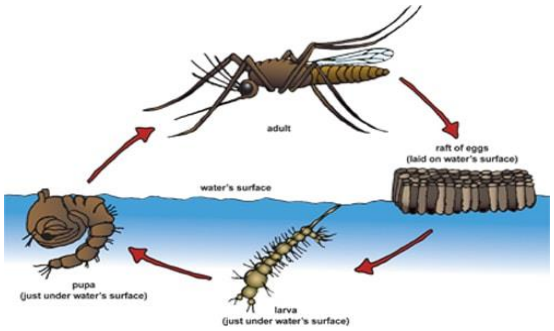

FIGURE 3.1.8: LIFE CYCLE OF ANOPHELES MOSQUITO

COURTESY; MONICA CHEESBROUGH TEXT BOOK OF MEDICAL MICROBIOLOGY

3.4 Life Cycle of *Simulium damnosum* (Black Fly)

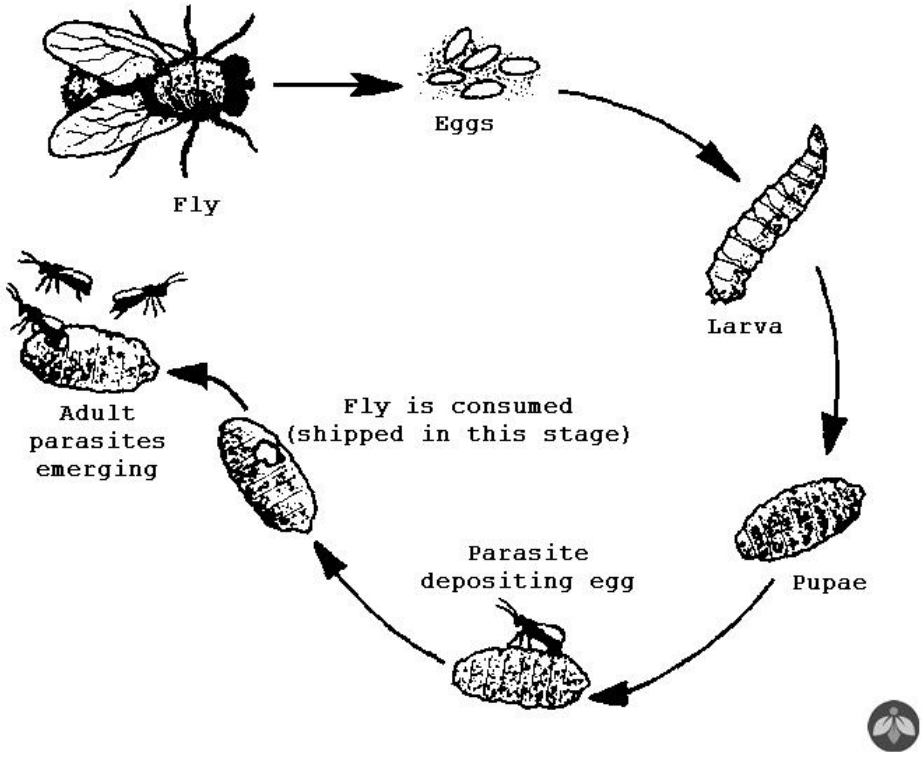

FIGURE 3.1.9 : Life Cycle of *Simulium damnosum* (Black Fly)

COURTESY; MONICA CHEESBROUGH TEXT BOOK OF MEDICAL MICROBIOLOGY

4.0 CONCLUSION

Arthropods belong to the phylum *Arthropoda*. They are animals with jointed legs, segmented bodies and chitinous exoskeletons. There are over one million species in the phylum Arthropoda (insects and their allies). They can transmit infection in the following ways: **Mechanical transmission,** in which the arthroipod vector merely transports the organisms from one host to another or from the environment to a host and is not a part of the life cycle of the organism. **Biological transmission,** requiring a period of incubation or development in this host. For example, most species of trypanosomes undergo a period of metacyclic multiplication in the arthropod before becoming infective for man. There are five main classes in the phylum Arthropoda. Insects and arachnids live in close association with man. Other classes include primarily parasities of the animals. Class Insecta includes mosquitoes, flies, fleas, bugs and lice; and class Arachnida includes ticks, mites, spiders and scorpions.

5.0 SUMMARY

In this unit, we have learnt the following

- Classification of Arthropods of public health relevance
- Types of insects of medical importance
- Life Cycle of Anopheles Mosquito
- Life Cycle of Simulium damnosum (Black Fly)

6.0 TUTOR MARKED ASSIGNMENT

I Describe the life cycles of the following insects

a) Anopheles mosquito
b) *Simulium damnosum* (black fly)

II Attempt the classification of arthropods of medical importance.

III Differentiate between hard tick and soft tick.

IV Write brief notes on the following insects

a. Sandflies
b. Reduviid bugs
c. Fleas
d. Lice

V What diseases are associated with the insects listed above clinically?

7.0 REFERENCES AND SUGGESTED FURTHER READINGS

Murray PR Baron EJ Jorgensen JH *et al*, editors, *Manual of clinical microbiology* **ed.** 8 Washington DC, 2003 ASM Press.

Monica Cheesbrough *Manual of clinical microbiology* **and** *Parasitology* volume 1 ed. .5 2007.

MODULE 4

PARASITOLOGY

UNIT; 1 MALARIA PARASITES

1.0 INTRODUCTION

2.0 OBJECTIVES

3.0 CONTENTS

3.1 GENERAL CONCEPTS OF MALARIA PARASITES

3.2 STRUCTURE AND LIFE CYCLE

3.3 PATHOGENESIS

3.4 HOST DEFENSES

3.5 EPIDEMIOLOGY

3.6 DIAGNOSIS

3.7 CONTROL

1.0 INTRODUCTION

Malaria has been a major disease of humankind for thousands of years. It is referred to in numerous biblical passages and in the writings of Hippocrates. Although drugs are available for treatment, malaria is still considered by many to be the most important infectious disease of humans: there are approximately 200 million to 500 million new cases each year in the world and the disease is direct cause of 1 million to 2.5 million deaths per year.

Malaria is caused by protozoa of the genus *Plasmodium.* Four species cause disease in humans: *P falciparum, P vivax, P ovale* and *P malariae.* Other species of plasmodia infect reptiles, birds and other mammals. Malaria is spread to humans by bite of female mosquitoes of the genus *Anopheles.*

2.0 OBJECTIVES

At the end of this unit you should be able to conveniently describe the following features of malaria parasites;

- General concepts of malaria parasites
- Structure and life cycle
- Pathogenesis
- Host defenses
- Epidemiology
- Diagnosis
- Control

3.1 General Concepts of Malaria Parasites

Clinical Manifestations

Initially patients have fever chills, sweating, headache, weakness and other symptoms mimicking a “viral syndrome”. Later, severe disease may develop with an abnormal level of consciousness, severe anemia, renal failure and multisystem failure.

Classification

Plasmodia are protozoa. Only the species *Plasmodium falciparum, P vivax, P malariae and P ovale* are usually infectious for humans. Of these, P falciparum is the most dangerous.

3.2 Structure and Life Cycle

In nature, uninucleate sporozoites in the salivary glands of infected mosquitoes are injected into a human host when the mosquito feeds. The sporozoites

rapidly invade liver parenchymal cells, where they mature into liver-stage schizonts, which burst to release 2,000 to 40,000 uninucleate merozoites. In *P vivax* and *P ovale* infections, maturation of the schizont may be delayed for 1-2 years.

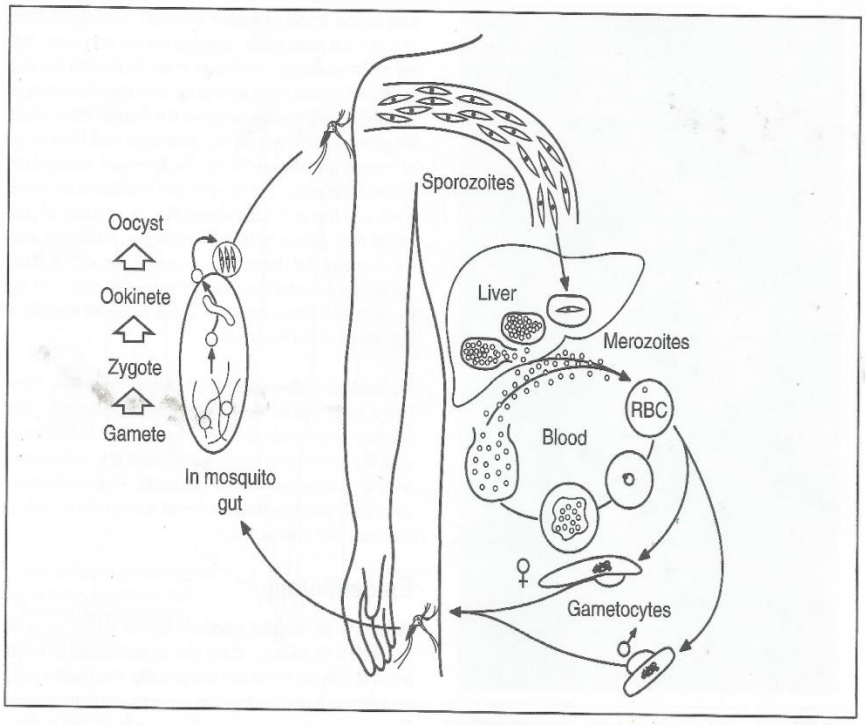

FIGURE 4.1.1: LIFE CYCLE OF THE MALARIA PARASITES IN MAN

Courtesy: Miller Ltd LH, Howard RJ Carter RJ Carter Research toward malaria vaccines.Science234:1350,1986.

Each merozoite can infect a red blood cell. Within the red cell, the merozoite matures either into a uninucleate gametocyte- the sexual stage, infectious for *Anopheles* mosquitoes- or, over 48 to 72 hours, into an erythrocytic- stage schizont containing 10 to 36 merozoites. Rupture of the schizont releases these merozoites, which infect other red cells. If a vector mosquito ingests gametocytes, the gametocytes develop in the mosquito gut to gametes, which undergo fertilization and mature in 2 to 3 weeks to sporozoites.

3.3 Pathogenesis

The fever and chills of malaria are associated with the rupture of erythrocytic-stage schizont. In severe falciparum malaria, parasitized red cells may obstruct capiilaries and postcapillary venules, leading to local hypoxia and the release of toxic cellular products. Obstruction of the microcirculation in the brain (cerebral malaria) and in other vital organs is thought to responsible for severe

complications. Cytokines (e.g. tumor necrosis factor) are also felt involved but at present their role in unclear.

3.4 Host Defenses

Both innate and acquired immunity occur. Innate immunity consists of various traits of erythrocytes that discourage infection. The sickle-cell trait protects against the development of severe *P falciparum* malaria and the absence of Duffy antigen prevents infection by *P vivax.* Recurrent infections lead to the development of humoral and cellular immune responses against all *Plasmodium* stages. Acquired immunity does not prevent reinfection but does reduce the severity of disease.

3.5 EPIDEMIOLOGY

Malaria is distributed worldwide throughout the tropics and subtropics. Malaria is transmitted primarily by the bite of infected anopheline mosquitoes. It can also be transmitted by inoculation of infected blood and congenitally. Anopheline feed at night and their breeding sites are primarily in rural areas. The greatest risk of malaria is therefore from dusk to dawn in rural areas. In much malaria-endemic area, there is little or no risk in urban areas. However, urban transmission is common in some parts of the world, especially Africa.

Anopheles mosquitoes capable of transmitting malaria are found in a number of areas of the United States, Local transmission may therefore occur when these mosquitoes feed upon malaria-endemic areas. Local transmission has recently occurred in Southern California, New Jersey, New York City and Houston, Texas. Malaria may also occur when infected mosquitoes are transported into non-endemic areas, such as by airplanes or ships.

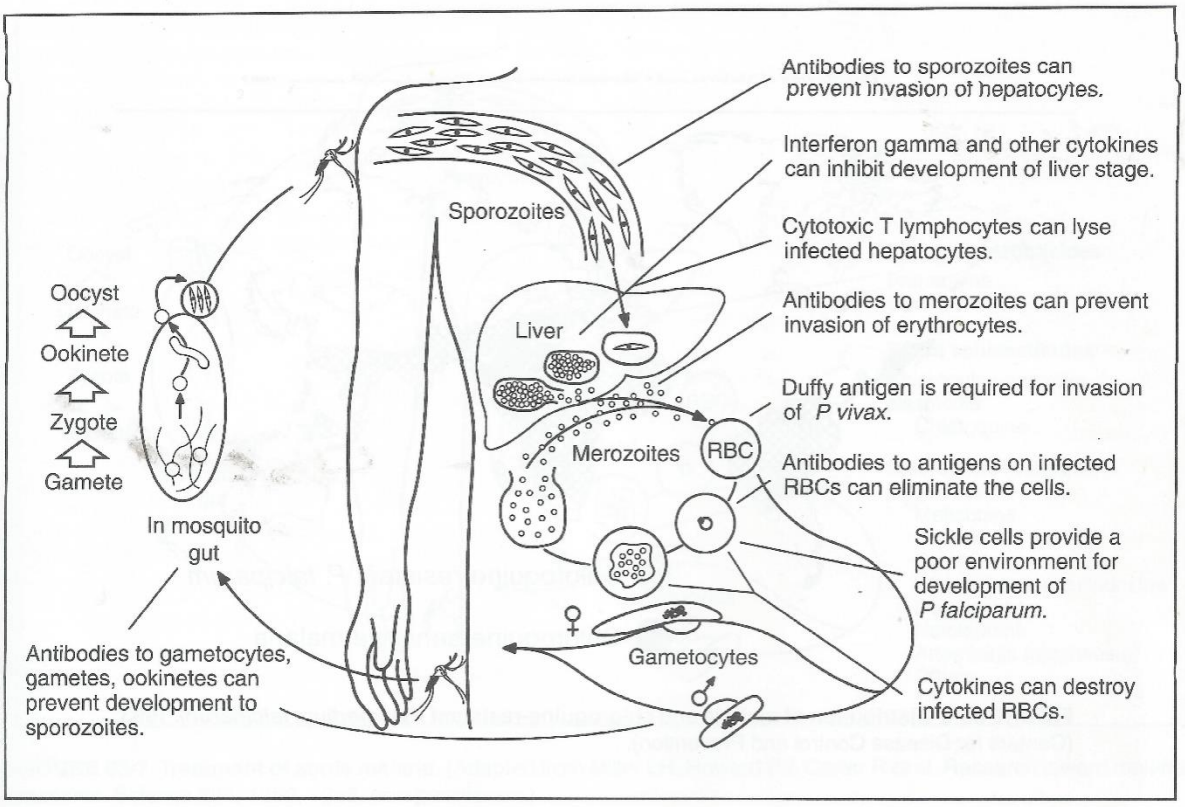

FIGURE 4.1.2: HOST DEFENCE AGAINST MALARIA

Courtesy: Courtesy: Miller Ltd LH, Howard RJ Carter RJ Carter Research toward malaria vaccines.Science234:1350,1986.

In the late 1950s and early 1960s, it was thought that malaria could be eradicated through the widespread use of insecticides such as DDT and by treatment of cases with chloroquine. Eradication is no longer thought possible, however, because of the development of drug resistance by both the mosquito and the parasite and because of deteriorating social and economic conditions in many malaria-endemic countries. These changes have resulted in a dramatic increase in the incidence of malaria in many parts of the world and an increase in malaria-related mortality in some of these areas.

3.6 DIAGNOSIS

Diagnosis depends primarily on the identification of plasmodia in thick and thin blood smears. The diagnosis of malaria requires a high index of suspicion; malaria should be considered in any individual who has a fever and has visited

an endemic area for malaria, received a blood transfusion or used intravenous drugs. Although 95% of individuals affected by malaria develop their primary illness within 6 weeks of exposure, some may have primary attacks up to a year after exposure and relapses of malaria can occur up to 2-3 years after exposure. Therefore, individuals having a febrile illness and a history of exposure in the last 2-3 years should be evaluated for malarial.

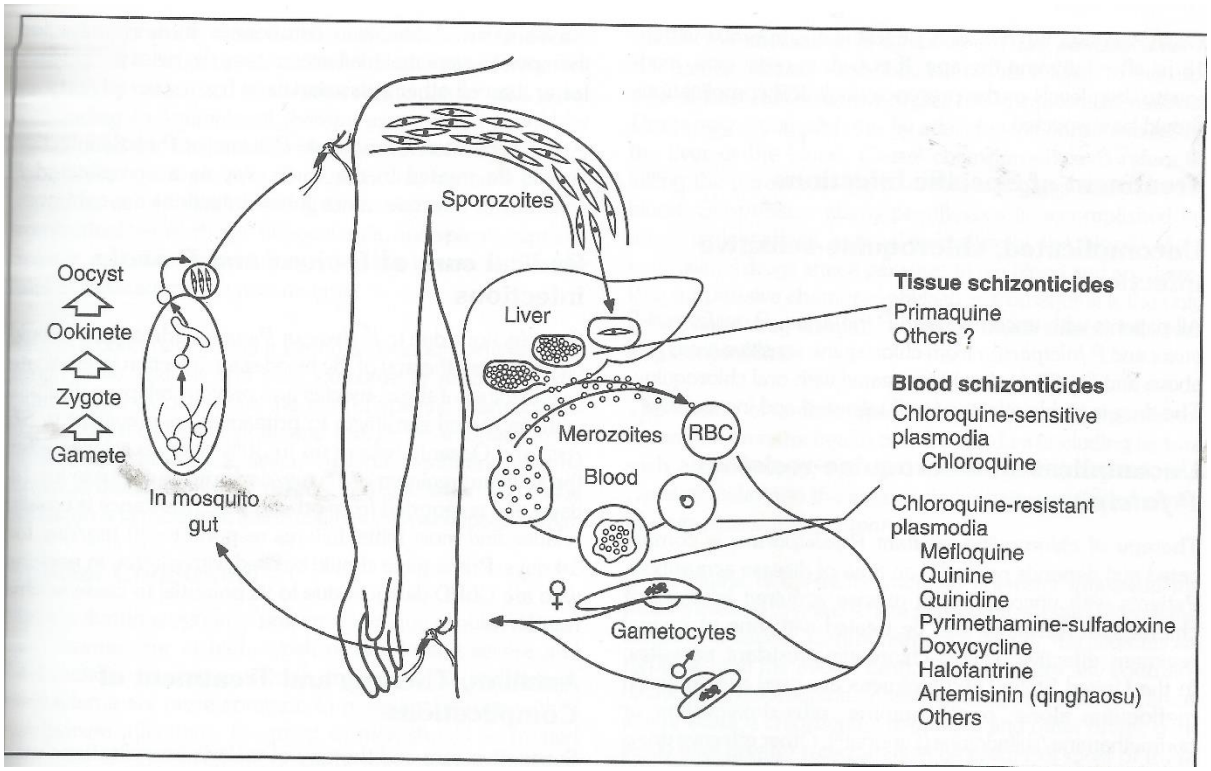

FIGURE 4.1.3: TREATMENT OF ACUTE MALARIA

COURTESY: MILLER LTD LH, HOWARD RJ CARTER RJ CARTER RESEARCH TOWARD MALARIA VACCINES.SCIENCE234:1350,1986.

Definitive diagnosis of malaria generally requires direct observation of malaria parasites in Giemsa-stained thick and thin blood smears. Thick blood smears are more difficult to interpret than thin blood smears but they are much more sensitive as more blood is examined. Thin blood smears, in which parasites are seen within erythrocytes are used to determine the species of the infecting parasite. The presence of diagnostic forms can vary markedly with the stage of the life cycle, especially early in disease. In falciparum malaria, most organisms are not present in the peripheral blood because they are sequestered in the microvascular tissue of internal organs. If malaria is suspected, blood smears should be examined every 6 to 12 hours for at least 2 days. New diagnostic methods include a rapid antigen-capture dipstick test and a technique for

detecting parasites with a fluorescent stain. Both of these tests are fast, easy to perform and are highly sensitive and specific.

Other diagnostic methods include assays to detect malaria antibodies and antigens and polymerase chain reaction/ DNA and RNA probe techniques. These techniques are used primarily in epidemiologic studies and immunization trials and rarely in the diagnosis of individual patients.

3.7 CONTROL

Malaria therapy is complicated by the fact that parasites may be present in the blood and the liver and that different drugs are required to eradicate each. Drugs which kill malaria parasites in the blood are called blood-stage schizonticides and those that kill them in the liver are called tissue schizonticides. A clinical cure refers to the elimination of parasites from the blood, which will relieve the signs and symptoms of disease. A radical cure is the eradication of all parasites from the body, both blood and liver. In cases of *P falciparum* and *P malariae,* which do not have latent liver forms (hypnozoites), an effective dose of a blood schizonticide to which the parasite is sensitive should lead to radical cure. In cases of *P vivax* and *Povale* malaria, which do form hypnozoites, radical cure requires therapy with both a blood schizonticide and a tissue schizonticide.

Recurrence of malaria infections after treatment is due either to recrudescence or to relapse. Recrudescence occurs when the blood schizonticide does not eliminate all parasites from the blood stream, either because the does was inadequate or because the parasite is resistant to the drug. Relapse occurs in *P vivax* and *P ovale* infections after the delayed development of liver-stage parasites that have not been treated adequately with a tissue schizonticide.

Resistance of malaria parasites to antimalarials may be complete or relative; relative resistance can be overcome by raising the dose of the antimalarial.

If ever in doubt as to infecting species or presence of resistance, clinicians should assume the infection to be chloroquine-resistant *P falciparum.* Such therapy will cover all malaria species, although side effects may be more common.

Control in Populations

Control of malaria is difficult and requires the sustained effort of many individuals from many disciplines. It is much more easily accomplished in some areas of the world than others. Control can be extremely difficult in areas where the *Anopheles* vector is numerous, long-lived and feeds only on humans.

Transmission of malaria requires the presence of three factors:

1. Malaria-infected humans carrying gametocytes that are infective to mosquitoes
2. *Anopheles* mosquitoes that live long enough for the malaria parasites to develop within them to the infective sporozoites stage and
3. Infected mosquitoes that bite non infected humans.

TREATMENT

The widespread resistance of *P falciparum* to chloroquine complicates treatment of falciparum malaria. Alternative drugs such as mefloquine, pyrimethamine/sulfadoxine (Fansidar^{R}), quinine, quinidine, halofantrine and artemisinin derivatives (qinghaosu) are used. Chloroquine remains highly effective against *P malariae* and *P ovale* malaria and against *P vivax* everywhere except Papua New Guinea and parts of Indonesia, where significant resistance has developed. Disease caused by *P vivax* and *P ovale* requires primaquine to eradicate latent liver forms of the parasite.

Uncomplicated, chloroquine-sensitive infections

All patients with uncomplicated *P malariae, P ovale* and *P vivax* and *P falciparum* from chloroquine sensitive areas should be treated with oral chloroquine. The drug is highly effective, well tolerated and inexpensive.

Uncomplicated, chloroquine-resistant *P falciparum*

Therapy of chloroquine-resistant *P falciparum* is complicated and depends primarily on area of disease acquired in areas of chloroquine resistance can be treated with one of several regimens effective against chloroquine-resistant parasites. In the United States, two regimens are used primarily:

1. Mefloquine alone
2. Quinine, plus doxycycline or pyrimethamine/sulfadoxine (Fansidar^{R}). Other effective drugs include halofantrine, artemisinin (qinghaosu) derivatives and clindamycin.

Halofantrine and artemisinin are used widely overseas.

Uncomplicated, chloroquine-resistant *P vivax*

Chloroquine-resistant *P vivax* is highly prevalent on the island of New Guinea (Papua New Guinea and Irian Jaya, Indonesia) and may be present elsewhere. Recent studies in Indonesia have shown halofantrine and chloroquine plus primaquine to be highly effective against these resistant strains. Although not specifically tested, the above regimens for chloroquine-resistant *P falciparum* should also be effective.

Complicated infections

Severe or complicated malaria is a medical emergency. It is caused almost exclusively by *P. falciparum.* Patients with complicated malaria should be treated with intravenous antimalarials and in an intensive care unit whenever possible. The drugs of choice are intravenous quinidine and quinine. Patients on these regimens must be observed closely for signs of hypotension or myocardial conduction abnormalities. Therapeutic plasma levels are 5 to 15 µg/ml for quinine and 5 to 10 µg/ml for quinidine. Oral quinine plus doxycycline or Fansidar^{R} is substituted as soon as there is clinical improvement. If acquired in an area of chloroquine-sensitive parasites, parenteral chloroquine may also be given. Artemisinin compounds show promise for therapy of severe malaria because they decrease parasitemia faster than all other antimalarials.

Any complicated *P malariae*, *P vivax* or *P ovale* infection should be treated in the same way as a complicated *P falciparum* infection, since mixed infections are common.

Special Conditions

Malaria during pregnancy presents a unique problem. Pregnant women are at higher risk of developing severe and fatal malaria. Hyperparasitemia, hypoglycemia and pulmonary edema are more common in pregnant women with *P falciparum* infections. Pregnant women should be treated promptly with appropriate doses of antimalarials. Quinine does not appear to induce labor as was once thought. Pregnant women with chloroquine-sensitive *P vivax* infections should be treated with chloroquine to eliminate the erythrocytic-stage infection and then placed on weekly chloroquine to prevent relapse, as the safety of primaquine in pregnancy is not known.

PREVENTION:

Malaria may be prevented by chemoprophylaxis and personal protective measures against the mosquito vector and by community-wide measures against to control the vector. Exposure to night-feeding anopheles mosquitoes is reduced by using protective clothing, insect repellents, insecticides, insecticide-impregnated bed nets, etc. mosquitoes may be reduced by destroying breeding places and by application of insecticides, vaccines are being developed.

4.0; CONCLUSION

Malaria is a pandemic all over the world. Humankind has suffered the scourge for thousands of years. It is referred to in numerous biblical passages and in the writings of Hippocrates. Drugs are available for treatment but it is rather challenging to develop vaccine for malaria being an intracellular parasite. There are approximately 200 million to 500 million new cases each year in the world and the disease is direct cause of 1 million to 2.5 million deaths per year. Malaria is caused by protozoa of the genus *Plasmodium.* Four species cause disease in humans: *P falciparum, P vivax, P ovale* and *P malariae.*

5.0 SUMMARY

In this unit we have learnt about the following important features of malaria parasites;

- General concepts of malaria parasites
- Structure and life cycle
- Pathogenesis
- Host defenses
- Epidemiology
- Diagnosis
- Control

6.0 TUTOR MARKED ASSIGNMENT

a) Fully describe the life cycle of the malaria parasite
b) Clearly differentiate between the various species of *plasmodium*
c) How would you diagnose malaria parasite in the laboratory?
d) What are the effective ways of controlling malaria disease?

7.0 SUGGESTED FURTHER READING AND RECOMMENDATIONS

University of Sothern Carolina: Online Microbiology Teaching Aids and Resources, 2013

Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby.

Andrew P. Waters, Chris J. Janse: Malaria Parasites: Genomes and Molecular Biology

Irwin W. Sherman: Malaria: Parasite Biology, Pathogenesis, and Protection

Courtesy: Miller Ltd LH, Howard RJ Carter RJ Carter Research toward malaria vaccines.Science234:1350,1986.New Edition 2016.

MODULE 5

SECTION E: **VIROLOGY**

UNIT 1: **HEPATITIS B**

1.0: INTRODUCTION

Hepatitis B is a liver infection caused by the hepatitis B virus (HBV). HBV is one of five types of viral hepatitis. The others are hepatitis A, C, D, and E. Each is a different type of virus, and types B and C are most likely to become chronic.

The Centers for Disease Control and Prevention (CDC) state that around 3,000 people in the United States die each year from complications caused by

hepatitis B. It's suspected that 1.4 million people in America have chronic hepatitis B.

HBV infection can be acute or chronic.

Acute hepatitis B causes symptoms to appear quickly in adults. Infants infected at birth rarely develop only acute hepatitis B. Nearly all hepatitis B infections in infants go on to become chronic.

Chronic hepatitis B develops slowly. Symptoms may not be noticeable unless complications develop.

2.0: OBJECTIVE

At the end of this unit you should be able to know:

- What hepatitis B is all about
- Structure of hepatitis B virus
- The mode of transmission of Hepatitis B
- The risk factors involved in Hepatitis B disease transmission
- The symptoms of Hepatitis B disease
- Mode of diagnosis of Hepatitis B disease
- Treatment of Hepatitis B disease
- Complications of Hepatitis B disease in public health practice

3.2 STRUCTURE

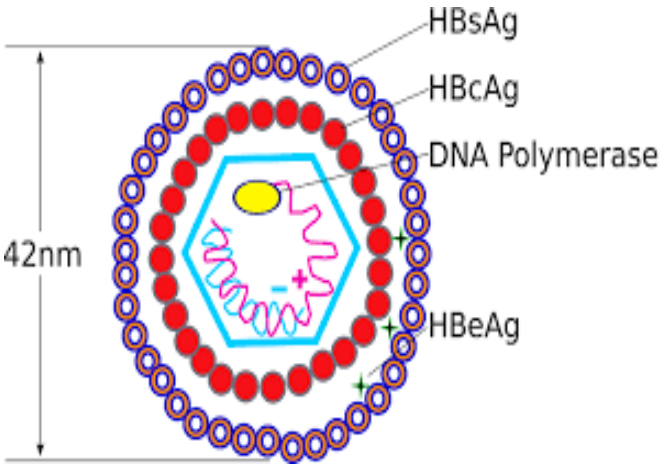

FIGURE 5.1.1 HEPATITIS VIRUS

Courtesy: CDC Online Resource Wikipedia

3.3 TRANSMISSION

Is hepatitis B contagious?

Hepatitis B is highly contagious. It spreads through contact with infected blood and certain other bodily fluids. Although the virus can be found in saliva, it's not spread through sharing utensils or kissing. It also doesn't spread through sneezing, coughing, or breastfeeding. Symptoms of hepatitis B may not appear for 3 months after exposure and can last for 2–12 weeks. However, you are still contagious, even without symptoms. The virus can live outside the body for up to seven days.

Possible methods of transmission include:

- direct contact with infected blood
- transfer from mother to baby during birth
- being pricked with a contaminated needle
- intimate contact with a person with HBV
- oral, vaginal, and anal sex
- using a razor or any other personal item with remnants of infected fluid

3.4 RISK FACTORS

Who is at risk for hepatitis B?

Certain groups are at particularly high risk of HBV infection. These include:

- healthcare workers
- men who have sex with other men
- people who use IV drugs
- people with multiple sex partners
- people with chronic liver disease
- people with kidney disease
- people over the age of 60 with diabetes
- those traveling to countries with a high incidence of HBV infection

3.5 SYMPTOMS

What are the symptoms of hepatitis B?

Symptoms of acute hepatitis B may not be apparent for months. However, common symptoms include:

- fatigue
- dark urine
- joint and muscle pain
- loss of appetite
- fever
- abdominal discomfort
- weakness
- yellowing of the whites of the eyes (sclera) and skin (jaundice)

Any symptoms of hepatitis B need urgent evaluation. Symptoms of acute hepatitis B are worse in people over the age of 60. Let your doctor know immediately if you have been exposed to hepatitis B. You may be able to prevent infection.

3.6 DIAGNOSIS

How is hepatitis B diagnosed?

Doctors can usually diagnose hepatitis B with blood tests. Screening for hepatitis B may be recommended for individuals who:

- have come in contact with someone with hepatitis B
- have travelled to a country where hepatitis B is common
- have been in jail
- use IV drugs
- receive kidney dialysis
- are pregnant
- are men who have sex with men
- have HIV

To screen for hepatitis B, your doctor will perform a series of blood tests.

Hepatitis B surface antigen test

A hepatitis B surface antigen test shows if you're contagious. A positive result means you have hepatitis B and can spread the virus. A negative result means you don't currently have hepatitis B. This test doesn't distinguish between chronic and acute infection. This test is used together with other hepatitis B tests to determine the state of a hepatitis B infection.

Hepatitis B core antigen test

The hepatitis B core antigen test shows whether you're currently infected with HBV. Positive results usually mean you have acute or chronic hepatitis B. It may also mean you're recovering from acute hepatitis B.

Hepatitis B surface antibody test

A hepatitis B surface antibody test is used to check for immunity to HBV. A positive test means you are immune to hepatitis B. There are two possible reasons for a positive test. You may have been vaccinated, or you may have recovered from an acute HBV infection and are no longer contagious.

Liver function tests

Liver function tests are important in individuals with hepatitis B or any liver disease. Liver function tests check your blood for the amount of enzymes made by your liver. High levels of liver enzymes indicate a damaged or inflamed liver. These results can also help determine which part of your liver may be functioning abnormally.

If these tests are positive, you might require testing for hepatitis B, C, or other liver infections. Hepatitis B and C viruses are a major cause of liver damage throughout the world. You will likely also require an ultrasound of the liver or other imaging tests.

3.7 TREATMENTS

What are the treatments for hepatitis B?

Hepatitis B vaccination and immune globulin

Talk to your doctor immediately if you think you have been exposed to hepatitis B within the last 24 hours. If you have not been vaccinated, it may be possible to prevent infection by receiving the hepatitis B vaccine and an injection of HBV immune globulin. This is a solution of antibodies that work against HBV.

Treatment options for hepatitis B

Acute hepatitis B usually doesn't require treatment. Most people will overcome an acute infection on their own. However, rest and hydration will help you recover. Antiviral medications are used to treat chronic hepatitis B. These help you fight the virus. They may also reduce the risk of future liver complications.

You may need a liver transplant if hepatitis B has severely damaged your liver. A liver transplant means a surgeon will remove your liver and replace it with a donor liver. Most donor livers come from deceased donors.

3.8 COMPLICATIONS

What are the potential complications of hepatitis B?

Complications of having chronic hepatitis B include:

- hepatitis D infection
- liver scarring (cirrhosis)
- liver failure

- liver cancer
- death

Hepatitis D infection can only occur in people with hepatitis B. Hepatitis D is uncommon in the United States but can also lead to chronic liver disease.

PREVENTION

How can I prevent hepatitis B?

The hepatitis B vaccine is the best way to prevent infection. Vaccination is highly recommended. It takes three vaccines to complete the series. The following groups should receive the hepatitis B vaccine:

- all infants, at the time of birth
- any children and adolescents who weren't vaccinated at birth
- adults being treated for a sexually transmitted infection
- people living in institutional settings
- people whose work brings them into contact with blood
- HIV-positive individuals
- men who have sex with men
- people with multiple sexual partners
- injection drug users
- family members of those with hepatitis B
- individuals with chronic diseases
- people traveling to areas with high rates of hepatitis B

In other words, just about everyone should receive the hepatitis B vaccine. It's a relatively inexpensive and very safe vaccine.

There are also other ways to reduce your risk of HBV infection. You should always ask sexual partners to get tested for hepatitis B. Use a condom or dental dam when having anal, vaginal, or oral sex. Avoid drug use. If you're traveling internationally, check to see if your destination has a high incidence of hepatitis B and make sure you are fully vaccinated prior to travel.

4.0: CONCLUSION

Hepatitis B is a liver infection caused by the hepatitis B virus (HBV). HBV is one of five types of viral hepatitis. The others are hepatitis A, C, D, and E. Around 3,000 people in the United States die each year from complications caused by hepatitis B. It's suspected that 1.4 million people in America have chronic hepatitis B. HBV infection can be acute or chronic.

5.0: SUMMARY

In this unit we have learnt the following:

General knowledge on hepatitis b disease.

Structure of hepatitis b virus.

The mode of transmission of Hepatitis B.

The risk factors involved in Hepatitis B disease transmission.

The symptoms of Hepatitis B disease.

Mode of diagnosis of Hepatitis B disease

Treatment of Hepatitis B disease

Complications of Hepatitis B disease in public health practice

6.0 Tutor Marked Assignment

a) What is Hepatitis B disease?

b) How is this disease transmitted?

c) Write briefly on the following:

i) laboratory diagnosis of Hepatitis B

ii) Symptoms of Hepatitis B disease

iii) Structure of Hepatitis B Virus

iv)Risk factors in Hepatitis B disease transmission

v)Treatment of Hepatitis b disease

7.0 REFERENCES AND SUGGESTED FURTHER READING

University of Sothern Carolina: Online Microbiology Teaching Aids and Resources, 2013

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R. Vasanthakumari: Textbook of Microbiology

V. Krishna: Textbook of Pathology, 2004.

UNIT 2: HUMAN IMMUNODEFICIENCY VIRUS

1.0 INTRODUCTION

2.0 OBJECTIVES

3.0 CONTENTS

3.1 WHAT ARE AIDS AND HIV?

3.2 CAUSES AND RISK FACTORS

3.3 MISCONCEPTIONS OF THE ILLNESS

3.4 SIGNS AND SYMPTOMS

3.5 DIAGNOSIS

3.6 TREATMENT

3.7 PREVENTION

3.8 COMPLICATIONS

3.9 NEXT STEPS AND RED FLAG

4.0 CONCLUSION

5.0 SUMMARY

6.0 TUTOR MARKED ASSIGNMENT

7.0 REFERENCES AND SUGGESTED FURTHER READING

University of Sothern Carolina: Online Microbiology Teaching Aids and Resources, 2013

Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby.

R. Vasanthakumari: Textbook of Microbiology

V. Krishna: Textbook of Pathology, 2004.

1.0 INTRODUCTION

The full form of AIDS is Acquired Immunodeficiency Syndrome, and HIV stands for Human Immunodeficiency Virus.

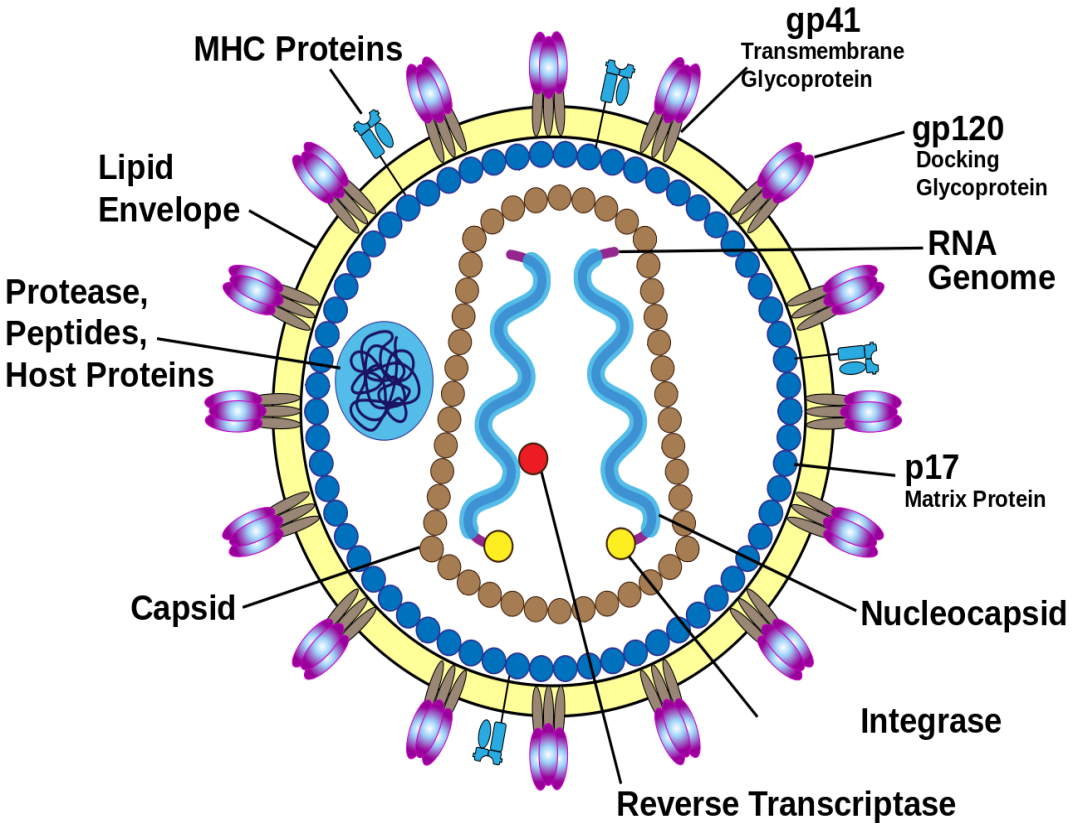

FIGURE 5.2.2 HIV AND AIDS VIRUS

Courtesy: CDC Online Resource Wikipedia

HIV is a virus and AIDS is a medical condition. AIDS appears in advanced stages of HIV infection, whereby there is a complete failure of the immune system in the body. Both the virus and the syndrome are often referred to together as HIV/AIDS.

In simple terms, it means that when the HIV attacks the body, the immune system gets compromised in a major way. The body is unable to fight numerous infections which a person with a healthy immune system can usually do. Therefore “opportunistic” infections set in, which the body is unable to fight and ultimately succumbs.

2.0 OBJECTIVES

At the end of this unit you should be able to know the following facts about HIV/AIDS:

- **Major causes and risk factors in HIV/AIDS**
- **Some misconceptions of the illness**
- **The signs and symptoms**
- **Diagnosis of HIV**
- **Treatment of HIV disease**
- **Prevention of HIV infection**
- **Complications in HIV infection**
- **Steps and red flag in HIV infection**

3.0 CONTENTS

3.1 Causes and risk factors

Causes

HIV which is found in the body fluids (semen, vaginal fluids, blood, and breast milk) of an infected person can enter the body of a healthy individual in the following ways:

- **Unprotected sex with a person who is HIV+ve:** Vaginal, oral, and anal sex or sharing sex toys, which causes contact with the infected sexual secretions.
- **From mother to baby:** This can happen during pregnancy, childbirth and through breastfeeding.
- **Blood transfusion**: Blood transfusion with infected blood which has not been properly screened and sharing infected syringes by drug users.

The thing to understand is that body fluids tend to have a very 'heavy virus load' and therefore, even a single episode of contact is enough for getting infected which is very rare. For example:

- While eating food, which has been pre-chewed by an HIV-infected person.

- When an HIV-infected person bites, there is a risk of transmission if the skin is broken.
- Contact between broken skin, wounds, or mucous membranes and HIV-infected blood or blood-contaminated body fluids.

3.2 MISCONCEPTIONS OF THE ILLNESS

There are a lot of misconceptions about HIV/AIDS. The disease cannot spread by:

- Casual touch like shaking hands, hugging, casual kissing, touching, etc.
- Sneezing
- Sharing towels
- Air or water
- Insects including mosquitoes or ticks
- Saliva, tears or sweat
- Toilet seats

3.3 SIGNS AND SYMPTOMS

Many people infected with HIV have no symptoms for several years and may, in fact, be even unaware that they are infected. Others may have flu-like symptoms usually two to six weeks after catching the virus and the symptoms can last up to four weeks.

Some of the symptoms of early HIV infection can be:

- Fever
- Body ache
- Sore throat
- Chills
- Sweating particularly at night

- Enlarged glands
- Red rashes
- Weakness
- Weight loss

A Symptomatic HIV infection: Sometimes these initial symptoms may disappear altogether for up to 10 years, and the person may look absolutely healthy and fine. But all the while the virus will silently damage the immune system and will suddenly flare up after so many years.

Late-stage HIV infection: At this point the HIV has destroyed the immune system and left it vulnerable to all infections and illnesses. This stage is termed as AIDS.

Signs and symptoms of late-stage HIV infection: This stage may include chronic and persistent diarrhoea, dry cough, blurred vision, shortness of breath, fever lasting for weeks, extreme weakness, night sweats, weight loss, swollen glands etc. This is also when the illnesses can set in, for instance, pneumonia, cancer, tuberculosis, etc.

3.4 DIAGNOSIS

If a person suspects that he/she may have come in contact with the virus, then it is crucial to get tested as soon as possible. Early diagnosis and management of the disease are of prime importance in ensuring a longer and better quality of life.

HIV testing is done through a virology test on blood samples. If the blood test is ‘positive’ then retesting is done several times over a period of time to confirm the diagnosis.

Retesting is necessary because sometimes it takes three weeks to three months for the virus to show up in the blood samples. So if a patient’s most ‘at risk moment’ of infection was within the last three months, then he/she should go for the test immediately

3.5 TREATMENT

Currently, there is no vaccine or cure for HIV/AIDS and the only aim of treatment is to control the spread of the virus.

- **HAART (Highly Active Antiretroviral Therapy):** It is a combination of drugs. The treatment is usually lifelong and in this way the person can hope to live for about 10-15 years.
- **Emergency HIV pills:** If the person feels that there has been exposure to the virus in the last 72 hours then post-exposure prophylaxis (PEP) medication (Which lasts up to 4 weeks) is to be taken immediately and it may help to stop the infection.

3.6 PREVENTION

There is a saying that prevention is better than cure. This proverb can be applied to AIDS with a slight variation- prevention is better as there is no cure.

Sexual Contact

- Sex education programmes in schools
- Easily accessible voluntary counselling and testing centres
- Promotion of safer sex practices (delaying sexual debut, condom use, and fewer sexual partners)
- Effective Antiretroviral Therapy treatment of HIV-infected individuals
- Pre-exposure prophylaxis for high-risk groups
- Male circumcision

Parenteral

- **Blood Product Transmission**: Donor questionnaire, routine screening of donated blood
- **Injection drug use**: Education, needle/syringe exchange, avoidance of 'shooting galleries', methadone maintenance programmes

Perinatal

- Measures to reduce vertical transmission Occupational
- Education/training: Universal precautions, needle stick injury avoidance
- Learn about birthing options (e.g., vaginal or caesarean).

- Breastfeeding: New mothers with HIV/AIDS may choose to use formula as an option to breastfeeding.

3.7 COMPLICATIONS

- Weakening immune system
- Vulnerability to certain cancers and infections
- Central nervous system complications
- Tumours

3.8 NEXT STEPS

Increased awareness will lead to changes in attitudes and behaviour pattern. The more people are educated about the ways that a person can get infected, the more precautions that he/she will be able to take to protect oneself. Education will also help in debunking the myths associated with the disease.

3.9 RED FLAGS

Red flags of HIV/AIDs include:

- Unprotected sex
- More than one sexual partner
- History of sexually transmitted diseases
- Exposure to infected needles or body piercing with unclean needles
- Blood transfusion from untested source

4.0 CONCLUSION

AIDS is Acquired Immunodeficiency Syndrome, and HIV stands for Human Immunodeficiency Virus. HIV is a virus and AIDS is a medical condition. AIDS appears in advanced stages of HIV infection, whereby there is a complete failure of the immune system in the body. The body is unable to fight

numerous infections which a person with a healthy immune system can usually do.

5.0: SUMMARY

In this unit we have learnt the following:

- **Major causes and risk factors in HIV/AIDS**
- **Some misconceptions of the illness**
- **The signs and symptoms**
- **Diagnosis of HIV**
- **Treatment of HIV disease**
- **Prevention of HIV infection**
- **Complications in HIV infection**
- **Steps and red flag in HIV infection**

6.0 : TUTORED MARKED ASSIGNMENT

a) Exhaustively discuss HIV/AIDS infection.

b) Enumerate "*red flag*" in HIV disease

7.0 REFERENCES AND SUGGESTED FURTHER READINGS

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UNIT 3: COMMON COLD

1.0 INTRODUCTION TO COMMON COLD

Infection of the nose and throat accompanied by a runny nose, sneezing, cough, pain or slight fever is known as a common cold.

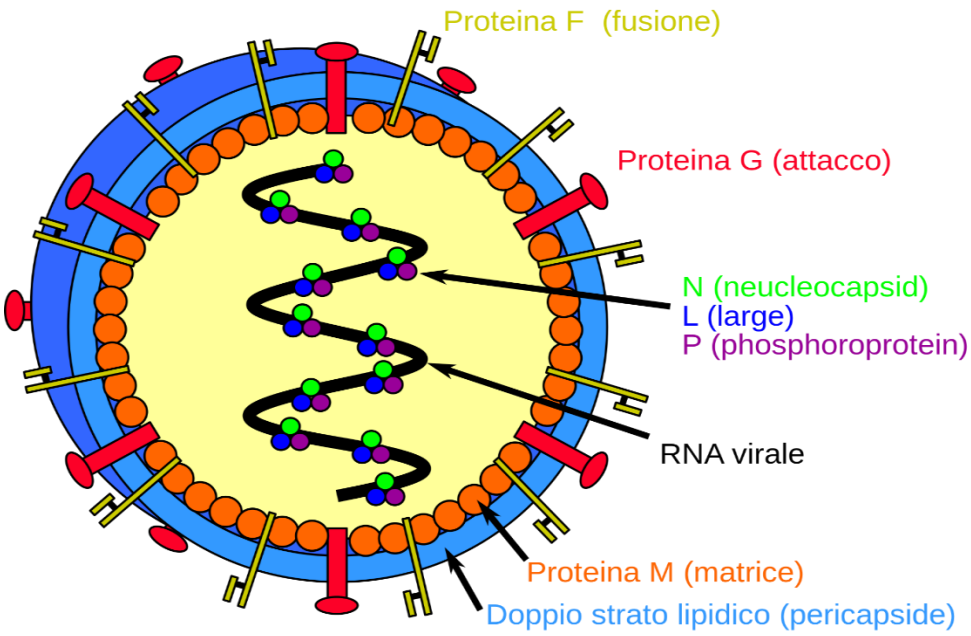

FIGURE 5.3.1: COMMON COLD VIRUS

COURTESY: CDC ONLINE RESOURCE WILKIPEDIA

2.0 OBJECTIVE

At the end of this unit you should be able to describe the following about the virus causing common cold:

- The structure of common cold virus
- Its mode of transmission
- Causes and risk factors
- Signs and symptoms
- Diagnosis
- Treatment
- Prevention

- Complications
- Next steps and red flag

3.0 CONTENTS

3.1 TRANSMISSION

How does one catch a cold?

A cold is passed on easily from an infected individual through:

- Body contact like a handshake or a hug.
- Sharing everyday objects: toys, drinking glasses, computer keyboards, etc.
- Sneezing or coughing without covering the mouth.

The virus first attacks the lining of the nose, throat and spreads the infection in the body. One can be prone to cold if one is tired due to emotional reasons and has prior allergies.

3.2 CAUSES AND RISK FACTORS

Minuscule organisms known as viruses are responsible for colds. Rhinovirus is the most common cause of common cold. Usually, kids below the age of 6 and individuals with general low immunity are prone to frequent episodes of the common cold.

Seasonal changes and smokers have also been found to be more susceptible. General exposure to an infected individual also puts a person at risk, irrespective of the above factors.

3.3 SIGNS AND SYMPTOMS

The commons signs and symptoms of common cold include:

- Soreness of the throat
- Runny or stuffy nose

- Cough
- Congestion
- Sneezing
- Fatigue
- Body aches
- Fever
- Nasal discharges with varying colour and consistency with the progression of the cold.

3.4 DIAGNOSIS

A common cold is usually diagnosed by the signs and symptoms, which are very distinct and universal. If your physician suspects a more widespread lung infection or any other underlying condition, he or she may ask for a chest X-ray or other tests.

3.5 TREATMENT

Over-the-counter (OTC) medicine for a runny nose, sore throat and pain are helpful in relieving these symptoms. Kids below the age of 4 should not be given over-the-counter medicines. The key reason being cold medicines are not been tested on kids but rather on adults. In serious cases on a physician's recommendation antivirals, antihistamines, decongestants based on the nature of the disease are prescribed.

Simple home remedies

- Drink lot of water to remain hydrated. This keeps the throat moistened and helps in decongestion.
- Use steam, taking care not to burn oneself. Breath slowly through the nose over a pot of steaming water with care.
- Take rest and stay warm indoors.
- Hot soups, herbal teas with ginger and cinnamon are soothing recipes for the throat.
- Hygiene: Wash your hands. The children must be taught to keep their hands clean.

- Use fresh tissues to sneeze and cough that will contain the germs from spreading in open and discard them.

3.6 PREVENTION

- Avoid sharing drinking glasses, towels, handkerchiefs, etc. among family members, to prevent the spread of the virus. Labels can be used to mark glasses for ease.
- Ensure the children go to a day-care centre/school that ensure sick children stay at home.
- Avoid contact with those suffering from a cold.
- Eat foods rich in antioxidants and vitamin C.

Why there is no vaccine or a potent medicine yet?

More than 200 viruses cause common cold with varying degree of symptoms and signs, which no single pill or medicine can tackle.

3.7 COMPLICATIONS

If the cold is left untreated, severe complications can result:

- Acute infection of the ear- Most commonly seen in children when the infection spreads behind the eardrum causing severe earache, nasal discharges and sometimes fever. Young children cry in pain and can't point it themselves.
- Sinusitis- Simple common cold can progress into infection and inflammation of the sinuses.
- Wheezing- Asthmatic people are at a greater risk of wheezing owing to cold.
- Other infections- Common colds could lead to other secondary infections, bronchiolitis, pneumonia requiring immediate medical attention.

3.8 NEXT STEPS

If you or your child has a runny or blocked nose, headache, mild fever, fatigue and body ache, contact your doctor.

3.9 RED FLAGS

If you or your child has:

- Fever over 38.5^{o}C (101.3^{o}F)
- Persistent ear pain
- Wheezing.
- Sick look and disorientation

seek immediate medical attention.

4.0 CONCLUSIONS

Infection of the nose and throat accompanied by a runny nose, sneezing, cough, pain or slight fever is known as a common cold. It is caused by an RNA virus called Rhino virus

5.0: SUMMARY

In this unit we have learnt the following about Rhino virus that causes the common cold:

- Structure of common cold virus
- Transmission of the common cold virus
- Causes and risk factors
- Signs and symptoms
- Diagnosis
- Treatment
- Prevention
- Complications
- Next steps and red flag in common cold infection

6.0 TUTORED MARKED ASSIGNMENT

a) Describe the structure of the common cold virus

b) Write brief notes on the following as far as common cold virus is concerned

i) Method available in the diagnosis of common cold virus

ii) Causes and risk factors in common cold virus infection.

iii) Prevention of common cold virus infection.

c) The name of common cold virus is Rhino virus **TRUE or FALSE?**

7.0 REFERENCES AND SUGGESTED FURTHER READINGS

University of Sothern Carolina: Online Microbiology Teaching Aids and Resources, 2013

Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby.

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UNIT 4: DENGUE FEVER

1.0 INTRODUCTION: DENGUE FEVER

WHAT IS DENGUE?

Dengue is a self-limited, mosquito-transmitted viral disease caused by dengue virus. Dengue is reported more often in the tropical parts of the world. Dengue fever also known as break bone fever can occur in infants, children and adults. The symptoms of dengue fever become evident after 3 to 14 days after a bite from an infected mosquito-*Aedes aegypti.*

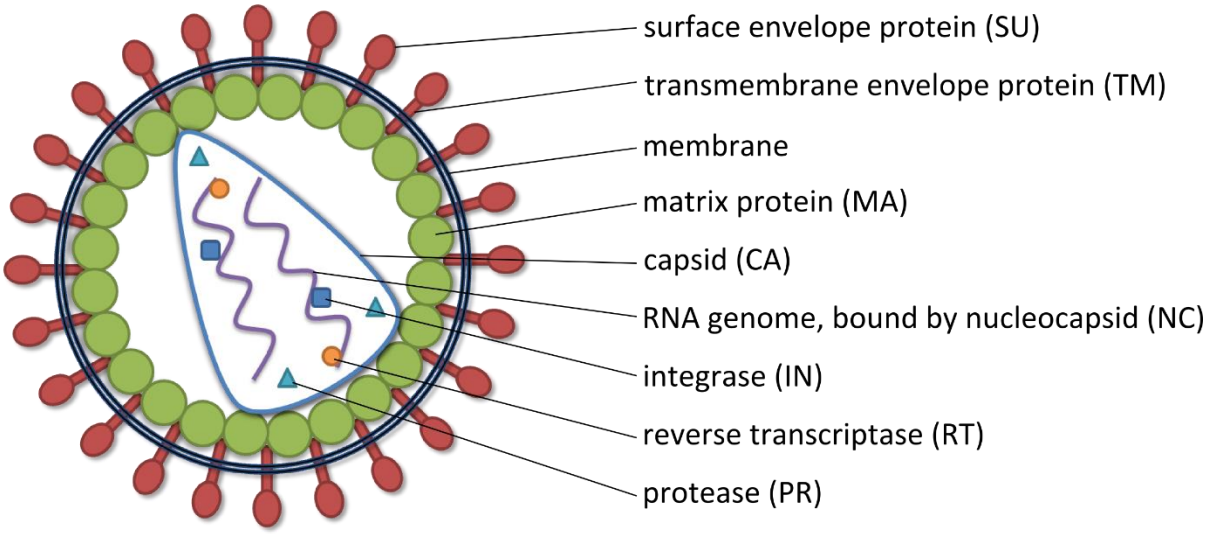

FIGURE 5.4.1 DENGUE VIRUS

Courtesy: Dr. Irwin Fox Emeritus Professor University of South Carolina USA.

The mild form of dengue fever is marked by high fever, joint pain, muscle pain and rash. Severe bleeding, abrupt fall in blood pressure, abdominal pain and even death can result from severe dengue fever (dengue haemorrhagic fever). Presently, there are no specific medicines or vaccines for dengue treatment.

Individuals with dengue fever benefit from a lot of fluid intake, rest and by use of non-aspirin medication for reducing the temperature. People with a severe form of dengue require hospitalisation. Early diagnosis and treatment of dengue fever can prevent the condition from taking a lethal form.

Around 50 to 100 million people are affected by dengue annually in over 100 countries, risking almost 50% of the world's population to dengue infection.

2.0 OBJECTIVES

At the end of this unit you should be able to know the following about Dengue virus disease:

- **What is dengue fever?**
- **Causes and risk factors in dengue virus infection**

- **Signs and symptoms**
- **Diagnosis**
- **Treatment**
- **Prevention**
- **Complications**
- **Next steps and red flag**

3.1 CAUSES AND RISK FACTORS

Causes

Aedes aegypti mosquitoes is a daytime mosquito, spreads the dengue virus. These mosquitoes may harbour any of the four dengue viruses (DENV1, DENV2, DENV3 and DENV4).

Dengue fever does not spread from one individual to another. When a female mosquito bites a non- infected person, the virus enters the skin through the mosquito's saliva. The virus enters the white blood cells (WBC) and multiplies in the WBC. This multiplication is responsible for the symptoms of Dengue virus. Once the viral load increases inside the WBCs, other organs get affected like liver and bone marrow which leads to dysfunctioning of bone marrow resulting in decreased platelets.

After individual recovers from dengue fever, one will be immune to the particular virus but not to the other 3 viruses. Individuals are at higher risk of developing dengue haemorrhagic fever if they have been infected multiple times with dengue earlier.

RISK FACTORS

- Sex- Women are more predisposed to developing dengue virus infection
- Diabetes and Bronchial Asthma- individuals with diabetes and bronchial asthma are more prone to develop dengue virus infection
- Genes- Individuals with defect in TNF (Tumour necrosis factor), G-6PD (Glucose -6 phosphate deficiency) are more likely to develop dengue virus infection

3.2 SIGNS AND SYMPTOMS

Symptoms of dengue fever usually arise within 3 to 14 days after a bite from an infected mosquito.

Signs and symptoms of dengue fever include:

- Fever- Saddleback fever. The temperature rises to 104ºF and associated with pain and muscle aches
- Muscle, joint and bone pain
- Headaches- due to pain behind the eyes
- Flushed, red skin
- Fatigue
- Rash (petechiae- small red dots on skin) which itches

Sometimes, the symptoms can worsen and be fatal in nature resulting in dengue haemorrhagic fever:

- The platelets (cells responsible for clots) decrease in the blood
- Minor bleeding from mouth and nose (from gastrointestinal tract)
- Abdominal pain
- Vomiting and nausea
- Issues of heart, lung and liver
- Severe dehydration

Signs

Drop in platelets, bleeding from mucosa, gastrointestinal tract, blood in vomitus (hematemesis) or blood in stools (melena), presence of petechiae on the legs, arms and other parts of the body which are itchy.

3.3 DIAGNOSIS

Often the diagnosis of dengue might be difficult as the symptoms and signs are also similar to those seen in malaria, typhoid, etc. Virus isolation and molecular diagnostic techniques are used for diagnosis of dengue:

The tests may include:

- Blood test to detect lowered levels of white blood cells, platelets and measure the haematocrits (platelet count, prothrombin time, partial thromboplastin time and thrombin time, Liver function tests)

- Tourniquet test – This test is used to detect the presence of petechiae. Blood pressure cuff is tied to the arm and inflated and kept between systolic and diastolic pressure for five minutes. If > 20 petechiae/ square inch develops, then the test is considered as positive
- Antibody tests
- Molecular tests
- Ultrasonography

Now rapid diagnostic tests to detect all the 4 serotypes of dengue virus are available.

3.4 TREATMENT

There are no specific medicines for the treatment of dengue fever. Physicians my advice rest, fluid intake in plenty to prevent dehydration from high fever and vomiting. Antibiotics are of no use as dengue fever is caused by a virus. Pain relievers like ibuprofen, aspirin and others which can lead to bleeding complications are avoided.

In case of severe dengue fever, hospitalisation requiring blood transfusion and intravenous fluids and electrolyte replacement may be needed.

3.5 PREVENTION

There are no vaccines to prevent dengue. Best way to prevent dengue is by avoiding mosquito bites. Use of mosquito repellents, mosquito nets, screens and wearing long-sleeved clothing, socks and boots when outdoors might prevent the mosquitoes from biting.

A. aegypti mosquitoes can breed in flower vases, water tanks, tyres, and their eggs can survive in dry state without water for a year. Measures must be taken to destroy the mosquito eggs and larvae by preventing water stagnation in pots, discarded tyres, etc. in the neighbourhood.

3.6 COMPLICATIONS

Severe dengue fever could be fatal and even cause death. Heart, liver and lungs may be damaged.

3.7 NEXT STEPS

Visit your doctor if:

- You have recently been to a region where dengue fever was prevalent and if you suspect dengue fever
- You have a severe backache and muscle ache with fever over 104°F
- You are noticing rashes which itch on your skin

3.8 RED FLAGS

Seek immediate medical attention if you have:

- Abdominal pain
- Bleeding from mucosa
- Blood in vomitus or blood in stools
- Petechiae (small red dots) on the skin which are itching
- Raised haematocrits and drop in platelet count
- Diminishing fever with increased distress

4.0 CONCLUSION

The mild form of dengue fever is marked by high fever, joint pain, muscle pain and rash. Severe bleeding, abrupt fall in blood pressure, abdominal pain and even death can result from severe dengue fever (dengue haemorrhagic fever). Presently, there are no specific medicines or vaccines for dengue treatment. Around 50 to 100 million people are affected by dengue annually in over 100 countries, risking almost 50% of the world's population to dengue infection.

5.0 SUMMARY

In this unit we have learnt the following about dengue haemoharrgic fever virus:

- **What dengue haemoharrgic fever virus truly is**
- **Causes and risk factors in dengue virus infection**

- **Signs and symptoms**
- **Diagnosis**
- **Treatment**
- **Prevention**
- **Complications**
- **Next steps and red flag**

6.0 TUTORED MARKED ASSIGNMENT

a) Fully describe Dengue haemorrhagic fever virus

b) How would you diagnose this in the laboratory?

7.0 REFERENCES AND SUGGESTED FURTHER READING

University of Sothern Carolina: Online Microbiology Teaching Aids and Resources, 2013

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UNIT 5: EBOLA VIRUS DISEASE

1.0 INTRODUCTION

2.0 OBJECTIVES

3.0 CONTENTS

3.1 THE FACTS

1.0 INTRODUCTION : EBOLA VIRUS DISEASE

Ebola Virus Disease

(Ebola, Ebola Haemorrhagic Fever)

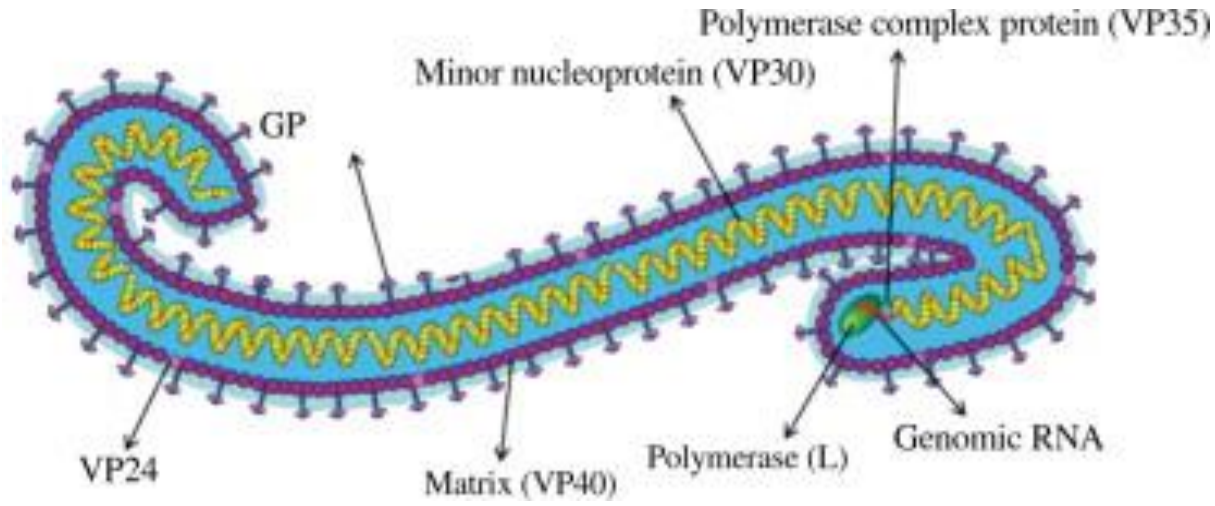

FIGURE 5.5.1: EBOLA VIRUS

Courtesy : **Tara C. Smith: Kent State University. Ebola resurgences in West Africa *www.kent.edu. Retrieved* 2018-07-26**

THE FACTS

The Ebola virus disease (EVD), previously referred to as Ebola haemorrhagic fever, is a severe and often fatal infection. It is spread through contact with infected blood or bodily fluids.

Ebola virus disease was first identified in 1976 in Sudan and the Democratic Republic of the Congo (formerly Zaire). It is named after a river in the Democratic Republic of the Congo. **Since its discovery, there have been several Ebola outbreaks, primarily limited to remote villages near tropical rainforests in Central and West Africa.** As a result of the remoteness of the locations in which most of the outbreaks have occurred, the number of victims has been limited. The 2014 outbreak of Ebola virus disease has been one of the largest in documented history, in terms of both the number of cases and the geographical spread.

There have been 5 identified species of the Ebola virus genus, with 3 of them having caused previous EVD outbreaks. The 2014 outbreak is caused by the Zaire species, the deadliest strain, with a historic fatality rate of up to 90%.

2.0 OBJECTIVES

At the end of this unit you should be able to know the following about Ebola virus disease:

- **The facts about Ebola virus**

- **Causes of Ebola virus infection**
- **Transmission**
- **Symptoms and complications**
- **Diagnosis**
- **Treatment and prevention**

3.0 CONTENTS

3.1 CAUSES OF EBOLA VIRUS INFECTION

Ebola outbreaks occur when the virus is transmitted first from an infected animal to a human and then between humans. The viral infection is spread from animals to humans through contact with infected wildlife such as fruit bats, chimps, and gorillas. Certain fruit bats are believed to be the natural hosts for the Ebola viruses.

3.2 TRANSMISSION

EVD is transmitted from person to person by direct contact (through broken skin and mucous membrane) via bodily fluids or secretions from infected people, such as:

- blood
- breast milk
- semen (up to 61 days after infection)
- sweat
- stool
- urine
- vomit

Transmission can also occur through contact with objects contaminated with these fluids and the bodies of the deceased with EVD. Since the bodies of the deceased can infect those who handle them, safe burial practises are extremely important in containing outbreaks. The infection can be spread further by cultural burial practises such as ritual washings that bring people into close contact with infected bodies.

3.3 SYMPTOMS AND COMPLICATIONS

The Ebola virus targets the host’s (infected person's) blood and immune system, which can lead to bleeding and a weakened immune system. After an incubation period (time between infection and the appearance of symptoms) of 2 to 21 days, EVD is characterized by a rapid onset of flu-like symptoms such as:

- fever
- headache
- muscle pains
- sore throat
- weakness

From there, many patients go on to develop:

- diarrhoea
- measles-like rash
- reduced liver and kidney function
- vomiting

30% to 50% of cases result in internal and external bleeding 4 to 5 days after the onset of symptoms. Although some people die as a result of shock due to multiple organ failures, most Ebola victims die as a result of severe dehydration from extensive vomiting and diarrhoea.

During outbreaks, those at greatest risk of getting the viral infection are health care workers and the family and friends of the infected who have close contact with the patients.

3.4 DIAGNOSIS

Making the Diagnosis

Due to the fact that most Ebola infection symptoms such as weakness, fever, headache, and muscle pains are not specific to the disease, more common diseases need to be ruled out first, especially during the early stages of the infection when diagnosis is difficult to make. Common diseases with similar symptoms include malaria, typhoid fever, and cholera. The person's medical history is also looked at, with particular interest in whether the person was in

contact with possible infected individuals or animals. People with suspected EVD should be quarantined while waiting for definitive diagnosis by laboratory tests.

There are many laboratory tests that can be used to diagnose Ebola virus disease. It is commonly and quickly done through detection of RNA and antibodies of the Ebola virus in the blood. In simple terms, these tests detect traces of the virus itself or our bodies' defence response against the virus.

3.5 TREATMENT AND PREVENTION

There is currently no cure for Ebola virus disease, nor are there any vaccines available to prevent infection. Treatment is supportive and typically involves rehydration, nutrition, and medications to manage symptoms (pain, fever, vomiting, etc.). The majority of people with EVD die from severe dehydration, so early supportive treatment is critical in improving the chances of survival.

Since there is no cure for the disease, the key in limiting outbreaks is to prevent transmission from animals to humans and between humans. There are several measures that need to be in place, including:

- rapid quarantine of suspected infected animals – these animals should then be buried or burned promptly
- handling all animals and their waste with gloves and other protective clothing
- cooking animal products (meat and blood) thoroughly before eating
- safe burial practises
- wearing protective gear such as gloves and other personal protective equipment (such as face protection and long-sleeved gowns) when dealing with infected patients
- safe injection practises
- regular hand washing
- sanitation and sterilization of the environment and instruments
- identification and isolation of infected individuals from the community
- tracing contacts, including those during the incubation period

*All medications have both common (generic) and brand names. The brand name is what a specific manufacturer calls the product (e.g., Tylenol®). The common name is the medical name for the medication (e.g., acetaminophen). A medication may have many brand names, but only one common name. This article lists medications by their common names. For information on a given medication, check our Drug Information database. For more information on brand names, speak with your doctor or pharmacist.

4.0 CONCLUSION

The Ebola virus disease (EVD), previously referred to as Ebola haemorrhagic fever, is a severe and often fatal infection. It is spread through contact with infected blood or bodily fluids. Ebola virus disease was first identified in 1976 in Sudan and the Democratic Republic of the Congo (formerly Zaire).There have been 5 identified species of the Ebola virus genus, with 3 of them having caused previous EVD outbreaks.

5.0 SUMMARY

In this unit we have learnt about the following as far as EBV disease is concerned:

- **The facts about Ebola virus**
- **Causes of Ebola virus infection**
- **Transmission**
- **Symptoms and complications**
- **Diagnosis**
- **Treatment and prevention**

6.0 TUTORED MARKED ASSIGNMENT

a) Succinctly write on Ebola virus disease and its scourge

7.0 REFERENCES AND SUGGESTED FURTHER READINGS

University of Sothern Carolina: Online Microbiology Teaching Aids and Resources, 2013

Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby.

Tara C. Smith: Kent State University. Ebola resurgences in West Africa *www.kent.edu. Retrieved* 2018-07-26

UNIT 6: LASSA FEVER

1.0 INTRODUCTION

2.0 OBJECTIVES

3.0 CONTENTS

3.1 THE FACTS

3.2 CAUSE AND MANIFESTATIONS

3.3 DIAGNOSIS

3.4 TREATMENT AND PREVENTION

4.0 CONCLUSION

5.0 SUMMARY

6.0 TUTOR MARKED ASSIGNMENT

7.0 REFERENCES

1.0 INTRODUCTION: LASSA FEVER

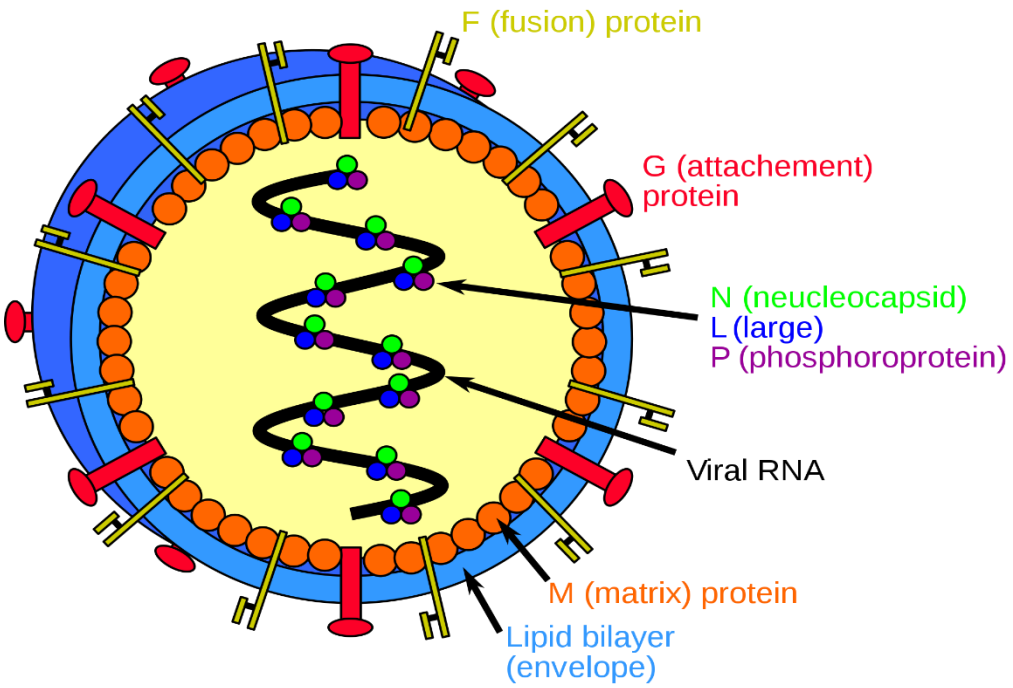

FIGURE 5.6.1: LASSA FEVER VIRUS

COURTESY : DR. ALVIN FOX UNIVERSITY OF SOUTH CAROLINA, U.S.A.

THE FACTS ABOUT LASSA FEVER

Lassa fever - Natural focal viral infection, occurs with kapillyarotoksikoz and multiple organ disorders. Clinical manifestations include fever, Lassa fever, intoxication, diarrhoea, bleeding, ulcerative pharyngitis, renal failure, e.t.c Laboratory diagnosis of Lassa fever is based on virus isolation from the patient's biological materials, as well as the determination of antiviral antibodies using ELISA IHA, RSK. Upon confirmation of Lassa fever is carried rehydration, detoxification, antiviral therapy; haemorrhagic syndrome relief, correction of metabolic disorders and so on.

2.0 OBJECTIVES

At the end of this unit you should be able to know the following facts about Lassa fever;

- **Cause and manifestations of Lassa fever infection**
- **Diagnosis of Lassa fever**
- **How to treat and prevent Lassa fever infection**

3.0 CONTENTS

3.1 CAUSE AND MANIFESTATIONS

Lassa fever, haemorrhagic fever caused by the same name arenaviruses and related to especially dangerous infections with natural foci. The first cases of fever have been reported in the medical staff of the hospital in the town of Lassa (Nigeria) in 1969 in the form of nosocomial infection. Name of the area later gave the name of the selected virus and the disease. Lassa fever is endemic to West and Central African countries: here haemorrhagic fever ill 300-500000 people per year. Imported cases of infection recorded in Europe, the USA, Israel and Japan. The mortality rate from Lassa fever reaches 15-50%; Infection is especially dangerous for pregnant women, because it leads to the death of the mother and the foetus in 80% of cases.
DIC, multiple organ disorders. Tropism of the virus to many organs and tissues causes necrotic changes in the endothelium of blood vessels of the liver, kidneys, myocardium, and so on.

In parallel with fever in 80% of patients develop angina or necrotizing ulcerative pharyngitis.

By the end of the first week of course Lassa fever have pains in the chest, back, abdomen; develop nausea, vomiting, watery diarrhoea, quickly leads to dehydration. At the beginning of the second week of disease associated rash petechial maculopapular, erythematous character. At the same time develop haemorrhagic manifestations: ecchymosis, epistaxis, gastrointestinal, lung, uterine bleeding. You may experience convulsions, meningeal symptoms, impaired consciousness, hearing loss. On examination of patients with Lassa fever detected dryness of the skin and mucous membranes, cervical adenopathy, hepatomegaly, bradycardia, hypotension. In the case of a favourable course of Lassa fever occurs in 2-3 weeks' lytic temperature decrease. For a long time saved post infectious asthenia, infection relapses. By late complications include uveitis, deafness, alopecia, orchitis.
Severe Lassa fever occurs in about 30-50% of patients. In these cases, against the backdrop of feverish intoxication and haemorrhagic syndrome unfolding picture of multiple organ injuries, which includes pneumonia, pleural effusion, myocarditis, pericarditis, hepatitis, ascites, serous meningitis, encephalitis, and so on. D. Fatalities are usually observed on the 2nd week disease from acute renal failure, infectious-toxic and hypovolemic shock, pulmonary oedema and

other causes. Special weight for Lassa fever is characterized in children under 2 years of age and pregnant women: the latter almost always ends disease intrauterine foetal death or maternal mortality. In connection with this infection pregnant Lassa fever is a direct indication for abortion.

Fever, ulcerative pharyngitis, rash and haemorrhagic syndrome; Stay in endemic foci, contact with patients. The hemogram marked leucocytosis and a sharp increase in the erythrocyte sedimentation rate; in urine - proteinuria, leucocyturia, red blood cell, cylindruria. With the help of X-ray light detected infiltrative changes and the presence of pleural effusion. On ECG revealed signs of diffuse myocardial damage.

3.2 DIAGNOSIS

Laboratory confirmation of the diagnosis of Lassa fever is a virus isolation from saliva swabs from the throat, blood, urine, exudates, cerebrospinal fluid; determination of antiviral antibodies using IFA, RNIF, IHA, RSK; detection of viral RNA by PCR. In its current Lassa fever is similar to many infectious diseases (herpes and streptococcal sore throat, diphtheria, measles, SARS, malaria, other haemorrhagic fevers, typhoid, leptospirosis). Differential diagnosis facilitates inspection of patient infectious diseases, pulmonology, gastroenterology, nephrology, neurology and others.

3.3 TREATMENT AND PREVENTION

At suspicion or confirmation of Lassa fever patients are hospitalized in the infectious department. Mandatory strict isolation of patients in special boxes and strict observance of anti-epidemic measures. Causal and vaccine formulations are in development, so the therapeutic measures when Lassa fever are reduced to the pathogenesis and therapy posindromnoy. Spent treatment directed at the correction of metabolic acidosis, the restoration of the BCC, the fight against haemorrhagic syndrome. Ongoing activities detoxification and rehydration infusion, blood transfusion; introduced vascular and respiratory analeptics, antipyretics, vitamins. When complications used antibiotics and steroids. In the case of renal failure, haemodialysis is shown. Early initiation of antiviral therapy with ribavirin can reduce the severity of clinical manifestations of Lassa fever and reduce mortality. In some cases, the observed positive effect of the introduction of convalescent plasma.

The forecast Lassa fever is extremely serious: even with hospitalization and treatment mortality rate reaches 15%. In order to prevent outbreaks of Lassa

fever required the immediate organization of quarantine measures (isolation of patients and contact persons, the burning of dead bodies, conducting the current and final disinfection in the hearth). Patients to be isolated for 30 days from the onset; contact persons - for 17 days (the maximum duration of the incubation period). Medical personnel caring for patients must comply with all the requirements to work with especially dangerous infections (personal protection, anti-epidemic mode). Alopecia prevention of Lassa fever involves the fight against rats, vector control, protection of food and water from contamination rodent excreta.

4.0 CONCLUSION

Natural focal viral infection occurs with multiple organ disorders. Clinical manifestations include fever, Lassa fever, intoxication, diarrhoea, bleeding, ulcerative pharyngitis, renal failure; e.t.c Laboratory diagnosis of Lassa fever is based on virus isolation from the patient's biological materials, as well as the determination of antiviral antibodies using ELISA IHA, RSK. Upon confirmation of Lassa fever is carried rehydration, detoxification, and antiviral therapy.

5.0 SUMMARY

In this unit we have learnt the following about haemoharrgic Lassa fever virus infection:

- Some key facts about Lassa haemoharrgic fever virus infection
- Cause and manifestations of Lassa fever infection
- Diagnosis of lassa fever
- How to treat and prevent lassa fever infection

6.0 TUTORED MARKED ASSIGNMENT

As a public health personnel, who had been invited by some group to give a brief lecture on haemorrhagic Lassa fever virus please write your submissions on this topic.

7.0 REFERENCES AND SUGGESTED FURTHER READINGS.

University of Sothern Carolina: Online Microbiology Teaching Aids and Resources, 2013
Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby.

UNIT 7: CHICKENPOX VIRUS

1.0 INTRODUCTION: THE FACTS ON CHICKEN POX

Chickenpox is a contagious illness characterised by a red, itchy, blistering rash that can cover the entire body.

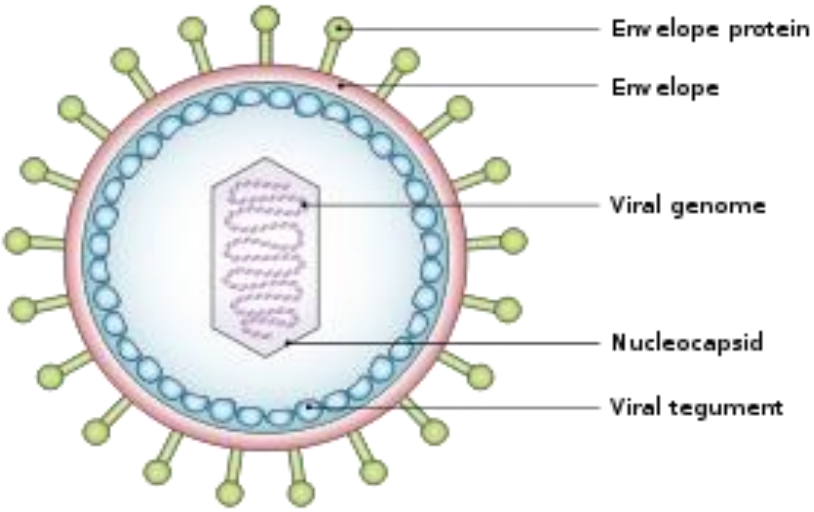

FIGURE 5.7.1 CHICKEN POX VIRUS, "*VARICELLA ZOSTER* VIRUS

COURTESY: CDC ONLINE RESOURCE WIKIPEDIA

It is caused by the varicella-zoster virus. As it is an airborne disease, chickenpox transmission occurs easily through coughing or sneezing by infected individuals who will remain contagious from 48 hours before the appearance of the rash until after the rash has scabbed over and dried out completely.

Symptoms usually come on after an incubation period of 10 to 21 days after the initial infection, and the disease is most common in childhood.

2.0 OBJECTIVES

At the end of this unit you should know the following about Chicken pox virus infection;

- Symptoms of chicken pox virus infection
- Diagnosis of chicken pox
- Treatment of chicken pox virus disease
- Prevention of chicken pox disease

3.0 CONTENTS

3.1 SYMPTOMS

What are its symptoms?

In addition to the tell-tale rash, which can last around two weeks, chickenpox symptoms include:

- A rash in the oral cavity
- Fever (this may last longer in adults)
- Tiredness and malaise
- Headache
- Nausea and aching muscles (particularly in adults)

The chickenpox rash will progress through three stages in the course of the infection:

1. Small red dots, usually beginning on the torso, face and upper limbs before spreading to the rest of the body.
2. Intensely itchy, fluid-filled blisters that rupture and leak.
3. Crusting and scabbing over of the blisters.

3.2 DIAGNOSIS

How is it diagnosed?

A doctor will usually come to a chickenpox diagnosis after examining the rash and taking note of any accompanying symptoms. A blood test or test of the fluid in the blisters can confirm the diagnosis, but this is usually not necessary. It's important to get a diagnosis from a healthcare professional in order to rule out any other more serious conditions, like meningitis.

3.3 TREATMENT

What are your treatment options?

Chickenpox treatment is largely symptomatic as the virus runs its course. Usually, while uncomfortable, the disease is relatively mild and resolves in five to 10 days. Over-the-counter painkillers can help reduce fever and pain, while an antihistamine may help with the itching.

The antiviral drug, acyclovir, can reduce the duration of the symptoms and may be prescribed in high-risk cases, such as in pregnant women or people with compromised immune systems where complications can include bacterial

infection, pneumonia, toxic shock syndrome and encephalitis. Because chickenpox is usually worse in adults, it's also advisable to start a cycle of acyclovir within 24 hours of the rash first developing.

3.4 PREVENTION

Can it be prevented?

It is essential for infected individuals to remain home from school or work until the blisters have scabbed over completely in order to avoid passing the virus to schoolmates or colleagues.

There is a chickenpox vaccine called the varicella vaccine that can help you prevent contracting the virus. It can be safely administered to babies older than nine months of age, adolescents and adults and is recommended for those who have not had the disease.

4.0 CONCLUSION

Chicken pox is caused by the *varicella-zoster virus*. As it is an airborne disease, chickenpox transmission occurs easily through coughing or sneezing by infected individuals who will remain contagious from 48 hours before the appearance of the rash until after the rash has scabbed over and dried out completely. The incubation period of this disease most common in childhood is 10 to 21 days after the initial infection.

5.0 SUMMARY

In this unit we have learnt the following about chicken pox virus disease;

- Symptoms of chicken pox virus infection
- Diagnosis of chicken pox
- Treatment of chicken pox virus disease
- Prevention of chicken pox disease

6.0 TUTORED MARKED ASSIGNMENT

a) With the aid of a well labelled diagram, describe the structure of the chicken pox virus.

b) What are the symptoms of chicken pox virus disease?

c) What is the scientific name of chicken pox virus?

d) Write brief notes on

i) Treatment

ii) Diagnosis

iii) Prevention of chicken pox virus disease.

7.0 REFERENCES AND SUGGESTED FURTHER READINGS

University of Sothern Carolina: Online Microbiology Teaching Aids and Resources, 2013

Bailey and Scott's: *Diagnostic Microbiology*, twelfth edition, St Louis, 2007, Mosby.

SECTION F: MODULE SIX: IMMUNOLOGY

UNIT 1: IMMUNOLOGY

1.0: INTRODUCTION TO IMMUNOLOGY

Immunology is the study of our protection from foreign agents or invading organisms and our responses to them. These invaders include viruses, bacteria, protozoa or even larger parasites. In addition, we develop immune responses against our own proteins (and other molecules) in autoimmunity and against our own aberrant cells in tumour immunity.

Our first line of defence against foreign organisms is barrier tissues such as the skin that stop the entry of organism into our bodies. If, however, these barrier layers are penetrated, the body contains cells that respond rapidly to the presence of the invader. These cells include macrophages and neutrophils that engulf foreign organisms and kill them without the need for antibodies. Immediate challenge also comes from soluble molecules that deprive the invading organism of essential nutrients (such as iron) and from certain

molecules that are found on the surfaces of epithelia, in secretions (such as tears and saliva) and in the blood stream. This form of immunity is the innate or non-specific immune system that is continually ready to respond to invasion.

A second line of defence is the specific or adaptive immune system which may take days to respond to a primary invasion (that is infection by an organism that has not hitherto been seen). In the specific immune system, we see the production of antibodies (soluble proteins that bind to foreign antigens) and cell-mediated responses in which specific cells recognize foreign pathogens and destroy them. In the case of viruses or tumours, this response is also vital to the recognition and destruction of virally-infected or tumorigenic cells. The response to a second round of infection is often more rapid than to the primary infection because of the activation of memory B and T cells. We shall see how cells of the immune system interact with one another by a variety of signal molecules so that a coordinated response may be mounted. These signals may be proteins such as lymphokines which are produced by cells of the lymphoid system, cytokines and chemokines that are produced by other cells in an immune response, and which stimulate cells of the immune system.

2.0 OBJECTIVES:

At the end of this unit you should know the following on immunology

- Nature of antigen-antibody reactions
- Affinity and Avidity
- Specificity and cross reactivity
- Tests for antigen-antibody reactions
- Applications
- Antibody formation

3.0 CONTENTS

3.10 NATURE OF ANTIGEN-ANTIBODY REACTIONS

3.11Lock and Key Concept

The combining site of an antibody is located in the Fab portion of the molecule and is constructed from the hypervariable regions of the heavy and light chains. X-Ray crystallography studies of antigen-antibody interactions show that the antigenic determinant nestles in a cleft formed by the combining site of the antibody as illustrated in Figure 6.1.1. Thus, our concept of antigen-

antibody reactions is one of a key (i.e. the antigen) which fits into a lock (i.e. the antibody).

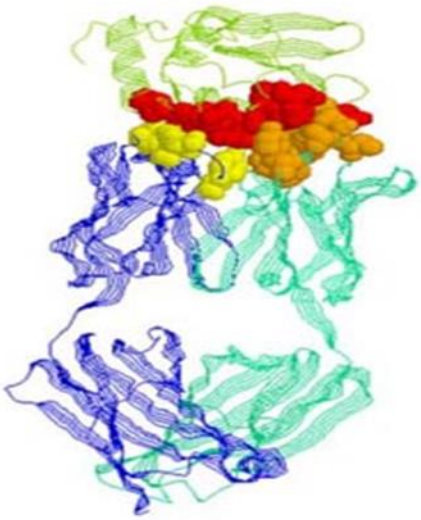

FIGURE 6.1.1: LOCK AND KEY CONCEPT OF ANTIGEN AND ANTIBODY REACTION

Courtesy : Li Y. LiH , Smith –Gill,,S.J Mariuzza R.A., Biochemistry 39, 6296,2000.

3.12 Non-covalent Bonds

The bonds that hold the antigen to the antibody combining site are all non-covalent in nature. These include hydrogen bonds, electrostatic bonds, Van der Waals forces and hydrophobic bonds. Multiple bonding between the antigen and the antibody ensures that the antigen will be bound tightly to the antibody.

3.13 Reversibility

Since antigen-antibody reactions occur via non-covalent bonds, they are by their nature reversible.

3.2 AFFINITY AND AVIDITY

3.21 Affinity

Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody. It is the sum of the attractive and repulsive forces operating between the antigenic determinant

and the combining site of the antibody as illustrated RESURGENCES IN WEST AFRICA *WWW.KENT.EDU. RETRIEVED* 2018-07-26

Affinity is the equilibrium constant that describes the antigen-antibody reaction as illustrated in Figure 6.1.2. Most antibodies have a high affinity for their antigens.

$$Ag + Ab \leftrightarrow Ag\text{-}Ab$$

Applying the Law of Mass Action:

$$K_{eq} = \frac{[Ag\text{-}Ab]}{[Ag] \times [Ab]}$$

FIGURE 6.1.2 AFFINITY AND AVIDITY

3.22 Avidity

Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies. Avidity is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities. This is illustrated in Figure 6.1.3

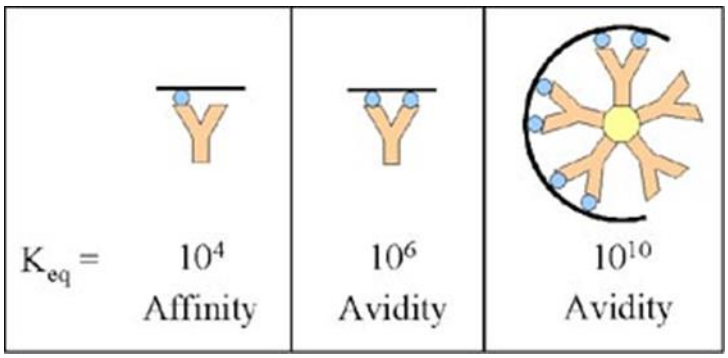

FIGURE 6.1.3 AVIDITY

All figures in this section:

Courtesy : **Gene Mayer, Ph.D** Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

To repeat, affinity refers to the strength of binding between a single antigenic determinant and an individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent antigens and antibodies.

3.3 SPECIFICITY AND CROSS REACTIVITY

3.31 Specificity

Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen. In general, there is a high degree of specificity in antigen-antibody reactions. Antibodies can distinguish differences in:

•The primary structure of an antigen

•Isomeric forms of an antigen

•Secondary and tertiary structure of an antigen

3.32 Cross reactivity

Cross reactivity refers to the ability of an individual antibody combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen. Figure 6.1.4 illustrates how cross reactions can arise. Cross reactions arise because the cross reacting antigen shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen (multispecificity).

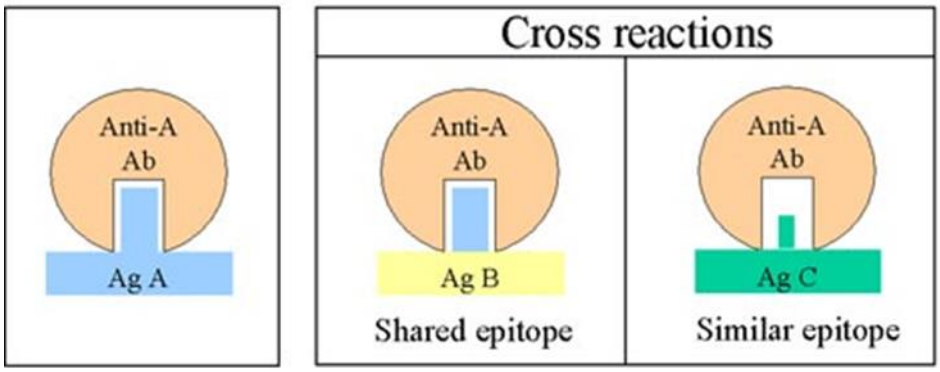

FIGURE 6.1.4 CROSS REACTIVITY

Courtesy:

Gene Mayer, Ph.D
Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

3.4 TESTS FOR ANTIGEN-ANTIBODY REACTIONS

3.41 Factors affecting measurement of antigen-antibody reactions

The only way that one knows that an antigen-antibody reaction has occurred is to have some means of directly or indirectly detecting the complexes formed between the antigen and antibody. The ease with which one can detect antigen-antibody reactions will depend on a number of factors.

3.42 Affinity

The higher the affinity of the antibody for the antigen, the more stable will be the interaction. Thus, the ease with which one can detect the interaction is enhanced.

3.43 Avidity

Reactions between multivalent antigens and multivalent antibodies are more stable and thus easier to detect.

3.44 Antigen to antibody ratio

The ratio between the antigen and antibody influences the detection of antigen-antibody complexes because the size of the complexes formed is related to the concentration of the antigen and antibody. This is depicted in Figure 6.1.5

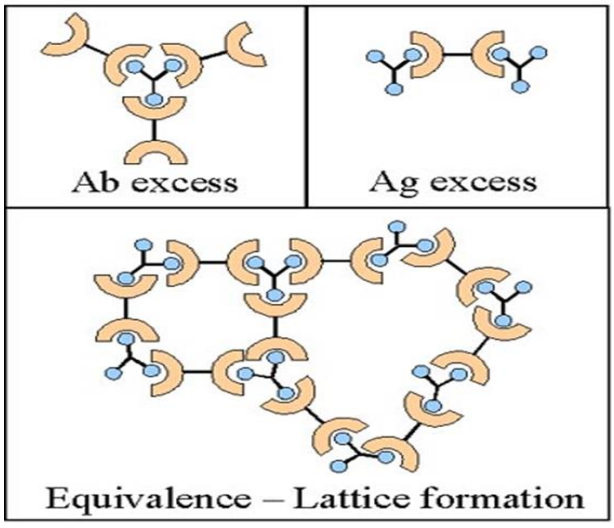

FIGURE 6.1.5: LEVELS OF ANTIGEN TO ANTIBODY RATIO MEASUREMENT

COURTESY: GENE MAYER, PH.D
EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY
UNIVERSITY OF SOUTH CAROLINA

3.45 Physical form of the antigen

The physical form of the antigen influences how one detects its reaction with an antibody. If the antigen is a particulate, one generally looks for agglutination of the antigen by the antibody. If the antigen is soluble one generally looks for the precipitation of the antigen after the production of large insoluble antigen-antibody complexes.

3.46 Agglutination Tests

Agglutination/Heamagglutination

When the antigen is particulate, the reaction of an antibody with the antigen can be detected by agglutination (clumping) of the antigen. The general term agglutinin is used to describe antibodies that agglutinate particulate antigens. When the antigen is an erythrocyte the term heamagglutination is used. All antibodies can theoretically agglutinate particulate antigens but IgM, due to its high valence, is particularly good agglutinin and one sometimes infers that an antibody may be of the IgM class if it is a good agglutinating antibody.

3.47 Qualitative agglutination test

Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody. The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen. (Figure 6.1.6).

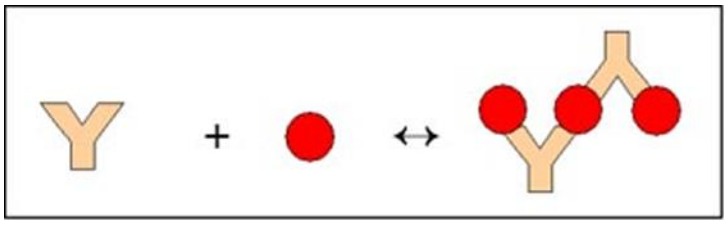

FIGURE 6.1.6: **AGGLUTINATION**

COURTESY: GENE MAYER, PH.D
EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY
UNIVERSITY OF SOUTH CAROLINA

For example, a patient's red blood cells can be mixed with antibody to a blood group antigen to determine a person's blood type. In a second example, a patient's serum is mixed with red blood cells of a known blood type to assay for the presence of antibodies to that blood type in the patient's serum.

3.48 Quantitative agglutination test

Agglutination tests can also be used to measure the level of antibodies to particulate antigens. In this test, serial dilutions are made of a sample to be tested for antibody and then a fixed number of red blood cells or bacteria or other such particulate antigen is added. Then the maximum dilution that gives agglutination is determined. The maximum dilution that gives visible agglutination is called the titer. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination. Figure 6.1.7 illustrates a quantitative heamagglutination test.

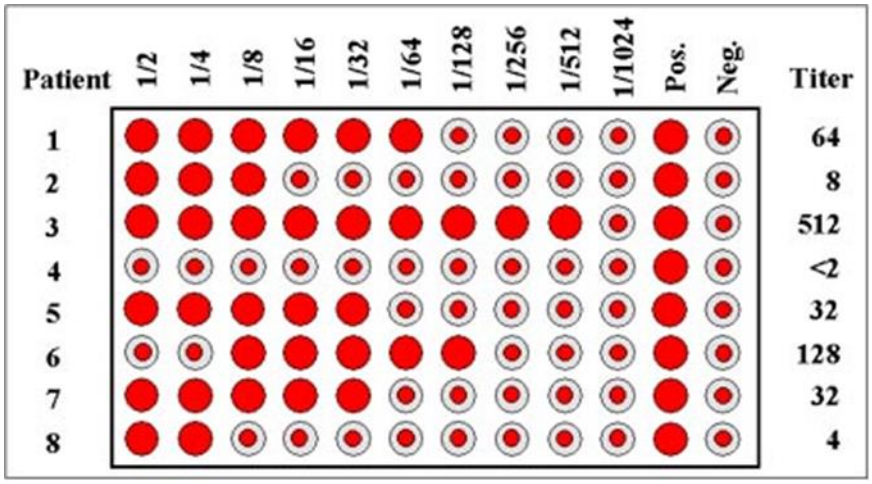

FIGURE 6.1.7: QUANTITATIVE AGGLUTINATION MEASUREMENT

Courtesy: Gene Mayer, Ph.D
Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

Prozone effect - Occasionally, it is observed that when the concentration of antibody is high (i.e. lower dilutions), there is no agglutination and then, as the sample is diluted, agglutination occurs (See Patient 6 in Figure 6.1.7). The lack of agglutination at high concentrations of antibodies is called the Prozone effect. Lack of agglutination in the Prozone is due to antibody excess resulting in very small complexes that do not clump to form visible agglutination.

3.5 Applications of agglutination tests

i. Determination of blood types or antibodies to blood group antigens.

ii. To assess bacterial infections

e.g. A rise in titer of an antibody to a particular bacterium indicates an infection with that bacterial type.

N.B. a fourfold rise in titer is generally taken as a significant rise in antibody titer.

3.51 Practical considerations

Although the test is easy to perform, it is only semi-quantitative.

3.52 Passive heamagglutination

The agglutination test only works with particulate antigens. However, it is possible to coat erythrocytes with a soluble antigen (e.g. viral antigen, a polysaccharide or a hapten) and use the coated red blood cells in an agglutination test for antibody to the soluble antigen (Figure 6.1.8). This is called passive heamagglutination. The test is performed just like the agglutination test. Applications include detection of antibodies to soluble antigens and detection of antibodies to viral antigens.

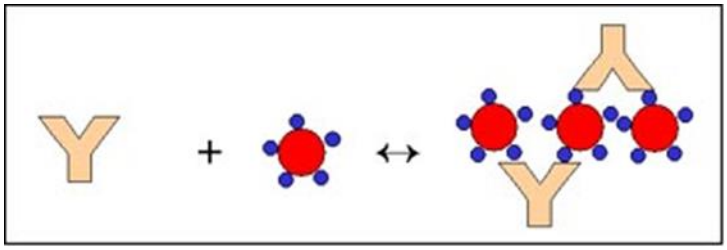

FIGURE 6.1.8: PASSIVE HAEMAGGLUTINATION

Courtesy: GENE MAYER, PH.D
EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY
UNIVERSITY OF SOUTH CAROLINA

3.53 Direct Coomb's Test

When antibodies bind to erythrocytes, they do not always result in agglutination. This can result from the antigen/antibody ratio being in antigen excess or antibody excess or in some cases electrical charges on the red blood cells preventing the effective cross linking of the cells. These antibodies that

bind to but do not cause agglutination of red blood cells are sometimes referred to as incomplete antibodies.

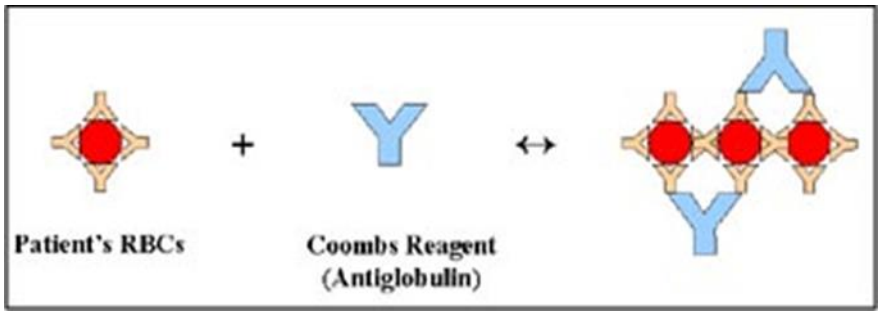

FIGURE 6.1.9 DIRECT COOMB'S TEST :

Courtesy: **Gene Mayer, Ph.D**
Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

In no way is this meant to indicate that the antibodies are different in their structure, although this was once thought to be the case. Rather, it is a functional definition only. In order to detect the presence of non-agglutinating antibodies on red blood cells, one simply adds a second antibody directed against the immunoglobulin (antibody) coating the red cells. This anti-immunoglobulin can now cross link the red blood cells and result in agglutination. This test is illustrated in Figure 6.1.9 and is known as the Direct Coomb's test.

3.54 Indirect Coomb's Test

If it is necessary to know whether a serum sample has antibodies directed against a particular red blood cell and you want to be sure that you also detect potential non- agglutinating antibodies in the sample, an Indirect Coomb's test is performed (Figure 6.1.10).

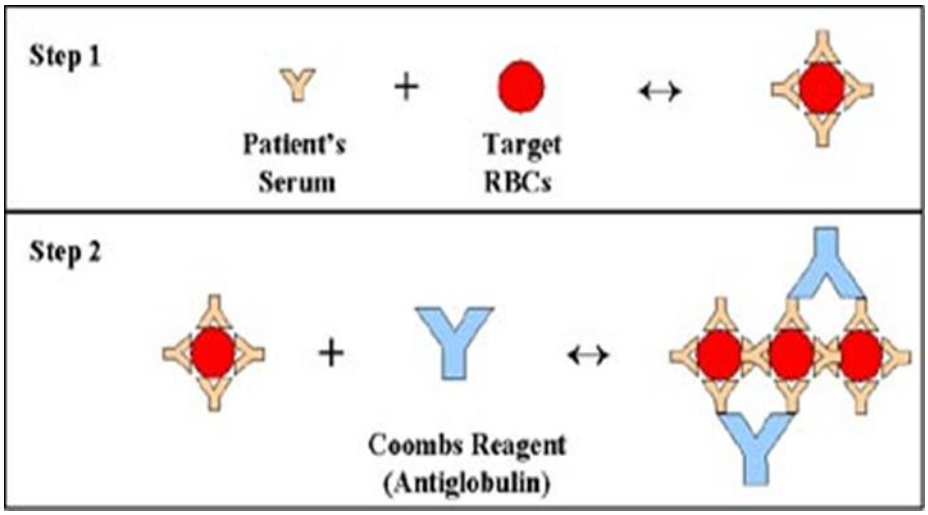

FIGURE 6.1.10: INDIRECT COOMB'S TEST

Courtesy: **Gene Mayer, Ph.D**
Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina.

This test is done by incubating the red blood cells with the serum sample, washing out any unbound antibodies and then adding a second anti-immunoglobulin reagent to cross link the cells.

3.6 APPLICATIONS

These include detection of anti-rhesus factor (Rh) antibodies. Antibodies to the Rh factor generally do not agglutinate red blood cells. Thus, red cells from Rh+ children born to Rh- mothers, who have anti-Rh antibodies, may be coated with these antibodies. To check for this, a direct Coombs test is performed. To see if the mother has anti-Rh antibodies in her serum an Indirect Coombs test is performed.

3.61 Heamagglutination Inhibition

The agglutination test can be modified to be used for the measurement of soluble antigens. This test is called heamagglutination inhibition. It is called heamagglutination inhibition because one measures the ability of soluble antigen to inhibit the agglutination of antigen-coated red blood cells by antibodies. In this test, a fixed amount of antibodies to the antigen in question is mixed with a fixed amount of red blood cells coated with the antigen (see passive heamagglutination above). Also included in the mixture are different amounts of the sample to be analysed for the presence of the antigen. If the sample contains the antigen, the soluble antigen will compete with the antigen

coated on the red blood cells for binding to the antibodies, thereby inhibiting the agglutination of the red blood cells. as illustrated in Figure 6.1.11.

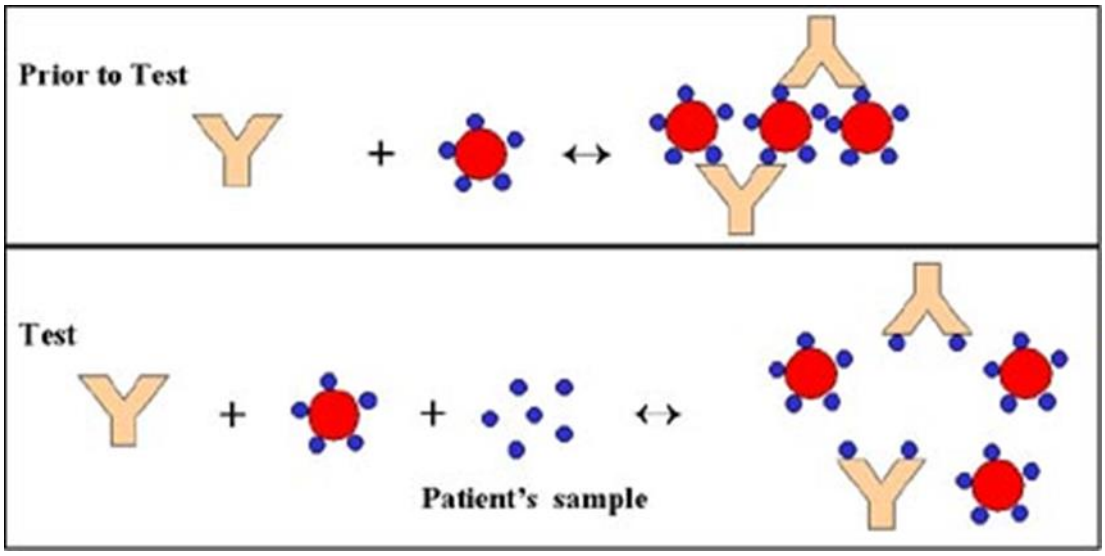

FIGURE 6.1.11: HEAMAGGLUTINATION INHIBITION TEST

Courtesy; **Gene Mayer, Ph.D**
Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

By serially diluting the sample, you can quantitate the amount of antigen in your unknown sample by its titer. This test is generally used to quantitate soluble antigens and is subject to the same practical considerations as the agglutination test.

3.62 Précipitation tests

Radial Immunodiffusion (Mancini)

In radial immunodiffusion antibody is incorporated into the agar gel as it is poured and different dilutions of the antigen are placed in holes punched into the agar. As the antigen diffuses into the gel, it reacts with the antibody and when the equivalence point is reached a ring of precipitation is formed as illustrated in Figure 6.1.12

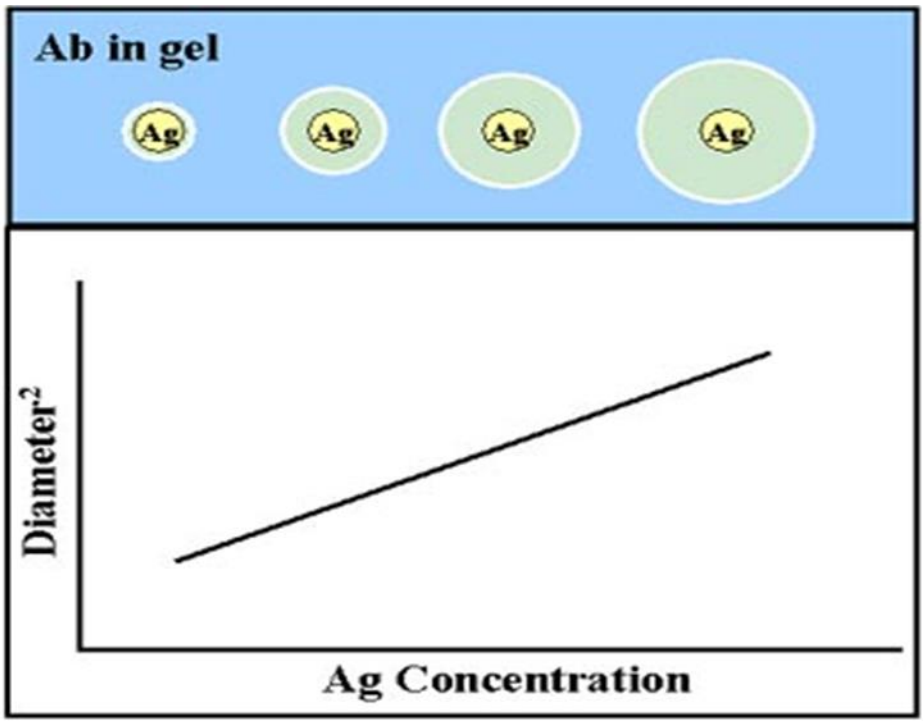

FIGURE 6.1.12 : RADIAL IMMUNODIFFUSION

Courtesy: **Gene Mayer, Ph.D**
Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

The diameter of the ring is proportional to the log of the concentration of antigen since the amount of antibody is constant. Thus, by running different concentrations of a standard antigen one can generate a standard cure from which one can quantitate the amount of an antigen in an unknown sample. Thus, this is a quantitative test. If more than one ring appears in the test, more than one antigen/antibody reaction has occurred. This could be due to a mixture of antigens or antibodies. This test is commonly used in the clinical laboratory for the determination of immunoglobulin levels in patient samples.

3.63 Immunoelectrophoresis

In Immunoelectrophoresis, a complex mixture of antigens is placed in a well punched out of an agar gel and the antigens are electrophoresed so that the antigen are separated according to their charge. After electrophoresis, a trough is cut in the gel and antibodies are added. As the antibodies diffuse into the agar, precipitin lines are produced in the equivalence zone when an antigen/antibody reaction occurs as illustrated in Figure 6.1.13

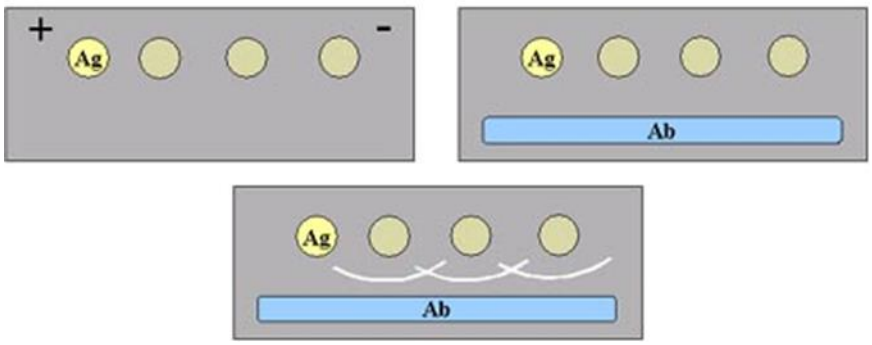

FIGURE 6.1.13: IMMUNOELECTROPHORESIS

Gene Mayer, Ph.D
Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

This tests is used for the qualitative analysis of complex mixtures of antigens, although a crude measure of quantity (thickness of the line) can be obtained. This test is commonly used for the analysis of components in a patient' serum. Serum is placed in the well and antibody to whole serum in the trough. By comparisons to normal serum, one can determine whether there are deficiencies on one or more serum components or whether there is an overabundance of some serum component (thickness of the line). This test can also be used to evaluate purity of isolated serum proteins.

3.64 Counter current electrophoresis

In this test the antigen and antibody are placed in wells punched out of an agar gel and the antigen and antibody are electrophoresed into each other where they form a precipitation line as illustrated in Figure 6.1.14. This test only works if conditions can be found where the antigen and antibody have opposite charges. This test is primarily qualitative, although from the thickness of the band you can get some measure of quantity. Its major advantage is its speed.

FIGURE 6.1.14: COUNTER CURRENT ELECTROPHORESIS

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Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

3.65 Radioimmunoassay (RIA)/Enzyme Linked Immunosorbent Assay (ELISA)

Radioimmunoassay (RIA) is assays that are based on the measurement of radioactivity associated with immune complexes. In any particular test, the label may be on either the antigen or the antibody. Enzyme Linked Immunosorbent Assays (ELISA) are those that are based on the measurement of an enzymatic reaction associated with immune complexes. In any particular assay, the enzyme may be linked to either the antigen or the antibody.

3.66 Competitive RIA/ELISA for Ag Detection

The method and principle of RIA and ELISA for the measurement of antigen is shown in Figure 6.1.16. By using known amounts of a standard unlabelled antigen, one can generate a standard curve relating radioactivity (cpm) (Enzyme) bound versus amount of antigen. From this standard curve, one can determine the amount of an antigen in an unknown sample.

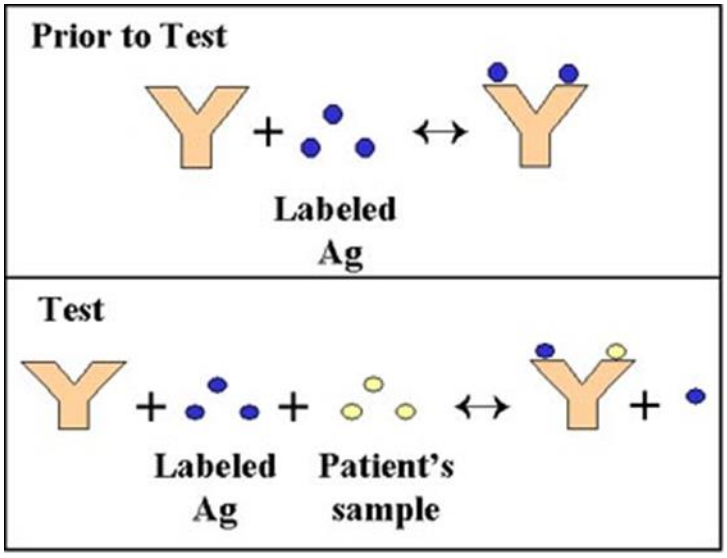

FIGURE 6.1.15: COMPETITIVE RIA/ELISA FOR AG DETECTION TEST

COURTESY: **Gene Mayer, Ph.D**
Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

The key to the assay is the separation of the immune complexes from the remainder of the components. This has been accomplished in many different ways and serves as the basis for the names given to the assay:

3.67 Precipitation with ammonium sulphate

Ammonium sulphate (33 - 50% final concentration) will precipitate immunoglobulins but not many antigens. Thus, this can be used to separate the immune complexes from free antigen. This has been called the Farr Technique

3.68 Anti-immunoglobulin antibody

The addition of a second antibody directed against the first antibody can result in the precipitation of the immune complexes and thus the separation of the complexes from free antigen.

3.69 Immobilization of the Antibody

The antibody can be immobilized onto the surface of a plastic bead or coated onto the surface of a plastic plate and thus the immune complexes can easily be separated from the other components by simply washing the beads or plate (Figure 6.1.16).

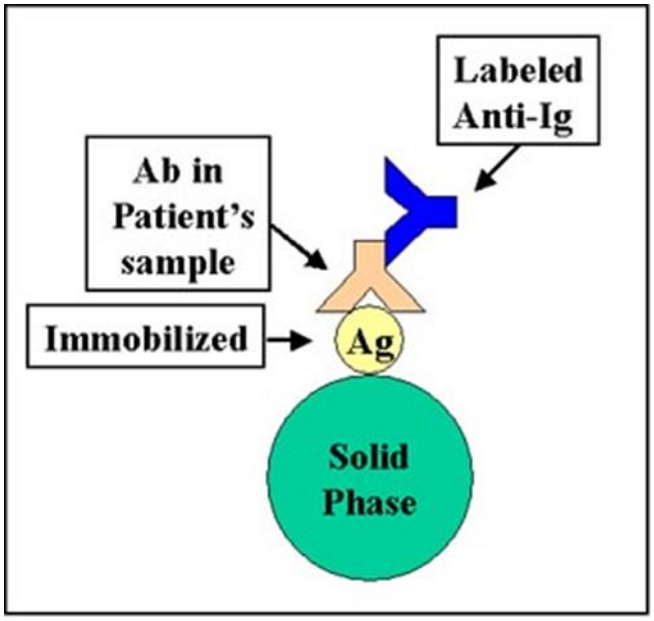

FIGURE 6.1.16 IMMOBILIZATION OF THE ANTIBODY

Courtesy: Gene Mayer, Ph.D
Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina.

This is the most common method used today and is referred to as solid phase RIA or ELISA. In the clinical laboratory, competitive RIA and ELISA are commonly used to quantitate serum proteins, hormones, drugs metabolites.

3.691 Non-competitive RIA/ELISA for Ag or Ab

Non-competitive RIA and ELISAs are also used for the measurement of antigens and antibodies. In Figure 6.1.17, the bead is coated with the antigen and is used for the detection of antibody in the unknown sample. The amount of labelled second antibody bound is related to the amount of antibody in the unknown sample.

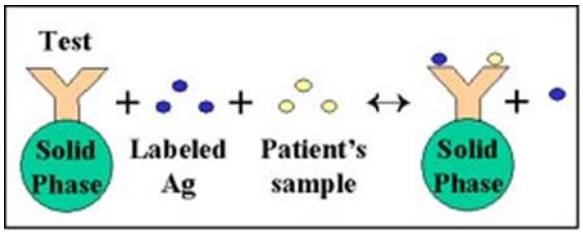

FIGURE 6.1.17: NON-COMPETITIVE RIA/ELISA FOR AG OR AB

This assay is commonly employed for the measurement of antibodies of the IgE class directed against particular allergens by using a known allergen as antigen and anti-IgE antibodies as the labelled reagent. It is called the RAST test (radioallergosorbent test). In Figure 6.1.18, the bead is coated with antibody and is used to measure an unknown antigen. The amount of labelled second antibody that binds is proportional to the amount of antigen that bound to the first antibody.

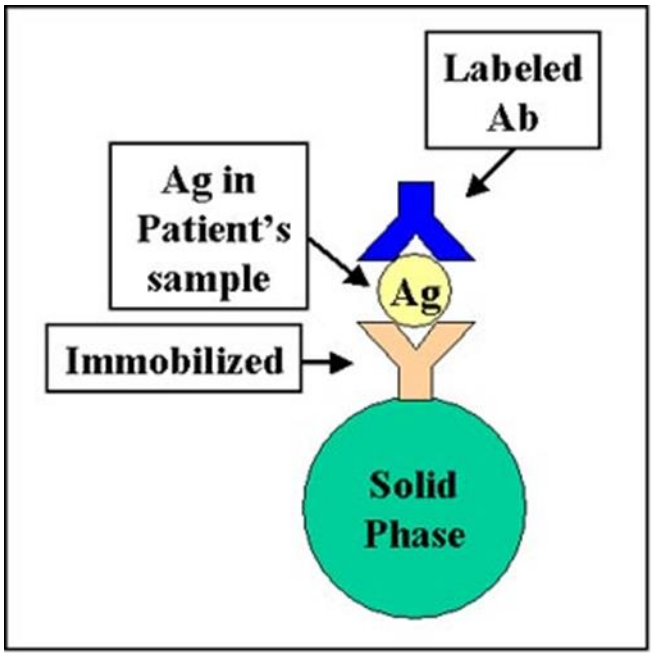

FIGURE 6.1.18: Radioallergosorbent Test

COURTESY : GENE MAYER, PH.D EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY UNIVERSITY OF SOUTH CAROLINA

3.693 Tests for Cell Associated Antigens

3.694 Immunofluorescence

Immunofluorescence is a technique whereby an antibody labeled with a fluorescent molecule (fluorescein or rhodamine or one of many other fluorescent dyes) is used to detect the presence of an antigen in or on a cell or tissue by the fluorescence emitted by the bound antibody.

3.695 Direct Immunofluorescence

In direct immunofluorescence, the antibody specific to the antigen is directly tagged with the Fluorochrome (Figure 6.1.19).

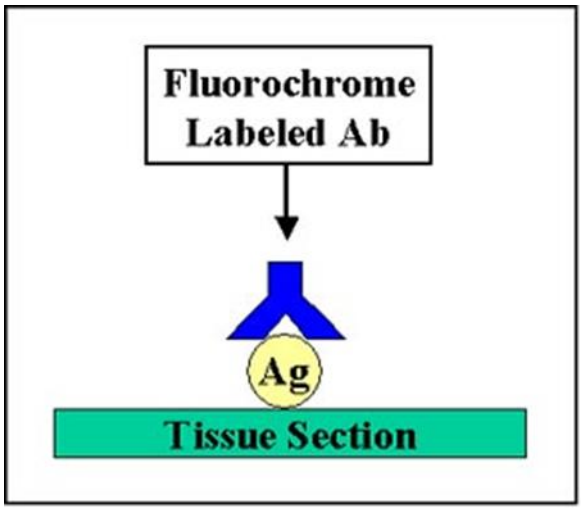

FIGURE 6.1.19: DIRECT IMMUNOFLUORESCENCE TEST

Courtesy : Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

3.696 Indirect Immunofluorescence

In indirect immunofluorescence, the antibody specific for the antigen is unlabelled and a second anti-immunoglobulin antibody directed toward the first antibody is tagged with the Fluorochrome (Figure 6.1.20). Indirect fluorescence is more sensitive than direct immunofluorescence since there is amplification of the signal.

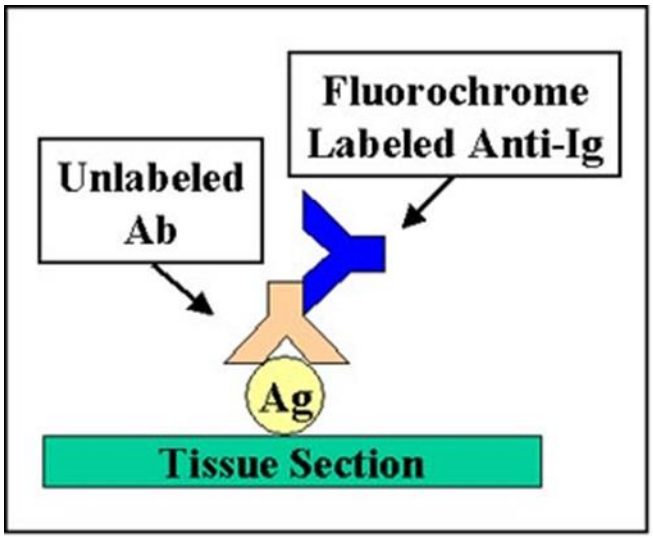

FIGURE 6.1.20 INDIRECT IMMUNOINFLUORESCENCE

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

3.697 Flow Cytometry

Flow cytometry is commonly used in the clinical laboratory to identify and enumerate cells bearing a particular antigen. Cells in suspension are labeled with a fluorescent tag by either direct or indirect immunofluorescence. The cells are then analysed on the flow cytometer.

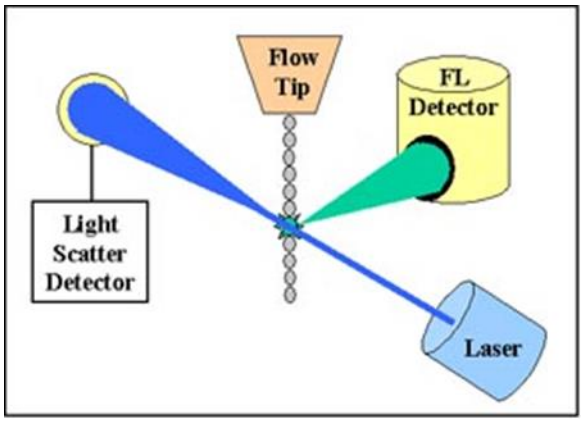

FIGURE 6.1.21: FLOW CYTOMETRY ASSAY

COURTESY: GENE MAYER, PH.D EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY UNIVERSITY OF SOUTH CAROLINA

Figure 6.1.21 illustrates the principle of flow cytometry. In a flow cytometer, the cells exit a flow cell and are illuminated with a laser beam. The amount of laser light that is scattered off the cells as they pass through the laser can be

measured, which gives information concerning the size of the cells. In addition, the laser can excite the Fluorochrome on the cells and the fluorescent light emitted by the cells can be measured by one or more detectors.

The type of data that is obtained from the flow cytometer is shown in Figure 6.1.22. In a one parameter histogram, increasing amount of fluorescence (e.g. green fluorescence) is plotted on the x axis and the number of cells exhibiting that amount of fluorescence is plotted on the y axis.

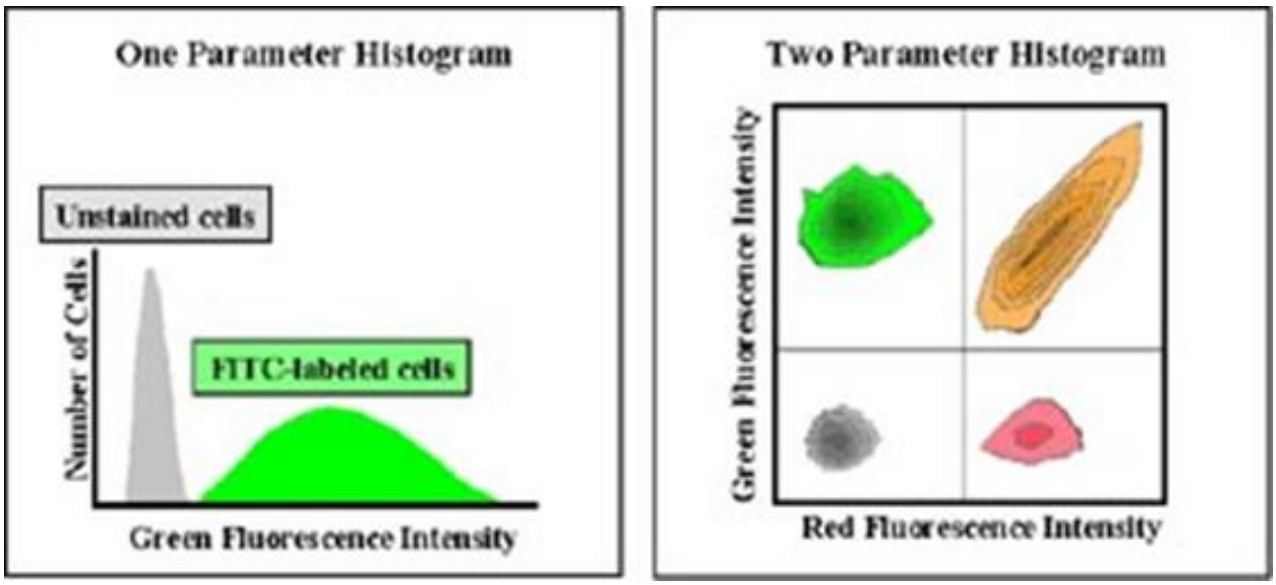

FIGURE 6.1.22: ONE AND TWO PARAMETER PRINCIPLES OF THE FLOW CYTOMETER

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

The fraction of cells that are fluorescent can be determined by integrating the area under the curve. In a two parameter histogram, the x axis is one parameter (e.g. red fluorescence) and the y axis is the second parameter (e.g. green fluorescence). The number of cells is indicated by the contour and the intensity of the colour.

3.697 Complement Fixation

Antigen/antibody complexes can also be measured by their ability to fix complement because an antigen/antibody complex will "consume" complement if it is present, whereas free antigens or antibodies do not. Tests for antigen/antibody complexes that rely on the consumption of complement

are termed complement fixation tests and are used to quantitate antigen/antibody reactions. This test will only work with complement fixing antibodies (IgG and IgM are best).

The principle of the complement fixation test is illustrated in Figure 6.1.24. Antigen is mixed with the test serum to be assayed for antibody and antigen/antibody complexes are allowed to form. A control tube in which no antigen is added is also prepared.

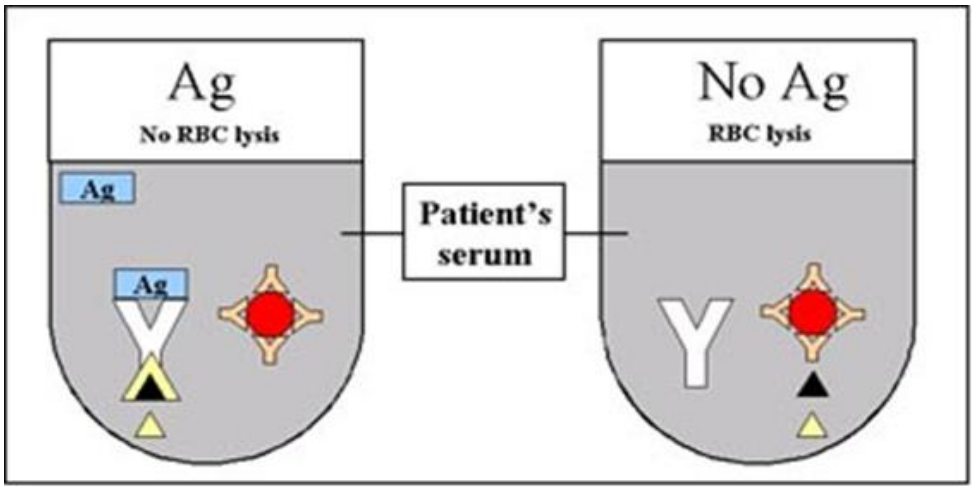

FIGURE 6.1.23: DEMONSTRATING PRINCIPLES OFTHE COMPLEMENT FIXATION TESTS

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

If no antigen/antibody complexes are present in the tube, none of the complement will be fixed. However, if antigen/antibody complexes are present, they will fix complement and thereby reduce the amount of complement in the tube. After allowing complement fixation by any antigen/antibody complexes, a standard amount of red blood cells, which have been pre-coated with anti-erythrocyte antibodies is added. The amount of antibody-coated red blood cells is predetermined to be just enough to completely use up all the complement initially added, if it were still there. If all the complement was still present (i.e. no antigen/antibody complexes formed between the antigen and antibody in question), all the red cells will be lysed. If antigen/antibody complexes are formed between the antigen and antibody in question, some of the complement will be consumed and, thus, when the antibody-coated red cells are added not all of them will lyse. By simply measuring the amount of red cell lysis by measuring the release of haemoglobin into the medium, one can indirectly quantitate antigen/antibody complexes in the tube. Complement fixation tests are most commonly used to

assay for antibody in a test sample but they can be modified to measure antigen.

3.7 ANTIBODY FORMATION

3.71 GENERAL CHARACTERISTICS OF THE ANTIBODY RESPONSE

3.72 Self / non-self-discrimination

One characteristic feature of the specific immune system is that it normally distinguishes between self and non-self and only reacts against non-self.

3.73 Memory

A second feature of the specific immune response is that it demonstrates memory. The immune system "remembers" if it has seen an antigen before and it reacts to secondary exposures to an antigen in a manner different than after a primary exposure. Generally, only an exposure to the same antigen will illicit this memory response.

3.74 Specificity

A third characteristic feature of the specific immune system is that there is a high degree of specificity in its reactions. A response to a particular antigen is specific for that antigen or a few closely related antigens.

N.B. These are characteristic of all specific immune responses.

3.75 Fate of the immunogen

Clearance after primary injection

The kinetics of antigen clearance from the body after a primary administration is depicted in Figure 6.1.24

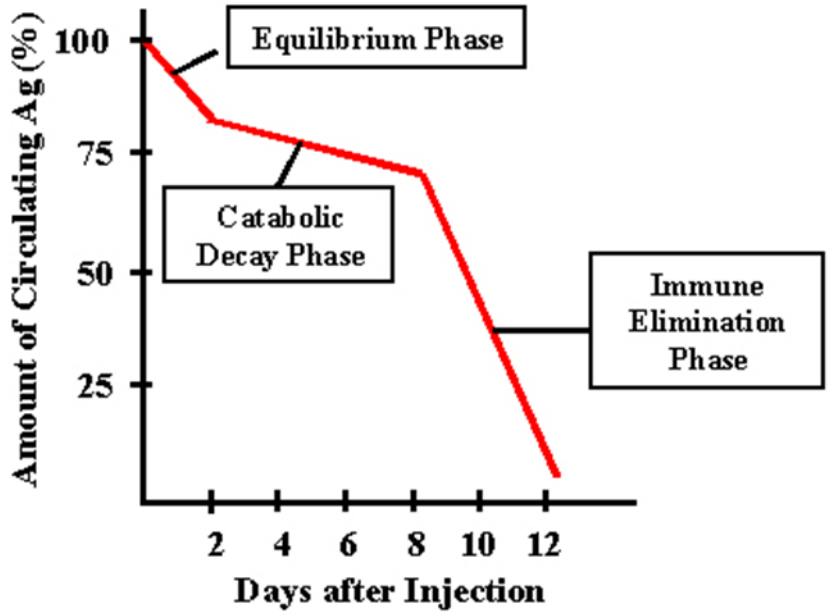

FIGURE 6.1.24: FATE OF THE IMMUNOGEN

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology
University of South Carolina

3.76 Equilibrium phase

The first phase is called the equilibrium or equilibration phase. During this time the antigen equilibrates between the vascular and extravascular compartments by diffusion. This is normally a rapid process. Since particulate antigens don't diffuse, they do not show this phase.

3.77 Catabolic decay phase

In this phase the host's cells and enzymes metabolize the antigen. Most of the antigen is taken up by macrophages and other phagocytic cells. The duration will depend upon the immunogen and the host.

3.78 Immune elimination phase

In this phase, newly synthesized antibody combines with the antigen producing antigen/antibody complexes which are phagocytosed and degraded. Antibody appears in the serum only after the immune elimination phase is over.

Clearance after secondary injection

If there is circulating antibody in the serum, injection of the antigen for a second time results in a rapid immune elimination. If the is no circulating

antibody, then injection of the antigen for a second time results in all three phases but the onset of the immune elimination phase is accelerated.

3.79 Primary (1^{o}) Antibody response

The kinetics of a primary antibody response to an antigen is illustrated in Figure 6.1.25

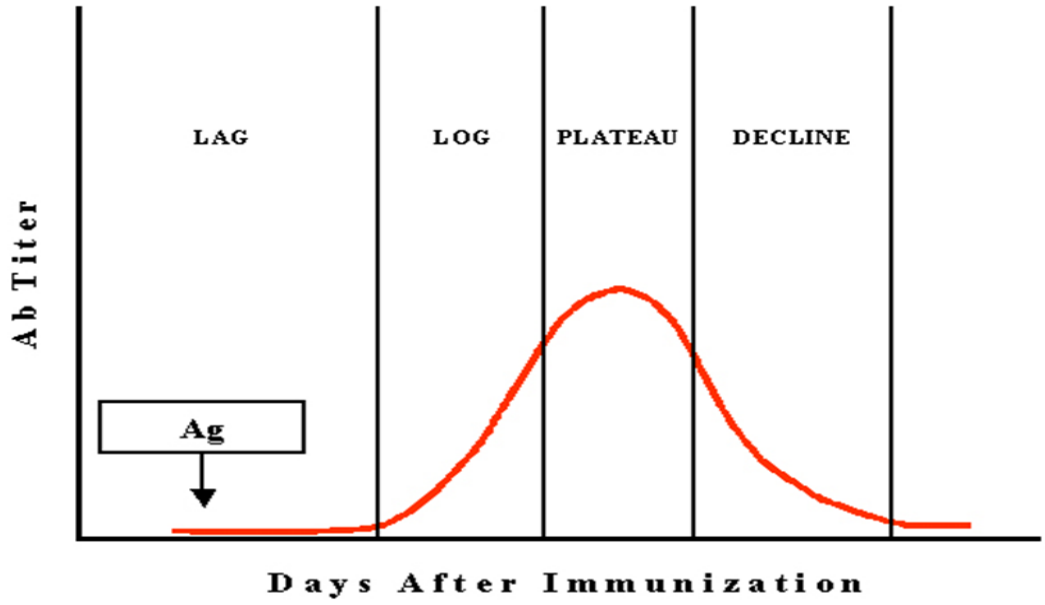

FIGURE 6.1.25: GRAPHICAL PRESENTATION OF PRIMARY (1^{o}) ANTIBODY RESPONSE

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

Inductive, latent or lag phase

In this phase, the antigen is recognized as foreign and the cells begin to proliferate and differentiate in response to the antigen. The duration of this phase will vary depending on the antigen but it is usually 5 to 7 days.

Log or Exponential Phase

In this phase, the antibody concentration increases exponentially as the B cells that were stimulated by the antigen differentiate into plasma cells which secrete antibody.

Plateau or steady-state phase

In this phase, antibody synthesis is balanced by antibody decay so that there is no net increase in antibody concentration.

Decline or decay phase

In this phase, the rate of antibody degradation exceeds that of antibody synthesis and the level of antibody falls. Eventually the level of antibody may reach base line levels.

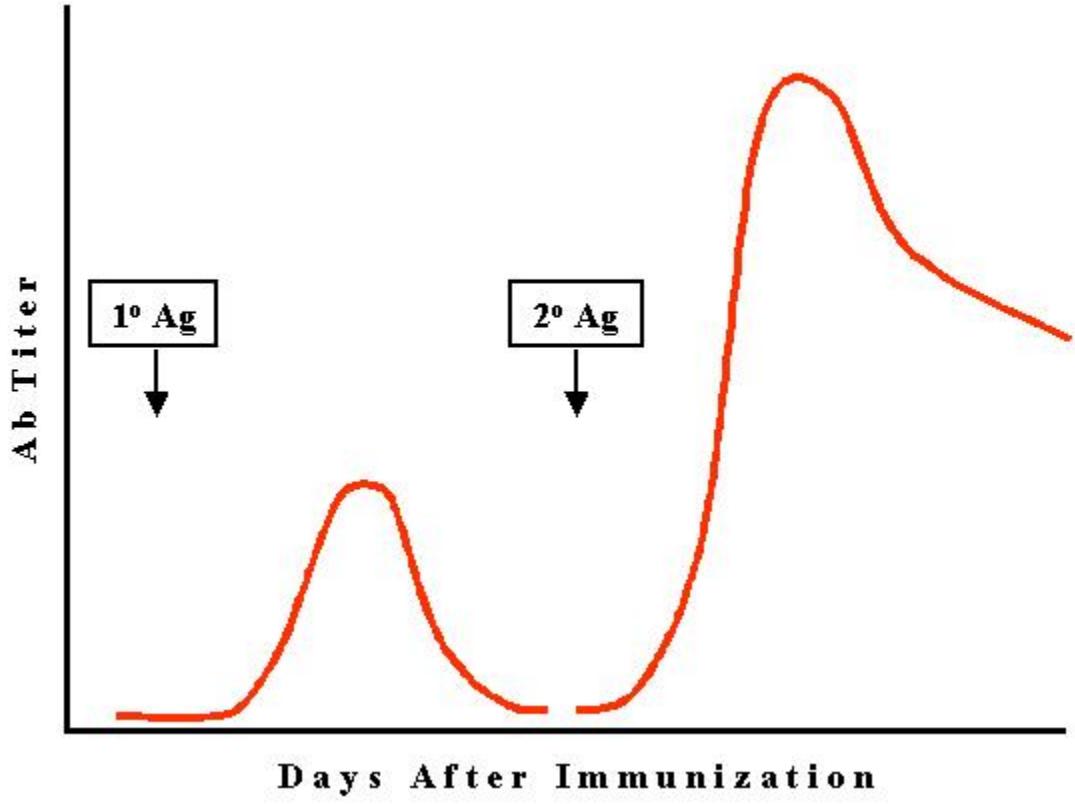

FIGURE 6.1.26: SECONDARY (2°), MEMORY OR ANAMNESTIC RESPONSE

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

Lag phase

In a secondary response, there is a lag phase by it is normally shorter than that observed in a primary response.

Log phase

The log phase in a secondary response is more rapid and higher antibody levels are achieved.

Steady state phase

Decline phase

The decline phase is not as rapid and antibody may persist for months, years or even a lifetime.

Specificity of primary and secondary responses

Antibody elicited in response to an antigen is specific for that antigen, although it may also cross react with other antigens which are structurally similar to the eliciting antigen. In general, secondary responses are only elicited by the same antigen used in the primary response. However, in some instances a closely related antigen may produce a secondary response, but this is a rare exception.

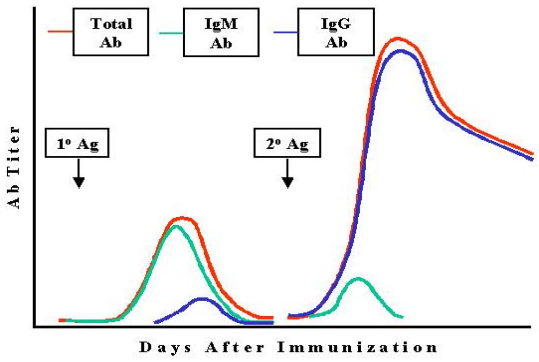

Figure 6.1.27 **: Specificity of primary and secondary responses**

Courtesy GENE MAYER, PH.D EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY UNIVERSITY OF SOUTH CAROLINA

Qualitative changes in antibody during primary and secondary responses

Immunoglobulin class variation

In the primary response, the major class of antibody produced is IgM whereas in the secondary response it is IgG (or IgA or IgE) (Figure 6.1.27). The antibodies that persist in the secondary response are the IgG antibodies.

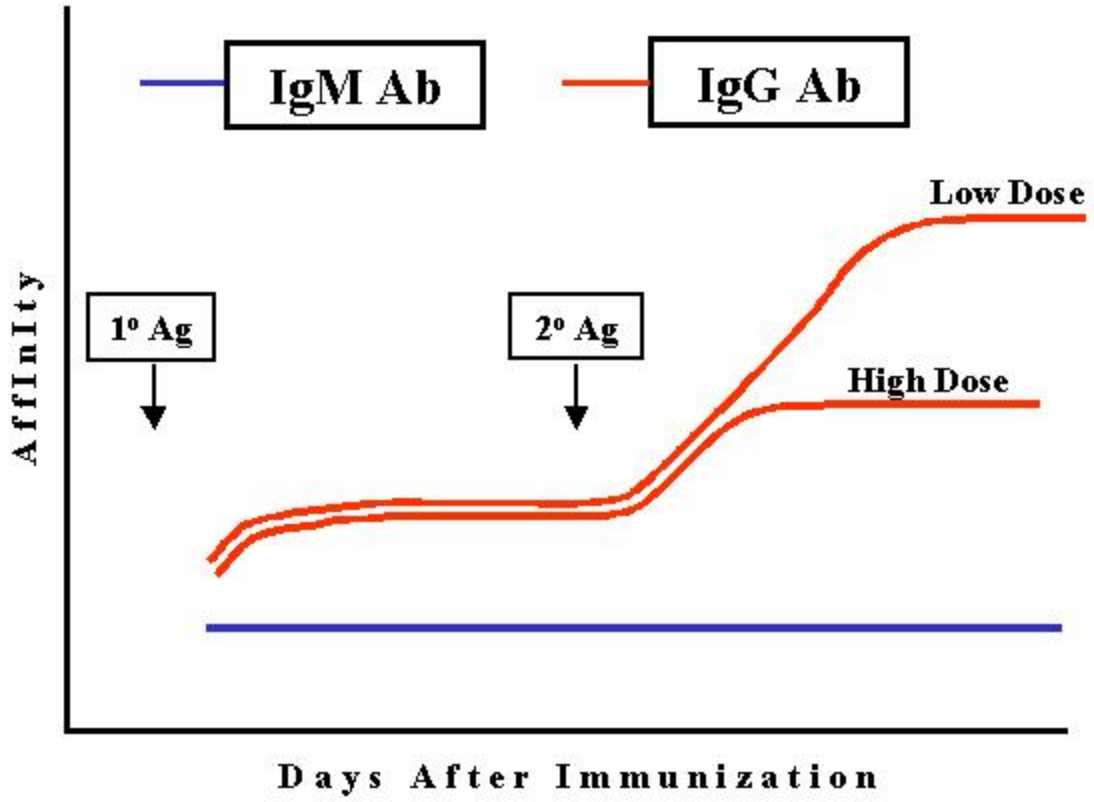

FIGURE 6.1.28: IMMUNOGLOBULIN CLASS VARIATION

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina :

Affinity

The affinity of the IgG antibody produced increases progressively during the response, particularly after low doses of antigen (Figure 6.1.28). This is referred to as affinity maturation. Affinity maturation is most pronounced after secondary challenge with antigen.

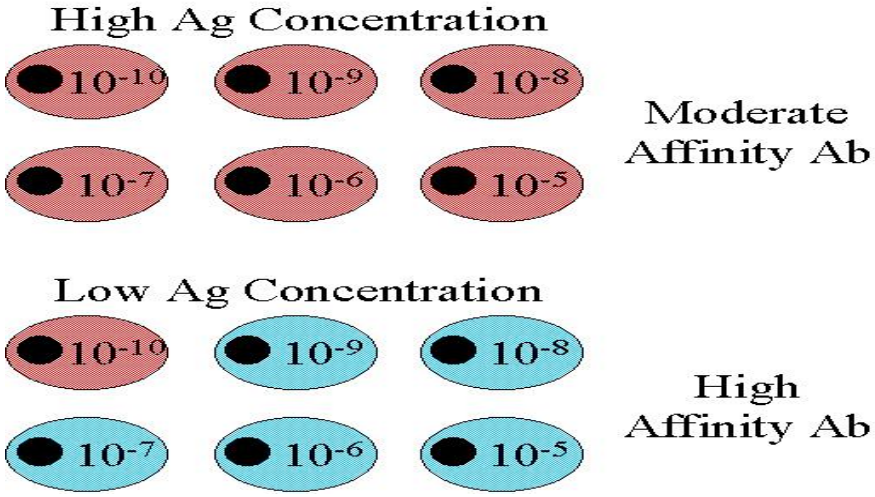

FIGURE 6.1.29: AFFINITY

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

One explanation for affinity maturation is clonal selection as illustrated in Figure 6.1.29. A second explanation for affinity maturation is that, after a class switch has occurred in the immune response, somatic mutations occur which fine tune the antibodies to be of higher affinity. There is experimental evidence for this mechanism, although it is not known how the somatic mutation mechanism is activated after exposure to antigen.

Avidity

As a consequence of increased affinity, the avidity of the antibodies increases during the response.

Cross-reactivity

As a result of the higher affinity later in the response, there is also an increase in detectible cross reactivity. An explanation for why increasing affinity results in an increase in detectible cross reactivity is illustrated by the following example.

Affinity of Ab for Ag

Early Late

Immunizing Ag 10^{-6} 10^{-9}

\+ ++

Cross reacting Ag 10^{-3} 10^{-6}

- +

If a minimum affinity of 10^{-6} is needed to detect a reaction, early in an immune response the reaction of a cross reacting antigen with an affinity of 10^{-3} will not be detected. However, late in a response when the affinities increase 1000 fold, the reaction with both the immunizing and cross reacting antigens will be detected.

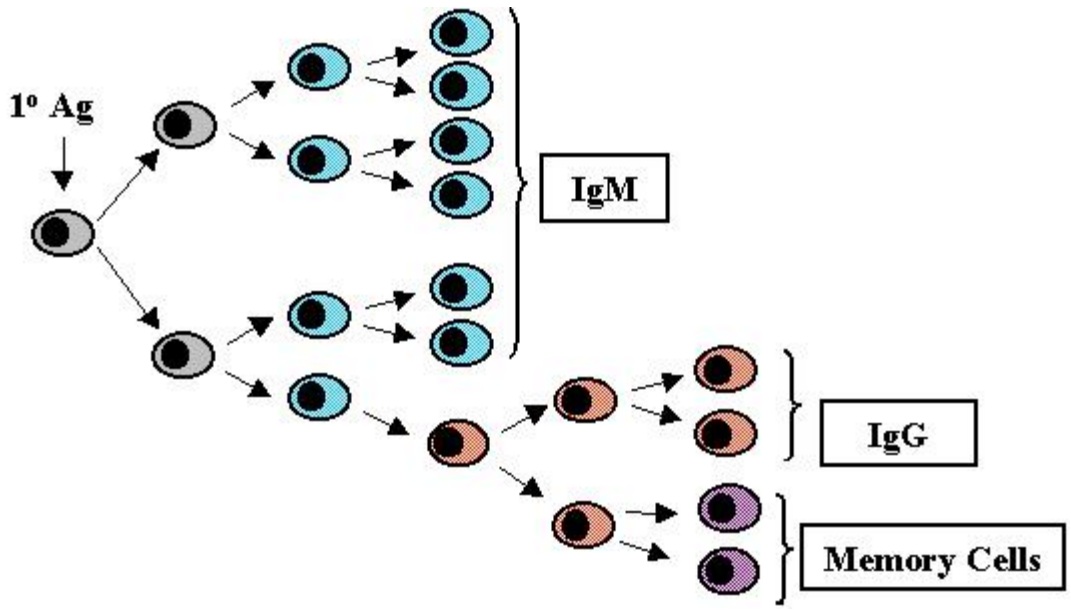

FIGURE 6.1.30: CELLULAR EVENTS DURING PRIMARY AND SECONDARY RESPONSES TO T-DEPENDENT ANTIGEN PRIMARY RESPONSE

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

Lag phase

Clones of T and B cells with the appropriate antigen receptors bind antigen, become activated and begin to proliferate. The expanded clones of B cells differentiate into plasma cells which begin to secrete antibody.

Log phase

The plasma cells initially secrete IgM antibody since the Cu heavy chain gene is closest to the rearranged VDJ gene. Eventually some B cells switch from making IgM to IgG, IgA or IgE. As more B cells proliferate and differentiate into antibody secreting cells the antibody concentration increases exponentially.

Stationary phase

As antigen is depleted, T and B cells are no longer activated. In addition, mechanisms which down regulate the immune response come into play. Furthermore, plasma cells begin to die. When the rate of antibody synthesis equals the rate of antibody decay the stationary phase is reached.

Decline phase

When no new antibody is produced because the antigen is no longer present to activate T and B cells and the residual antibody slowly is degraded, the decay phase is reached.

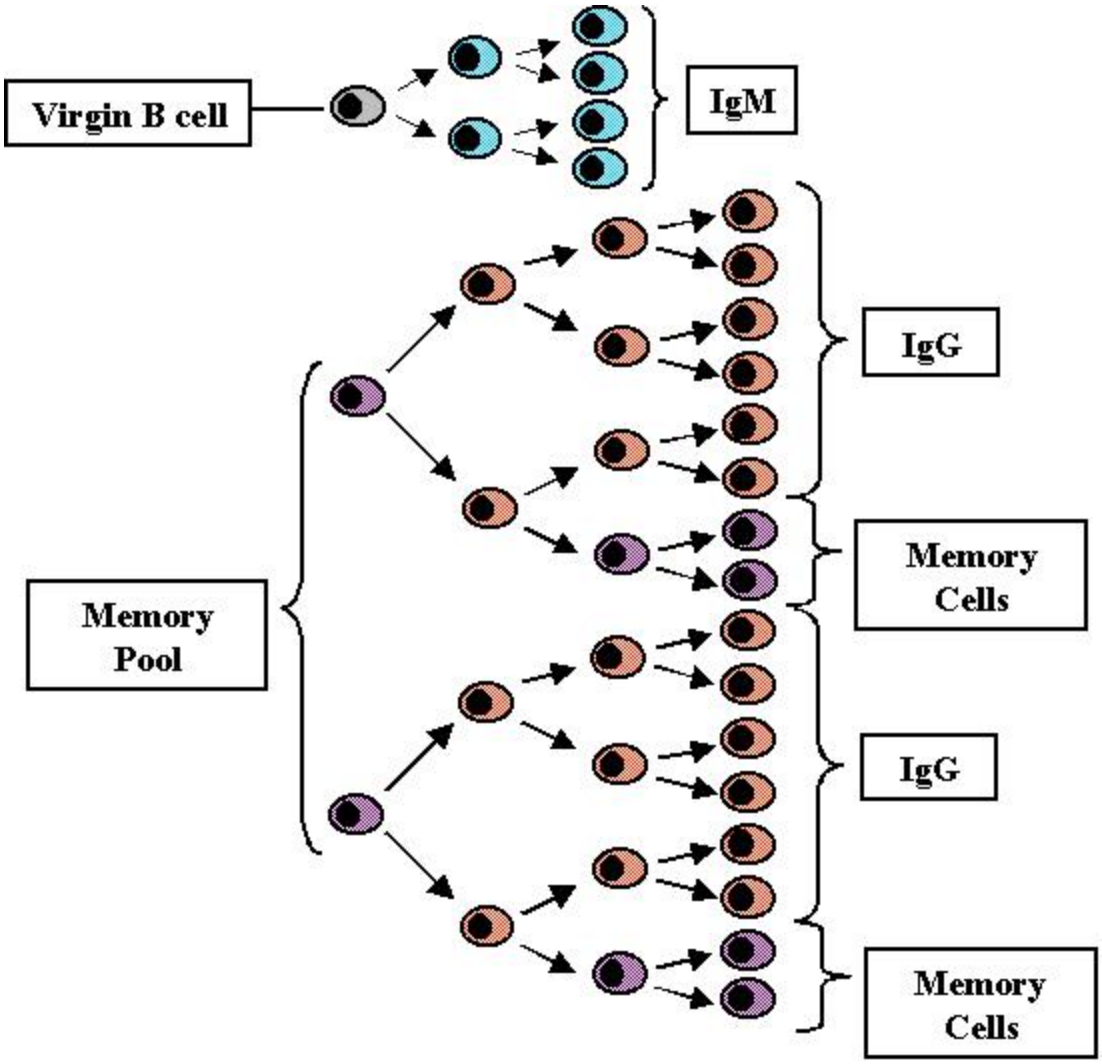

FIGURE 6.1.31 DECLINE PHASE

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

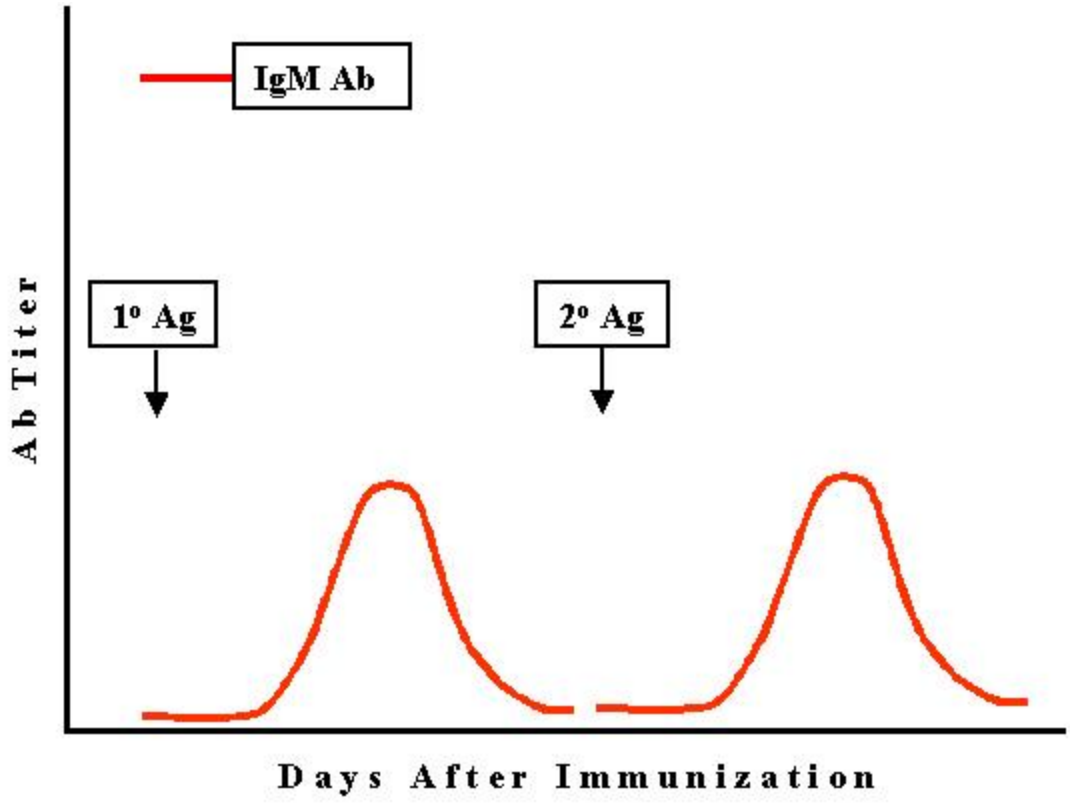

FIGURE 6.1.32

Courtesy: GENE MAYER, PH.D EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY UNIVERSITY OF SOUTH CAROLINA

Secondary response (Figure 6.1.32)

Not all of the T and B cells that are stimulated by antigen during primary challenge with antigen die. Some of them are long lived cells and constitute what is referred to as the memory cell pool. Both memory T cells and memory B cells are produced and memory T cells survive longer than memory B cells. Upon secondary challenge with antigen not only are virgin T and B cells activated, the memory cells are also activated and thus there is a shorter lag time in the secondary response. Since there is an expanded clone of cells being stimulated the rate of antibody production is also increased during the log phase of antibody production and higher levels are achieved. Also, since many if not all of the memory B cells will have switched to IgG (IgA or IgE) production, IgG is produced earlier in a secondary response. Furthermore, since there is an expanded clone of memory T cells which can help B cells to switch to IgG (IgA or IgE) production, the predominant class of Ig produced after secondary challenge is IgG (IgA or IgE).

Ab response to T-independent antigen

Responses to T-independent antigen are characterized by the production of almost exclusively IgM antibody and no secondary response. Secondary exposure to the antigen results in another primary response to the antigen as illustrated in Figure 6.1.33.

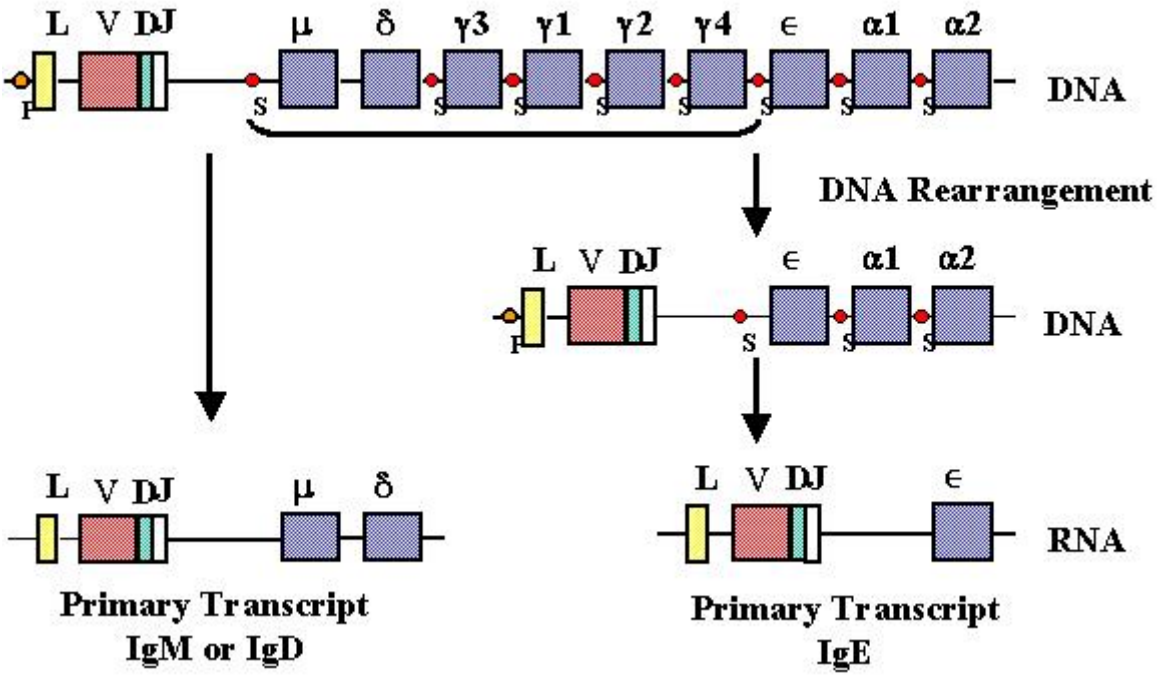

FIGURE 6.1.33 AB RESPONSE TO T-INDEPENDENT ANTIGEN

Courtesy : Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

Class switching

During an antibody response to a T-dependent antigen a switch occurs in the class of Ig produced from IgM to some other class (except IgD). Our understanding of the structure of the immunoglobulin genes, helps explain how class switching occurs (Figure 6.1.34).

During class switching another DNA rearrangement occurs between a switch site (Sμ) in the intron between the rearranged VDJ regions and the Cμ gene and another switch site before one of the other heavy chain constant region genes. The result of this recombination event is to bring the VDJ region close to

one of the other constant region genes, thereby allowing expression of a new class of heavy chain. Since the same VDJ gene is brought near to a different C gene and since the antibody specificity is determined by the hypervariable regions within the V region, the antibody produced after the switch occurs will have the same specificity as before.

Cytokines secreted by T helper cells can cause the switch to certain isotypes.

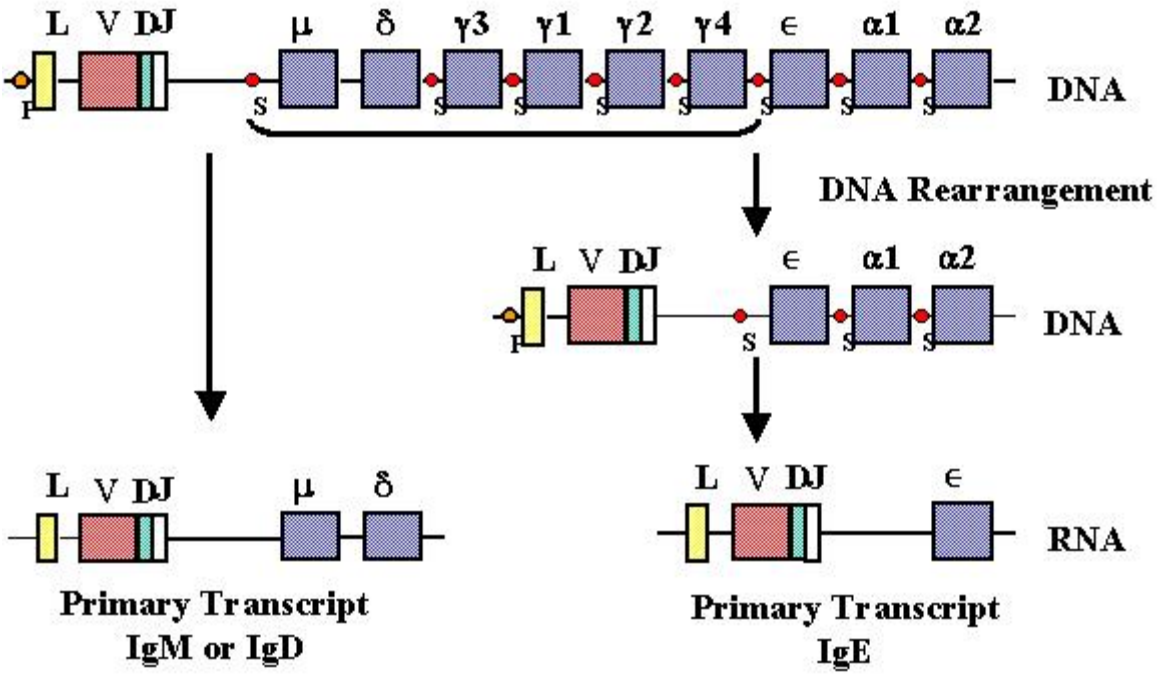

FIGURE 6.1.34 :

Courtesy :GENE MAYER, PH.D EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY UNIVERSITY OF SOUTH CAROLINA

Membrane and secreted immunoglobulin

The specificity of membrane immunoglobulin on a B cell and the Ig secreted by the plasma cell progeny of a B cell is the same. An understanding of how the specificity of membrane and secreted Ig from an individual B cell can be the same comes from an understanding of immunoglobulin genes (Figure 6.1.35).

There are two potential polyA sites in the immunoglobulin gene. One after the exon for the last heavy chain domain and the other after the exons that code

for the trans- membrane domains. If the first polyA site is used, the pre-mRNA is processed to produce a secreted protein. If the second polyA site is used, the pre-mRNA is processed to produce a membrane form of the immunoglobulin. However, in all cases the same VDJ region is used and thus the specificity of the antibody remains the same. All C regions genes have these additional membrane pieces associated with them and thus after class switching other classes of immunoglobulins can be secreted or expressed on the surface of B cells.

CELLS INVOLVED IN IMMUNE RESPONSES AND ANTIGEN RECOGNITION

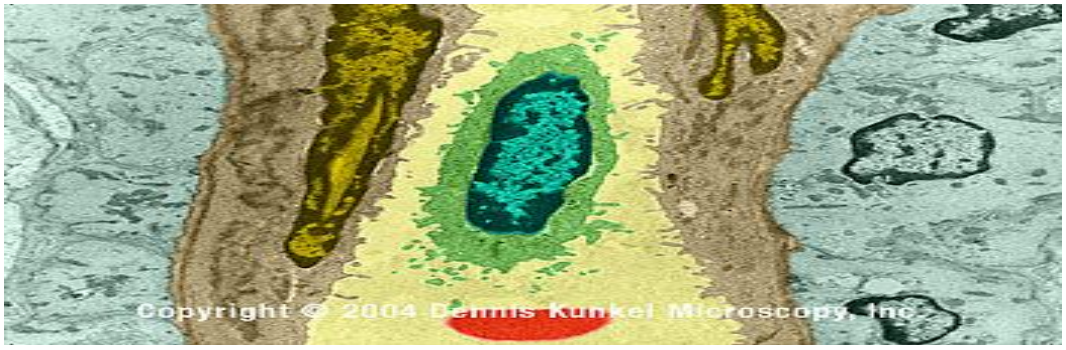

FIGURE6.1.35 : WHITE BLOOD CELL (LYMPHOCYTE) IN CAPILLARY (TEM X16,210)

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

OVERVIEW

The immune system has developed to protect the host from pathogens and other foreign substances. Self/non-self-discrimination is one of the hallmarks of the immune system. There are two main sites where pathogens may reside: extracellularly in tissue spaces or intracellularly within a host cell, and the immune system has different ways of dealing with pathogens at these sites. Although immune responses are tailored to the pathogen and to where the pathogen resides, most pathogens can elicit both an antibody and a cell-mediated response, both of which may contribute to ridding the host of the pathogen. However, for any particular pathogen an antibody or a cell-mediated response may be more important for defence against the pathogen.

Extracellular pathogens Antibodies are the primary defence against extracellular pathogens and they function in three major ways:

•Neutralization (Figure 6.1.36a)

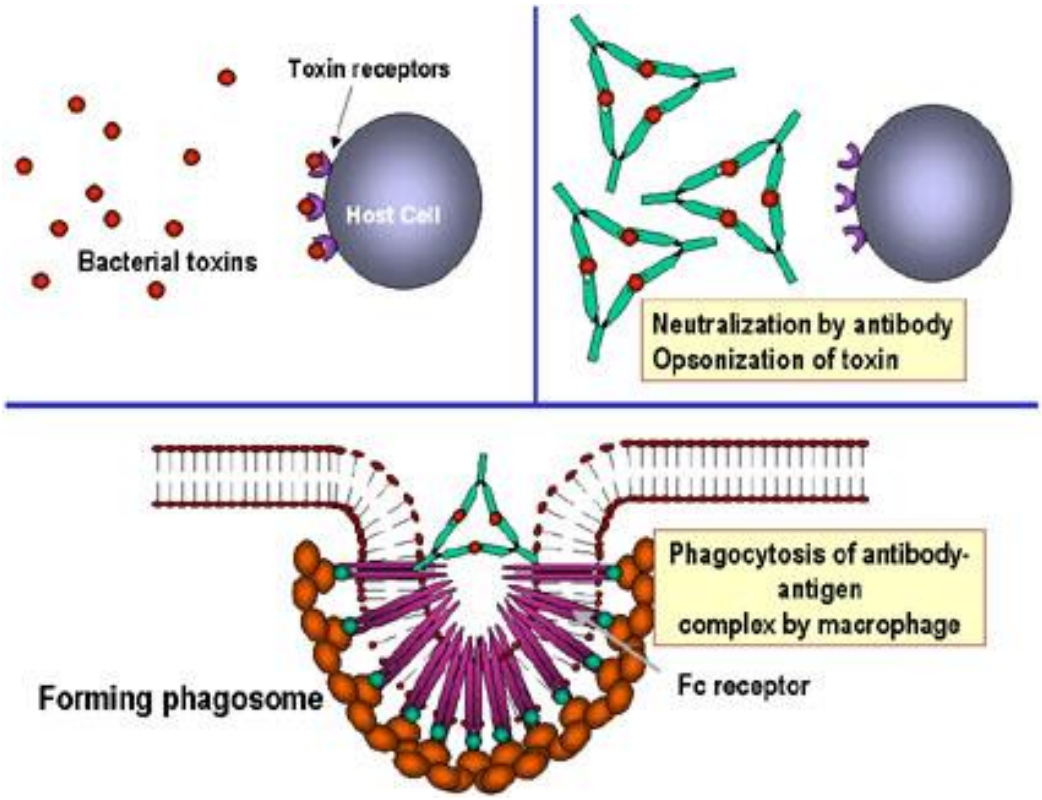

Figure 6.1.36: Neutralization

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

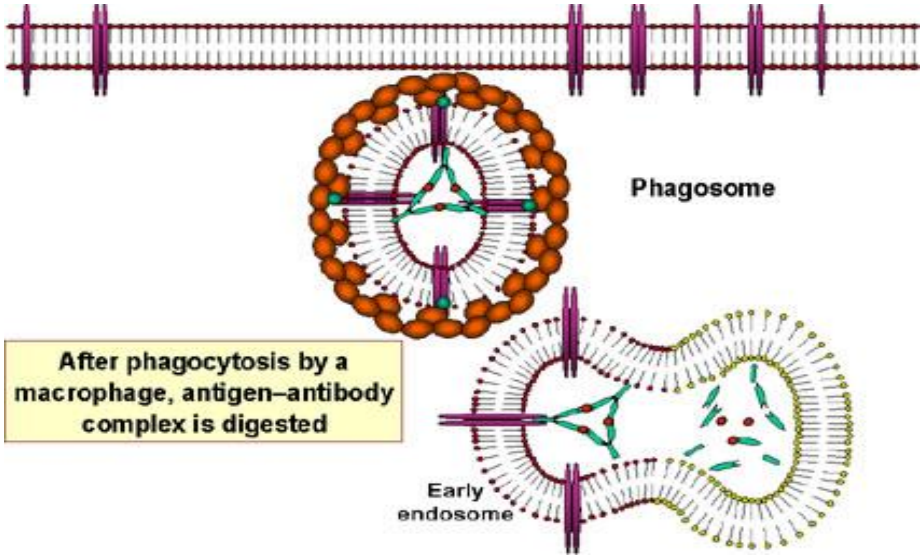

FIGURE 6.1.37A

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

By binding to the pathogen or foreign substance antibodies, can block the association of the pathogen with their targets. For example, antibodies to bacterial toxins can prevent the binding of the toxin to host cells thereby rendering the toxin ineffective. Similarly, antibody binding to a virus or bacterial pathogen can block the attachment of the pathogen to its target cell thereby preventing infection or colonization.

Opsonization (Figure 6.1.37b)

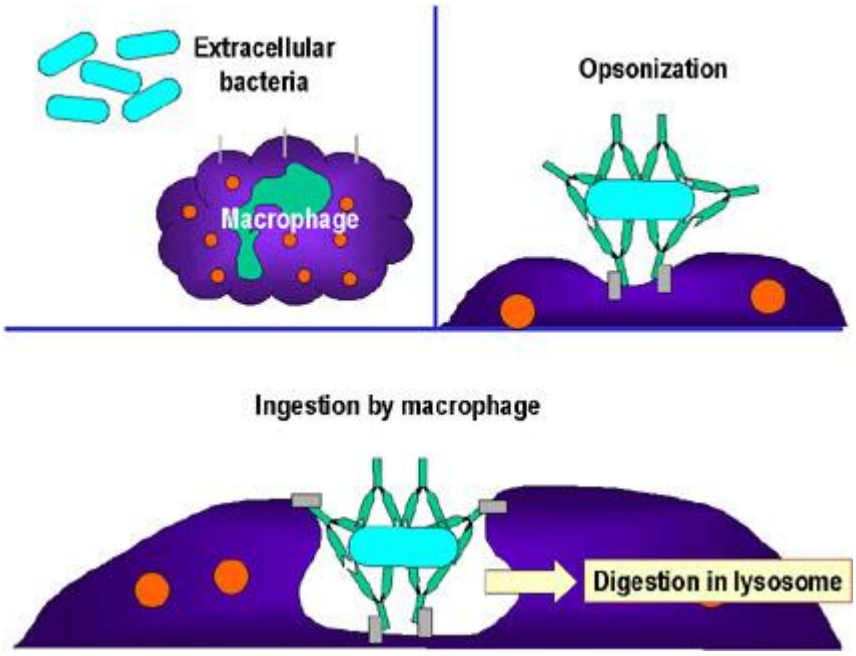

FIGURE 6.1.37B: COURTESY: GENE MAYER, PH.D EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY UNIVERSITY OF SOUTH CAROLINA

Antibody binding to a pathogen or foreign substance can opsonize the material and facilitate its uptake and destruction by phagocytic cells. The Fc region of the antibody interacts with Fc receptors on phagocytic cells rendering the pathogen more readily phagocytosed.

•Complement activation (Figure 6.1.37c)

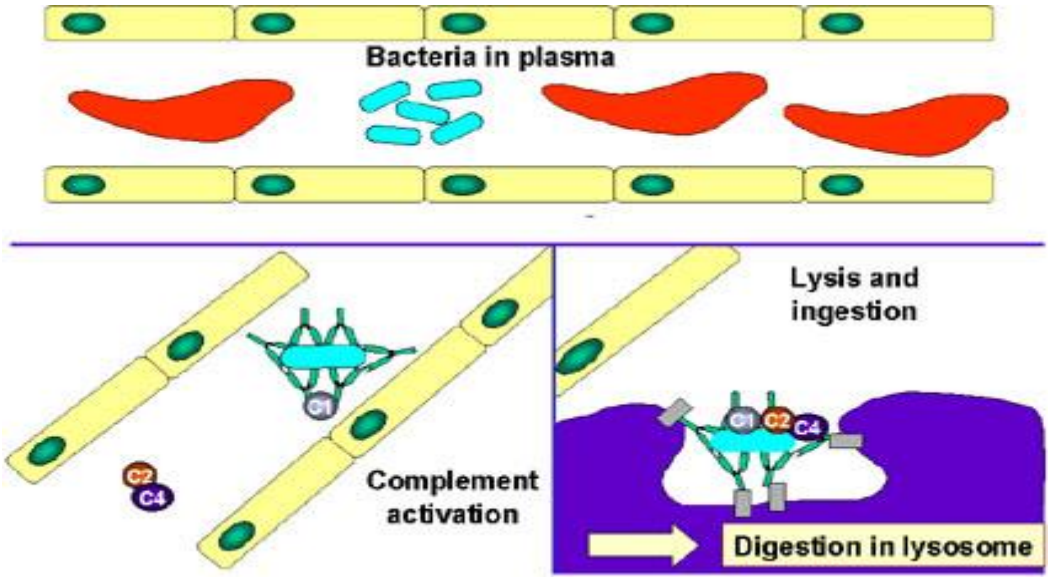

FIGURE 6.1.37C: Complement activation

Courtesy: **GENE MAYER, PH.D EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY UNIVERSITY OF SOUTH CAROLINA**

Activation of the complement cascade by antibody can result in lysis of certain bacteria and viruses. In addition, some components of the complement cascade (e.g. C3b) opsonize pathogens and facilitate their uptake via complement receptors on phagocytic cells.

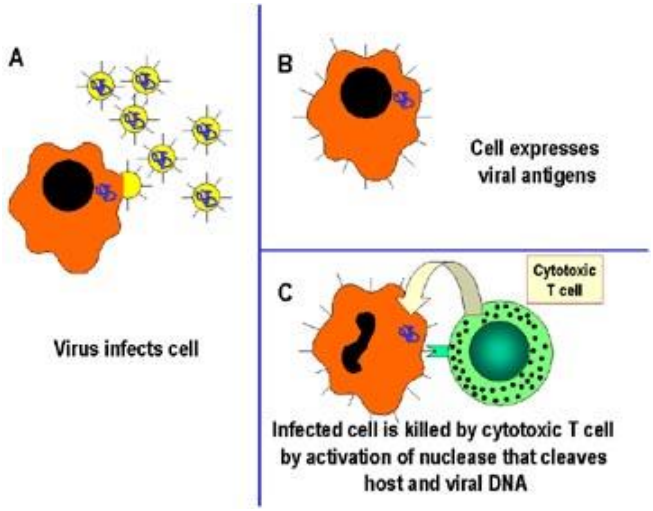

FIGURE 6.1.38

Courtesy : Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

Mechanism of host defence against intracellular infection by viruses. Cells infected by viruses are recognized by specialized T cells called cytotoxic T lymphocytes (CTLs), which kill the infected cells directly. The killing mechanism involves the activation of nucleases in the infected cell, which cleave host and viral DNA.

Courtesy Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

Intracellular pathogens

Because antibodies do not get into host cells, they are ineffective against intracellular pathogens. The immune system uses a different approach to deal with these kinds of pathogens. Cell-mediated responses are the primary defence against intracellular pathogens and the approach is different depending upon where the pathogen resides in the host cell (i.e., in the cytosol or within vesicles). For example, most viruses and some bacteria reside in the cytoplasm of the host cell, however, some bacteria and parasites actually live within endosomes in the infected host cell. The primary defence against pathogens in the cytosol is the cytotoxic T lymphocyte (Tc or CTL). In contrast, the primary defence against a pathogen within vesicles is a subset of helper T lymphocytes (Th1).

•Cytotoxic T lymphocytes (Figure 6.1.38)

CTLs are a subset of T lymphocytes that express a unique antigen on their surface called CD8. These cells recognize antigens from the pathogen that are displayed on the surface of the infected cell and kill the cell thereby preventing the spread of the infection to neighbouring cells. CTLs kill by inducing apoptosis in the infected cell.

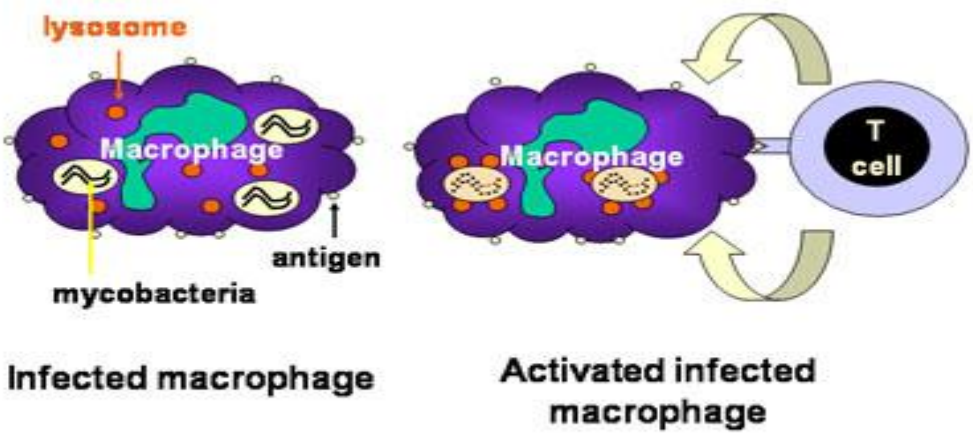

FIGURE 6.1.39: CYTOTOXIC T LYMPHOCYTES

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Mechanism of host defence against intracellular infection by mycobacteria. Mycobacteria infecting macrophages live in cytoplasmic vesicles that resist fusion with lysosomes and consequent destruction of the bacteria by macrophage bactericidal activity. However, when the appropriate T cell recognizes an infected macrophage it releases macrophage-activating molecules that induce lysosomal fusion and the activation of macrophage bactericidal activities

•Th1 Helper T cells (Figure 6.1.39)

Th cells are a subset of T cells that express a unique antigen on their surface called CD4. A subpopulation of Th cells, Th1 cells, is the primary defence against intracellular pathogens that live within vesicles. Th1 cells recognize antigen from the pathogen that are expressed on the surface of infected cells and release cytokines that activate the infected cell. Once activated, the infected cell can then kill the pathogen. For example, Mycobacterium tuberculosis, the causative agent of tuberculosis, infects macrophages but is not killed because it blocks the fusion of lysosomes with the endosomes in which it resides. Th1 cells that recognize *M. tuberculosis* antigens on the surface of an infected macrophage can secrete cytokines that activate macrophages. Once activated the lysosomes fuse with endosomes and the *M. tuberculosis* bacteria are killed.

Although immune responses are tailored to the pathogen and to where the pathogen resides, most pathogens can elicit both an antibody and a cell-mediated response, both of which may contribute to ridding the host of the pathogen. However, for any particular pathogen an antibody or a cell-mediated response may be more important for defence against the pathogen.

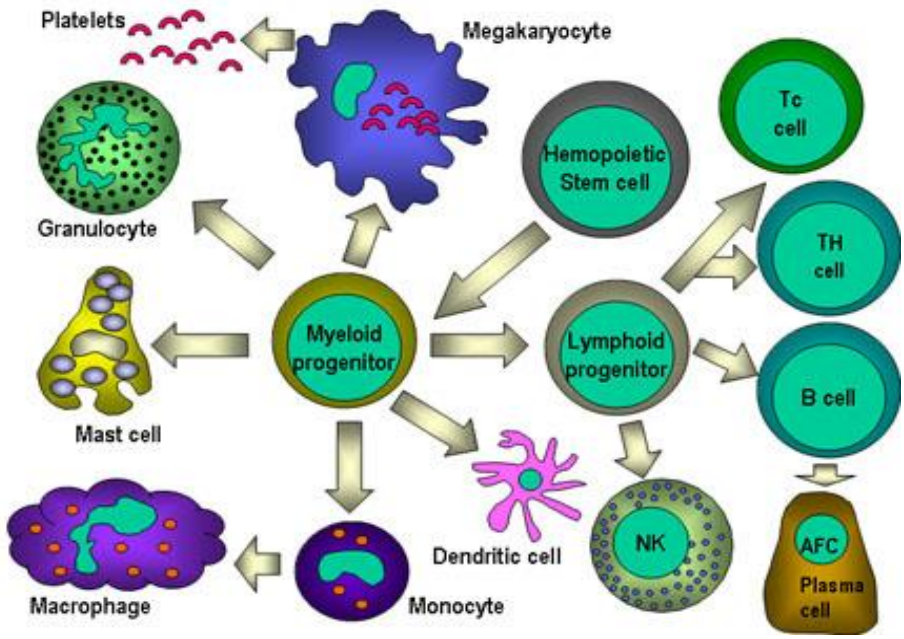

FIGURE 6.1.40 TH1 HELPER T CELLS

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All hematopoietic cells are derived from pluripotent stem cells which give rise to two main lineages: one for lymphoid cells and one for myeloid cells. The common lymphoid progenitor has the capacity to differentiate into either T cells or B cells depending on the microenvironment to which it homes. In mammals, T cells develop in the thymus while B cells develop in the foetal liver and bone marrow. An AFC is an antibody-forming cell, the plasma cell being the most differentiated AFC. NK cells also derive from the common lymphoid progenitor cell. The myeloid cells differentiate into the committed cells on the left. The collective name "granulocyte" is used for eosinophils, neutrophils and basophils.

Cells of the Immune System

All cells of the immune system originate from a hematopoietic stem cell in the bone marrow, which gives rise to two major lineages, a myeloid progenitor cell and a lymphoid progenitor cell (Figure 6.1.40). These two progenitors give rise to the myeloid cells (monocytes, macrophages, dendritic cells, megakaryocytes

and granulocytes) and lymphoid cells (T cells, B cells and natural killer (NK) cells), respectively. These cells make up the cellular components of the innate (non-specific) and adaptive (specific) immune systems.

Cells of the innate immune system

Cells of the innate immune system include phagocytic cells (monocyte/macrophages and PMNs), NK cells, basophils, mast cells, eosinophils and platelets. The roles of these cells have been discussed previously (see non-specific immunity). The receptors of these cells are pattern recognition receptors (PRRs) that recognize broad molecular patterns found on pathogens (pathogen associated molecular patterns, PAMPS).

Cells that link the innate and adaptive immune systems

A specialized subset of cells called antigen presenting cells (APCs) are a heterogeneous population of leukocytes that play an important role in innate immunity and also act as a link to the adaptive immune system by participating in the activation of helper T cells (Th cells). These cells include dendritic cells and macrophages. A characteristic feature of APCs is the expression of a cell surface molecule encoded by genes in the major histocompatibility complex, referred to as class II MHC molecules. B lymphocytes also express class II MHC molecules and they also function as APCs, although they are not considered as part of the innate immune system. In addition, certain other cells (e.g., thymic epithelial cells) can express class II MHC molecules and can function as APCs.

Cells of the adaptive immune system

Cells that make up the adaptive (specific) immune system include the B and T lymphocytes. After exposure to antigen, B cells differentiate into plasma cells whose primary function is the production of antibodies. Similarly, T cells can differentiate into either T cytotoxic (Tc) or T helper (Th) cells of which there are two types Th1 and Th2 cells.

There are a number of cell surface markers that are used in clinical laboratories to distinguish B cells, T cells and their subpopulations. These are summarized in table below:

Main distinguishing makers of T and B cells			
Maker	B cells	Tc	Th
CD3	-	+	+
CD4	-	-	+
CD8	-	+	-
CD19 and/or CD20	+	-	-
CD40	+	-	-
Ag receptor	BCR (surface Ig)	TCR	TCR

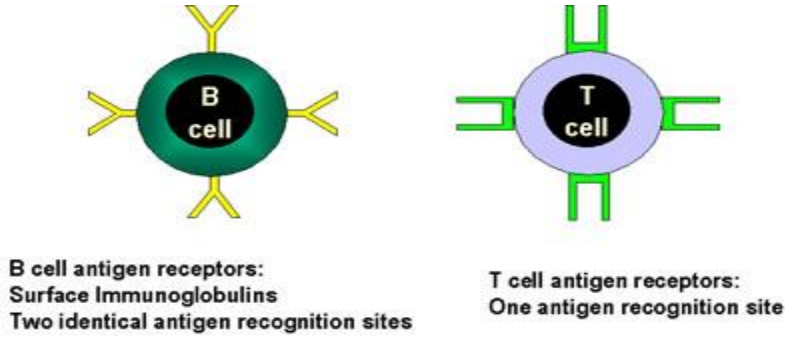

FIGURE 6.1.41

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

The antigen receptors of B cells have two antigen-recognition sites whereas those of T cells have only one

Specificity of the Adaptive Immune Response

Specificity on the adaptive immune response resides in the antigen receptors on T and B cells, the TCR and BCR, respectively. The TCR and BCR are similar in that each receptor is specific for one antigenic determinant but they differ in that BCRs are divalent while TCRs are monovalent (Figure 6.1.41). A consequence of this difference is that while B cells can have their antigen receptors cross-linked by antigen, TCRs cannot. This has implications as to how B and T cells can become activated.

Each B and T cells has a receptor that is unique for a particular antigenic determinant and there are a vast array of different antigen receptors on both B and T cells. The question of how these receptors are generated was the major

focus of immunologists for many years. Two basic hypotheses were proposed to explain the generation of the receptors: the instructionist (template) hypothesis and the clonal selection hypothesis.

Instructionist hypothesis

The instructionist hypothesis states that there is only one common receptor encoded in the germline and that different receptors are generated using the antigen as a template. Each antigen would cause the one common receptor to be folded to fit the antigen. While this hypothesis was simple and very appealing, it was not consistent with what was known about protein folding (i.e. protein folding is dictated by the sequence of amino acids in the protein). In addition, this hypothesis did not account for self/non-self-discrimination in the immune system. It could not explain why the one common receptor did not fold around self-antigens.

Clonal selection hypothesis

The clonal selection hypothesis states that the germline encodes many different antigen receptors - one for each antigenic determinant to which an individual will be capable of mounting an immune response. Antigen selects those clones of cells that have the appropriate receptor. The four basic principles of the clonal selection hypothesis are:

•Each lymphocyte bears a single type of receptor with a unique specificity.

•Interaction between a foreign molecule and a lymphocyte receptor capable of binding that molecule with a high affinity leads to lymphocyte activation.

•The differentiated effector cells derived from an activated lymphocyte will bear receptors of an identical specificity to those of the parental cell from which that lymphocyte was derived.

•Lymphocytes bearing receptors for self-molecules are deleted at an early stage in lymphoid cell development and are therefore absent from the repertoire of mature lymphocytes.

The clonal selection hypothesis is now generally accepted as the correct hypothesis to explain how the adaptive immune system operates. It explains many of the features of the immune response:

1) the specificity of the response;

2) the signal required for activation of the response (i.e. antigen);

3) the lag in the adaptive immune response (time is required to activate cells and to expand the clones of cells); and

4) self/non-self-discrimination.

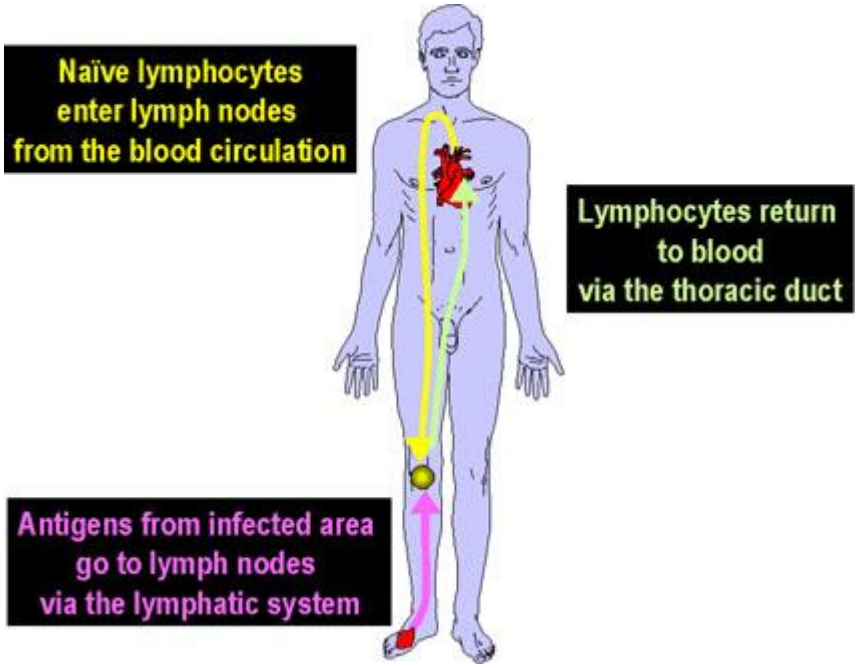

FIGURE 6.1.42 CIRCULATING LYMPHOCYTES ENCOUNTER ANTIGEN IN PERIPHERAL LYMPHOID TISSUES

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

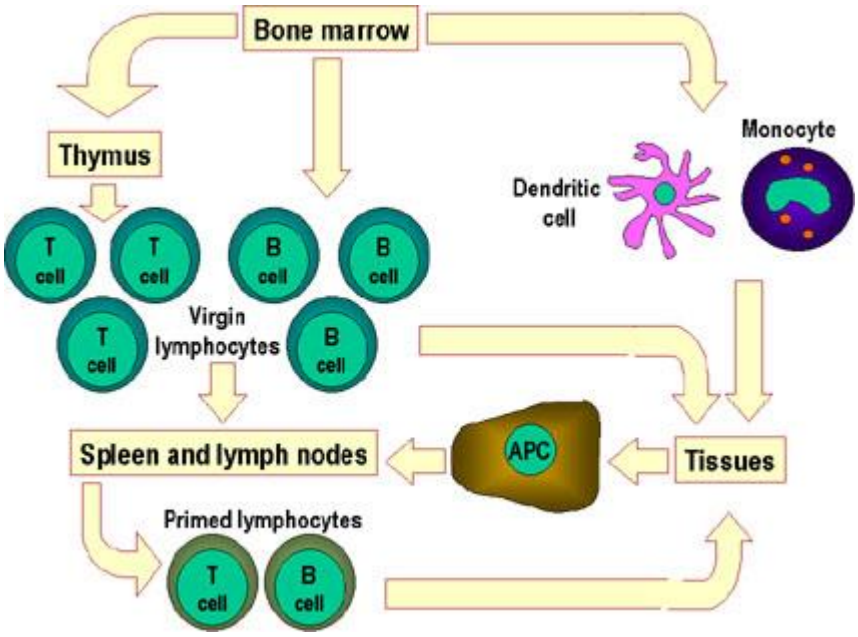

FIGURE 6.1.43

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Virgin lymphocytes from the primary lymphoid tissues such as bone marrow migrate to secondary lymphoid tissues, i.e. the spleen and lymph nodes. Antigen-presenting cells (APCs), including dendritic cells and mononuclear phagocytes (monocytes), also derive from bone marrow stem cells. These APCs enter tissues, take up antigen and transport it to the lymphoid tissues to be presented to T cells and B cells. Primed lymphocytes then migrate from the lymphoid tissues and accumulate preferentially at sites of infection and inflammation

Lymphocyte Recirculation

Since there are relatively few T or B lymphocytes with a receptor for any particular antigen (1/10,000 – 1/100,000), the chances for a successful encounter between an antigen and the appropriate lymphocyte are slim. However, the chances for a successful encounter are greatly enhanced by the recirculation of lymphocytes through the secondary lymphoid organs. Lymphocytes in the blood enter the lymph nodes and percolate through the lymph nodes (Figure 6.1.42). If they do not encounter an antigen in the lymph node, they leave via the lymphatics and return to the blood via the thoracic duct. It is estimated that 1-2% of lymphocytes recirculate every hour. If the lymphocytes in the lymph nodes encounter an antigen, which has been

transported to the lymph node via the lymphatics, the cells become activated, divide and differentiate to become a plasma cell, Th or Tc cell. After several days the effector cells can leave the lymph nodes via the lymphatics and return to the blood via the thoracic duct and then make their way to the infected tissue site.

Naive (virgin) lymphocytes enter the lymph nodes from the blood via High Endothelial Venules (HEVs) Homing receptors on the lymphocytes direct the cells to the HEVs. In the lymph nodes, lymphocytes with the appropriate antigen receptor encounter antigen, which has been transported to the lymph nodes by dendritic cells or macrophages. After activation the lymphocytes express new receptors that allow the cells to leave the lymph node and re-enter the circulation. Receptors on the activated lymphocytes recognize cell adhesion molecules expressed on endothelial cells near the site of an infection and chemokines produced at the infection site help attract the activated cells (Figure 6.1.43).

IMMUNITY: CONTRASTS BETWEEN NON-SPECIFIC AND SPECIFIC

Non-specific (natural, native, innate)

- System in place prior to exposure to antigen
- Lacks discrimination among antigens
- Can be enhanced after exposure to antigen through effects of cytokines

Specific (acquired, adaptive)

- Induced by antigen
- Enhanced by antigen
- Shows fine discrimination

The hallmarks of the specific immune system are memory and specificity.

- The specific immune system "remembers" each encounter with a microbe or foreign antigen, so that subsequent encounters stimulate increasingly effective defence mechanisms.

•The specific immune response amplifies the protective mechanisms of non-specific immunity, directs or focuses these mechanisms to the site of antigen entry, and thus makes them better able to eliminate foreign antigens.

CELLS OF THE IMMUNE SYSTEM

All cell types in the immune system originate from the bone marrow.

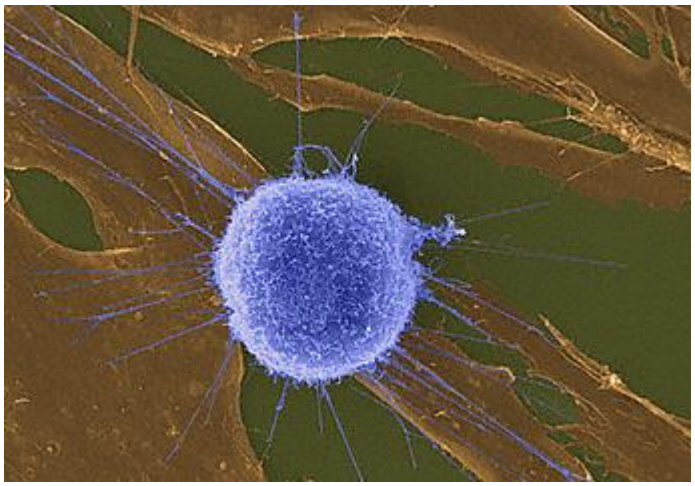

FIGURE 6.1.44A HUMAN T-LYMPHOCYTE ATTACKING FIBROBLAST TUMOUR / CANCER CELLS (SEM X4,000)

Courtesy: Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

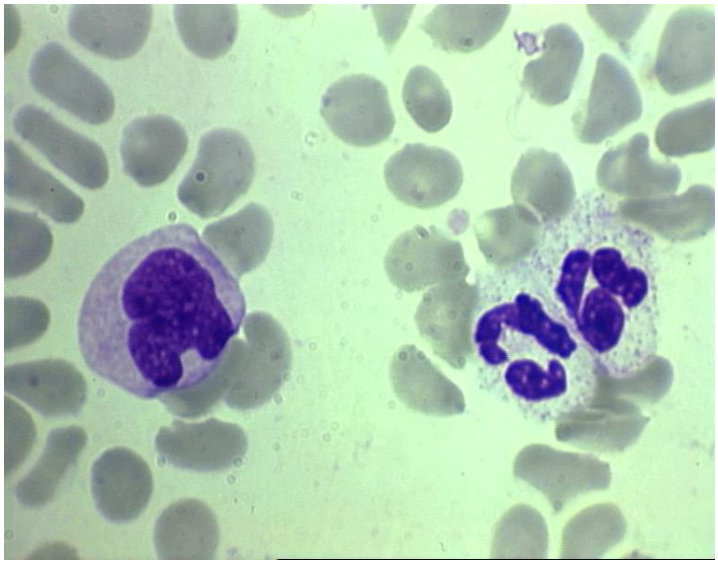

FIGURE 6.1.44B BLOOD FILM SHOWING A MONOCYTE (LEFT) AND TWO NEUTROPHILS

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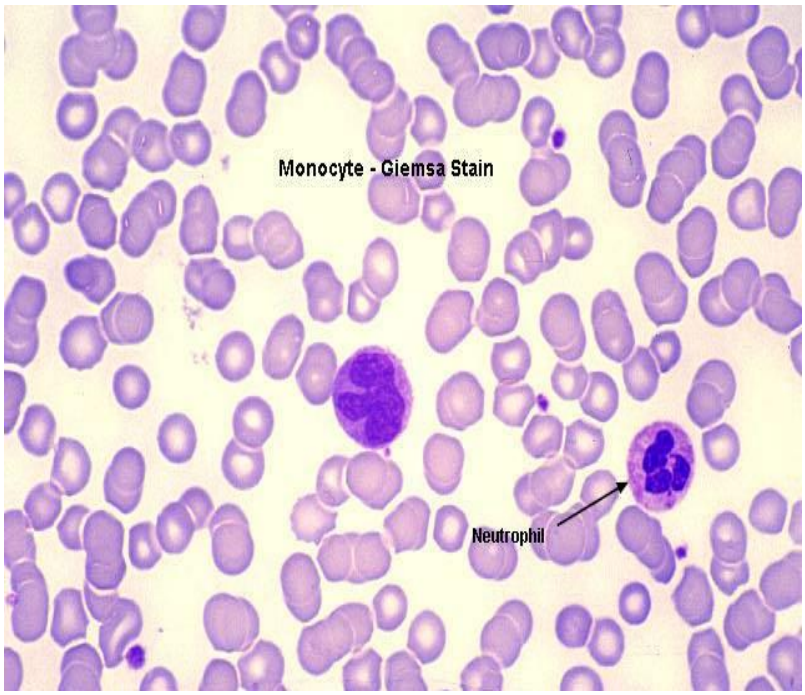

FIGURE 6.1.44C MONOCYTE, GIEMSA STAINED PERIPHERAL BLOOD FILM

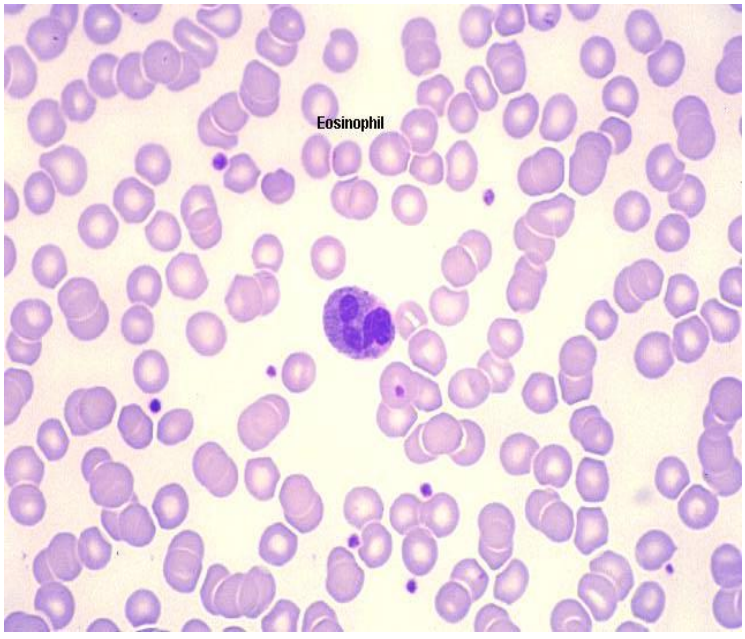

FIGURE 6.1.44D EOSINOPHIL, GIEMSA STAINED PERIPHERAL BLOOD FILM

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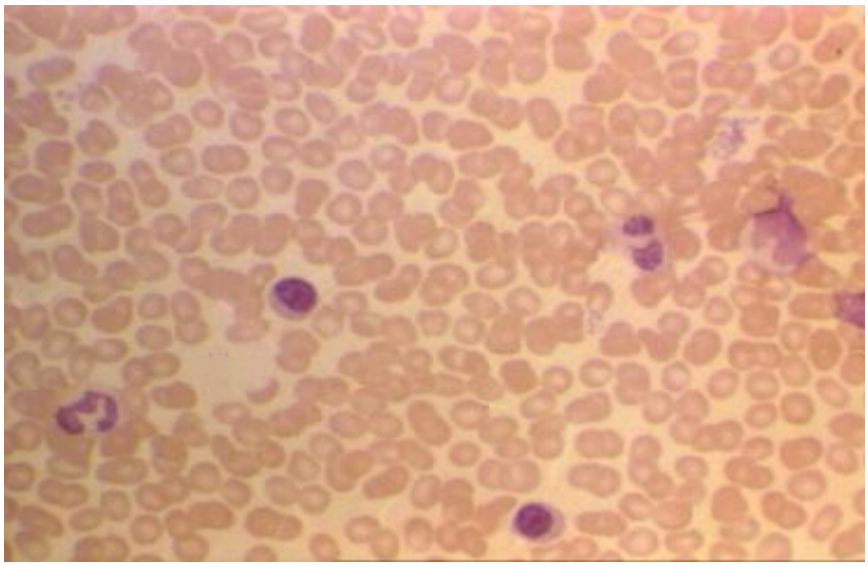

FIGURE 6.1.44E BLOOD FILM SHOWING SMALL LYMPHOCYTES

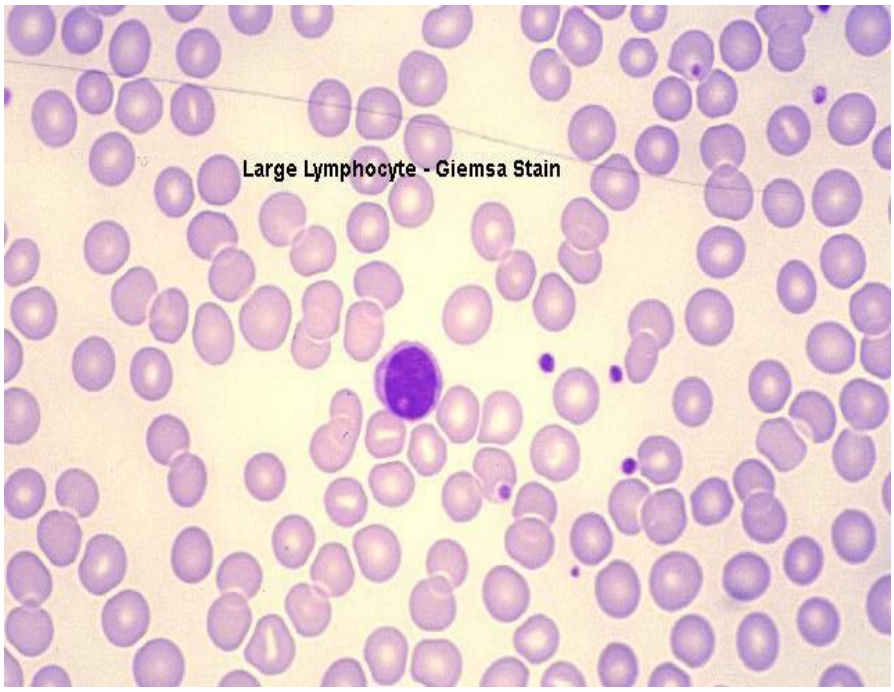

FIGURE 6.1.44F LARGE LYMPHOCYTE, GIEMSA STAINED PERIPHERAL BLOOD FILM

Courtesy Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

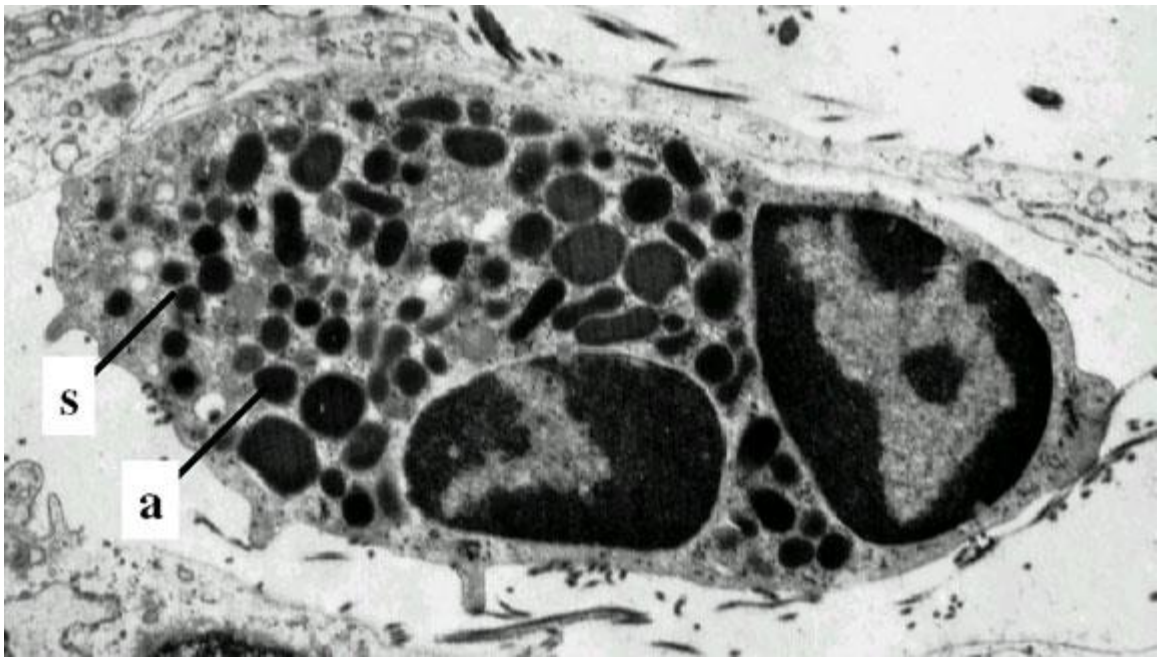

FIGURE 6.1.44G NEUTROPHIL - ELECTRON MICROGRAPH. NOTE THE TWO NUCLEAR LOBES AND THE AZUROPHILIC GRANULES

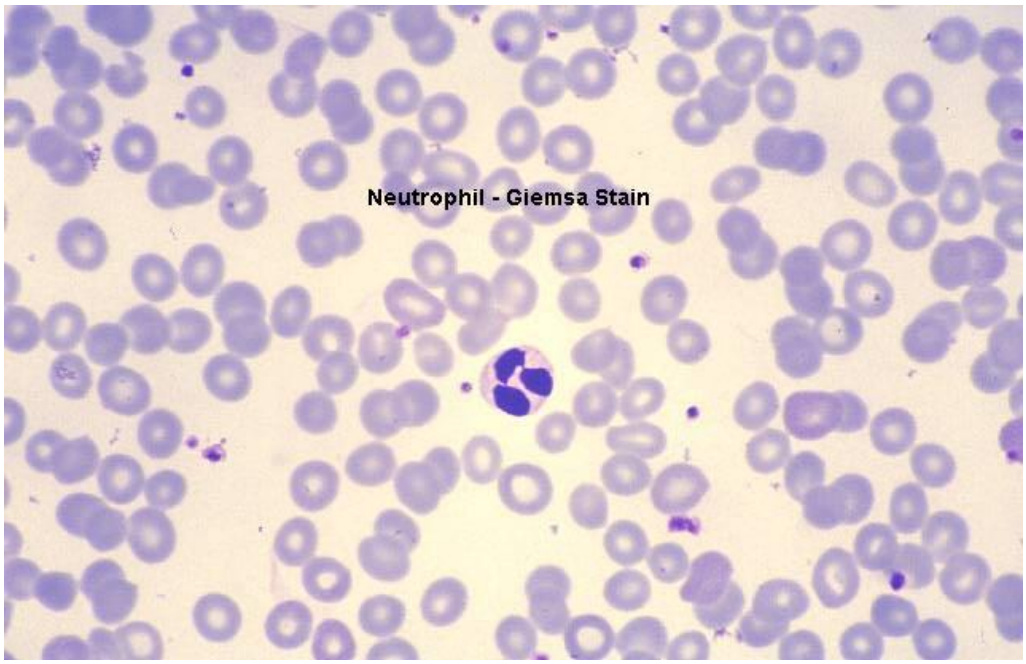

FIGURE 6.1.44H NEUTROPHIL, GIEMSA STAINED PERIPHERAL BLOOD FILM

Courtesy Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

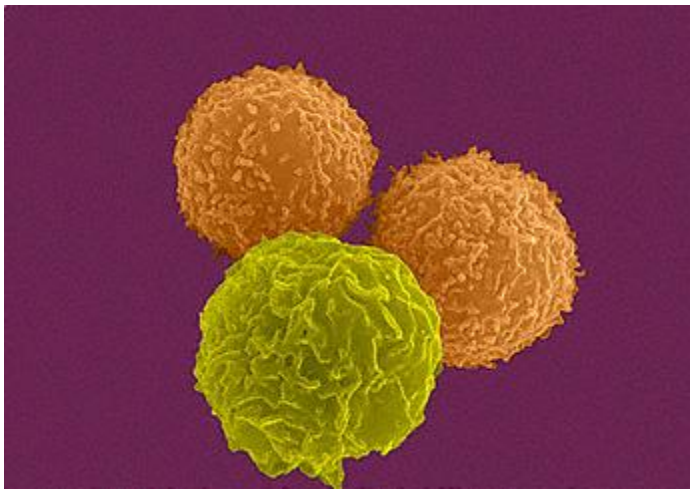

FIGURE 6.1.44I T LYMPHOCYTES (PRE-T CELLS) AND GRANULOCYTE (NEUTROPHIL).

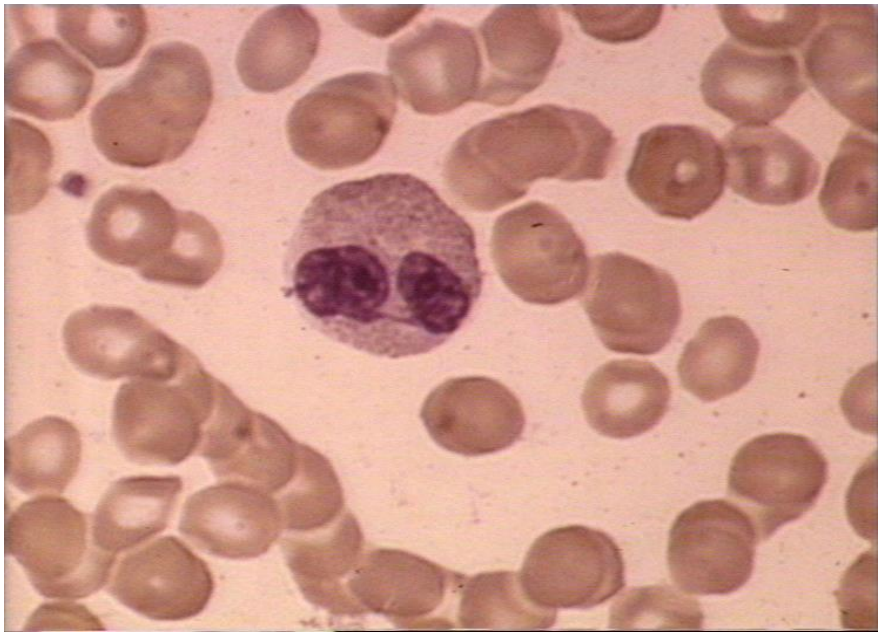

FIGURE 6.1.44J EOSINOPHIL IN BLOOD FILM

COURTESY GENE MAYER, PH.D EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY UNIVERSITY OF SOUTH CAROLINA

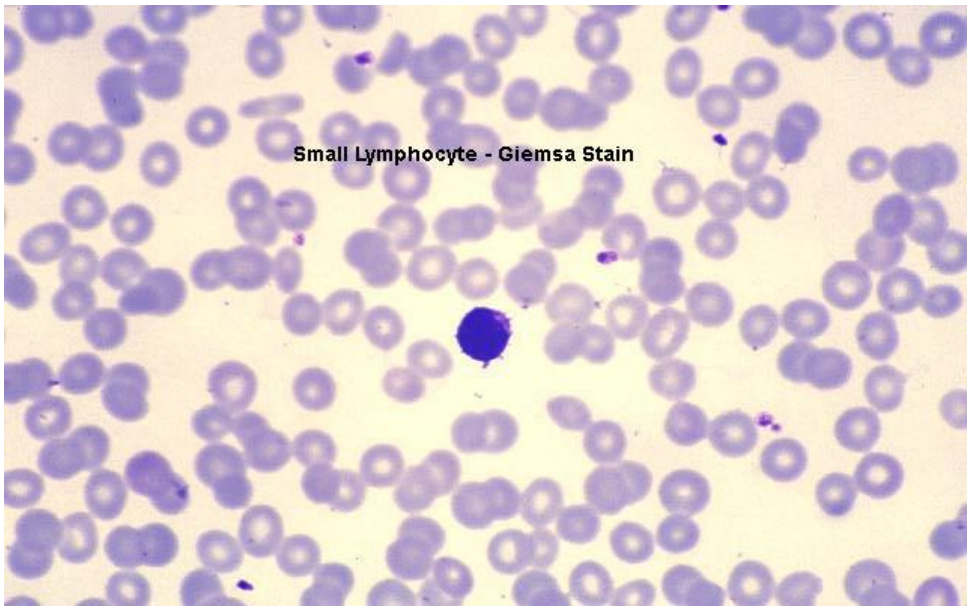

FIGURE 6.1.44 SMALL LYMPHOCYTE, GIEMSA STAINED PERIPHERAL BLOOD FILM

COURTESY GENE MAYER, PH.D EMERTIUS PROFESSOR OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY UNIVERSITY OF SOUTH CAROLINA

There are two main lineages that derive from the haemopoietin stem cell:

- The lymphoid lineage
  - T lymphocytes (T cells)
  - B lymphocytes (B cells)
  - Natural killer cells (NK cells)

- The myeloid lineage
  - Monocytes, macrophages
  - Langerhans cells, dendritic cells
  - Megakaryocytes
  - Granulocytes (eosinophils, neutrophils, basophils)

Clonal selection The four basic principles of the clonal selection hypothesis
Each lymphocyte bears a single type of receptor of a unique specificity
Interaction between a foreign molecule and a lymphocyte receptor capable of binding that molecule with high affinity leads to lymphocyte activation
The differentiated effector cells derived from an activated lymphocyte will bear receptors of an identical specificity to those of the parental cell from which that lymphocyte was derived
Lymphocytes bearing receptors specific for self-molecules are deleted at an early stage in lymphoid cell development and are therefore absent from the repertoire of mature lymphocytes

4.0 CONCLUSION

Immunology is the study of our protection from foreign macromolecules or invading organisms and our responses to them. These invaders include viruses, bacteria, protozoa or even larger parasites. In autoimmunity we develop immune responses against our own proteins (and other molecules) and against our own aberrant cells in tumour immunity. Our first line of defence against foreign organisms is barrier tissues such as the skin that stop the entry of organism into our bodies. A second line of defence is the specific or adaptive immune system which may take days to respond to a primary invasion. In the specific immune system, we see the production of antibodies (soluble proteins that bind to foreign antigens) and cell-mediated responses in which specific cells recognize foreign pathogens and destroy them.

5.0 SUMMARY

In this unit we have studied the following on immunology

- Nature of antigen-antibody reactions
- Affinity and Avidity
- Specificity and cross reactivity
- Tests for antigen-antibody reactions
- Applications
- Antibody formation

6.0 TUTORED MARKED ASSIGNMENT

a) Clearly differentiate Affinity from Avidity
b) Specificity from cross reactivity in immunological terms
c) Describe the various tests available to demonstrate/exemplify Antigen –antibody reactions
d) Concisely discuss the haematological application of this Health and public health establishments
e) How are antibodies formed?

7.0 REFERENCES AND SUGGESTED FURTHER READINGS

Benjamin E. Sunshine G. Leskowitz S: *Immunology*: a short course, ed 3, New York, 1996.Willey-Liss.

Constantine NT, Lana DP: Immunoassay for the diagnosis of infectious diseases. In Murray PR, Baron EJ Jorgensen JH, et al editors, *Manuals of clinical microbiology* ed. 8 Washington DC. 2003. ASM Press.

Detrick B. Hamilton RG Folds JD: *Manual of molecular and clinical laboratory immunology*, ed 7 , Washington , DC, 2006, ASM Press.

Dr Peter Darben, Queensland University of Technology clinical parasitology collection.

Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

SECTION G MYCOLOGY **MODULE 7**

UNIT 1: **THE ACTINOMYCES**

1.0: INTRODUCTION TO THE ACTINOMYCETES

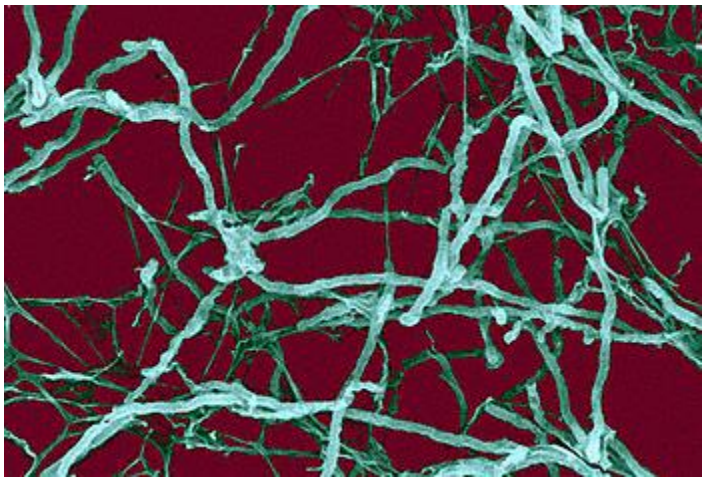

FIGURE 7.1.1 STREPTOMYCETES (ACTINOMYCES)

Courtesy: Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control

Streptomyces spp. -Gram-positive, filamentous or irregular-shaped prokaryote; used in the production of the antibiotic streptomycin. Causes Madura foot and myeloma. In this section, we shall discuss three genera of actinomycetes: Actinomyces, Nocardia, and Streptomyces. These organisms have been shown to be higher bacteria, but they were thought to be fungi for many years because they have filamentous forms, 0.5 to 0.8 microns in diameter, which appear to branch (figure 6.2.1). Some species form aerial mycelia in culture. The clinical manifestations of infection are similar to those of a systemic fungal

infection. It is now clear that they are not fungi but are closely related to the mycobacteria. Some properties of these genera include:

Actinomyces are anaerobic, while Nocardia and Streptomyces are aerobic.

Nocardia stain partially acid-fast, Actinomyces and Streptomyces are not acid-fast.

Actinomyces and Streptomyces produce granules. Most actinomycetes in tissue do not stain with the H & E stain commonly used for general histopathology. All genera may produce granules. Actinomyces almost always produce granules.

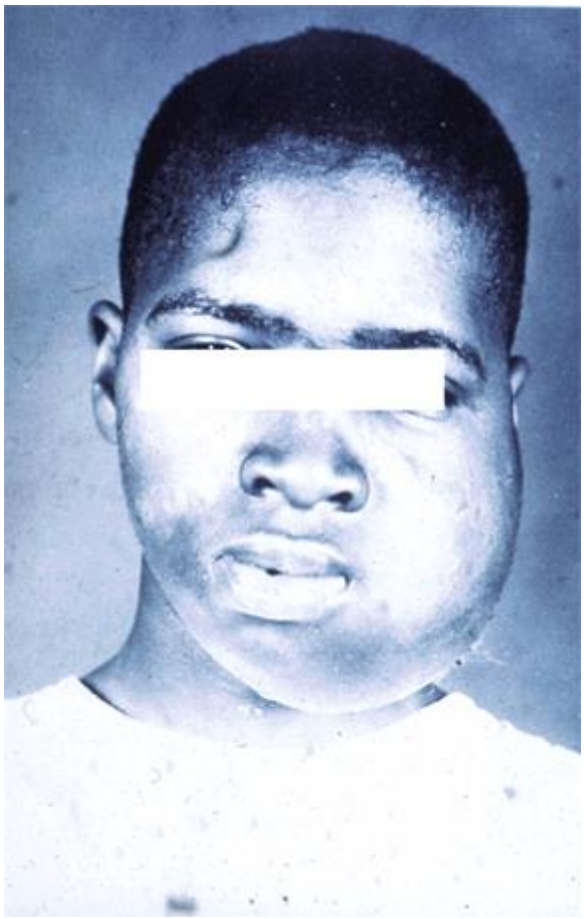

FIGURE 7.1.2

Actinomycosis, Cervicofacial

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control

2.0: OBJECTIVE

At the end of this unit you should be able to describe the following on actinomycetes genera:

- Know the Actinomyces
- Nocardia
- Streptomyces
- Actinomycosis
- Nocardiosis
- Streptomycosis

3.0: CONTENTS

3.1: INTRODUCTION TO The Genera – “Actinomyces”

3.2: Actinomycosis

3.3: Nocardiosis

3.4: Streptomycosis

3.2: ACTINOMYCOSIS

Actinomycosis is a chronic suppurative and granulomatous disease of the cervico-facial, thoracic or abdominal areas.

The most common cause of Actinomycosis is the organism Actinomyces israelii which infects both man and animals. In cattle, the disease is called "lumpy jaw" (figure 7.1.1) because of the huge abscess formed in the angle of the jaw. In man, A. israelii is an endogenous organism that can be isolated from the mouths of healthy people. Frequently, the infected patient has a tooth abscess or a tooth extraction and the endogenous organism becomes established in the traumatized tissue and causes a suppurative infection. These abscesses are not confined to the jaw and may also be found in the thoracic area and abdomen. The patient usually presents with a pus-draining lesion, so the pus will be the clinical material sent to the laboratory. This diagnosis can be made on the hospital floor. When the vial of pus is rotated, the yellow sulphur granules, characteristic of this organism, can be seen with the naked eye. These granules can also be seen by running sterile water over the gauze used to cover the lesion. The water washes away the purulent material leaving the golden granules on the gauze. This organism, which occurs worldwide, can be seen histologically as "sulphur granules" (figure 7.1.2 and 7.1.3) surrounded by

polymorphonuclear cells (PMN) forming the purulent tissue reaction. The organism is a gram positive rod that frequently branches. The laboratory must specifically be instructed to culture for this anaerobic organism. These lesions must be surgically drained prior to antibiotic therapy and the drug of choice is large doses of penicillin.

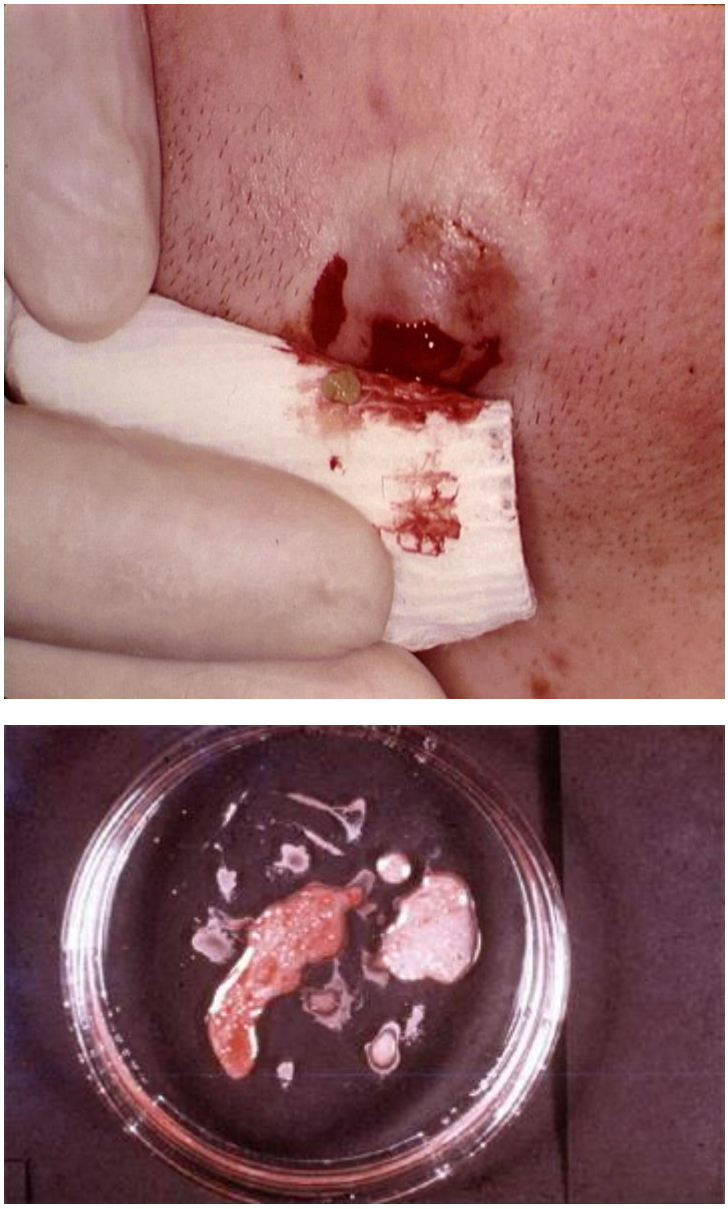

Figure 7.1.3:Sulphur granules in Actinomycosis

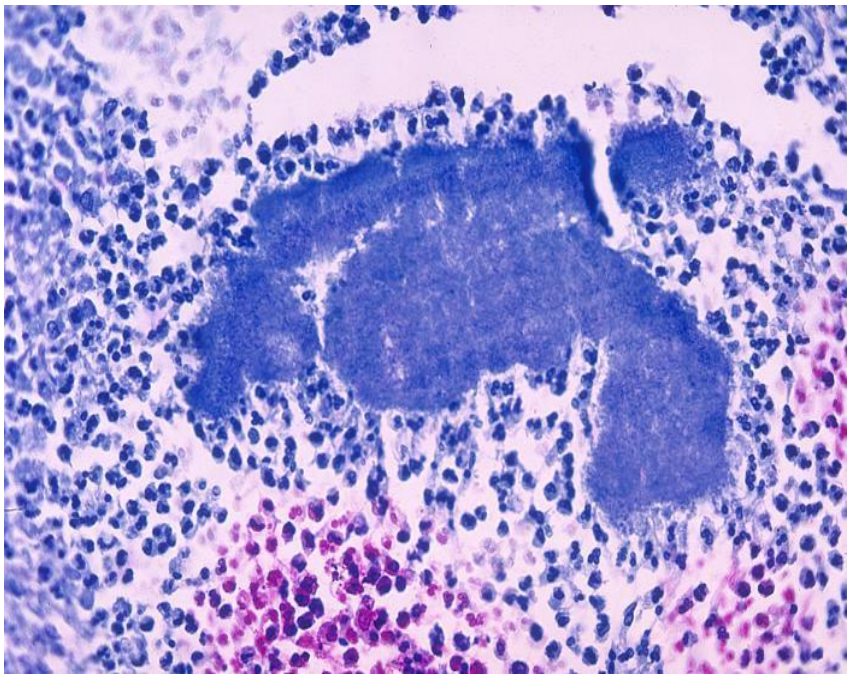

Figure 7.1.4a Histopathologic changes due to the gram-positive organism, Actinomyces israelii. Using a modified Fite-Faraco stain, a “sulphur granule” is shown in the middle of the image. These granules actually represent colonies of A. israelii, a gram-positive, anaerobic filamentous bacterium.

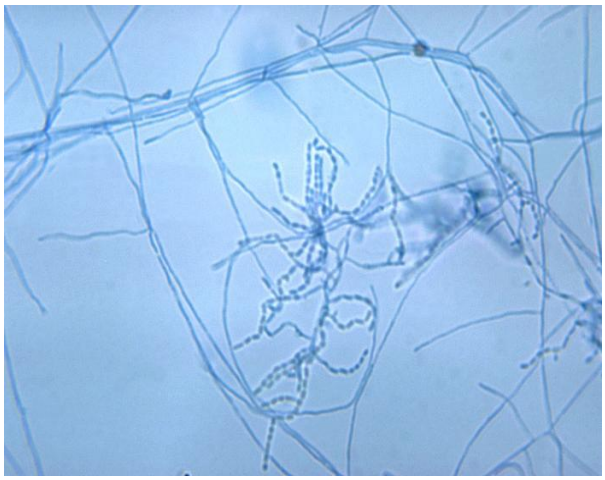

FIGURE 7.1.4B GRAM-POSITIVE AEROBIC NOCARDIA ASTEROIDS SLIDE CULTURE REVEALS CHAINS OF AMONGST AERIAL MYCELIA.

COURTESY: DR ART DISALVO
EMERITUS DIRECTOR, NEVADA STATE LABORATORY
EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL

3.3 NOCARDIOSIS

Nocardiosis primarily presents as a pulmonary disease or brain abscess in the U.S. In Latin America, it is more frequently seen as the cause of a subcutaneous infection, with or without draining abscesses. It can even present as a lesion in the chest wall that drains onto the surface of the body similar to Actinomycosis. Brain abscesses are frequent secondary lesions.

The most common species of Nocardia that cause disease in human beings are N. brasiliensis and N. asteroides. These are soil organisms which can also be found endogenously in the sputum of apparently healthy people. N. asteroides (figure 7.1.4a) is usually the etiologic agent of pulmonary nocardiosis (figure 7.1.4b) while N. brasiliensis (figure 7.1.5) is frequently the cause of sub-cutaneous lesions. The material sent to the lab, depending on the presentation of the disease, is sputum, pus, or biopsy material. These organisms rarely form granules. The Nocardia are aerobic, gram-positive rods and stain partially acid-fast (i.e., the acid-fast staining is not uniform) (figure 7.1.6). There are no serological tests, and the drug of choice is Bactrim (Trimethoprim plus sulfamethoxazole). The Nocardia grow readily on most bacteriologic and TB media. The geographic distribution of these organisms is worldwide.

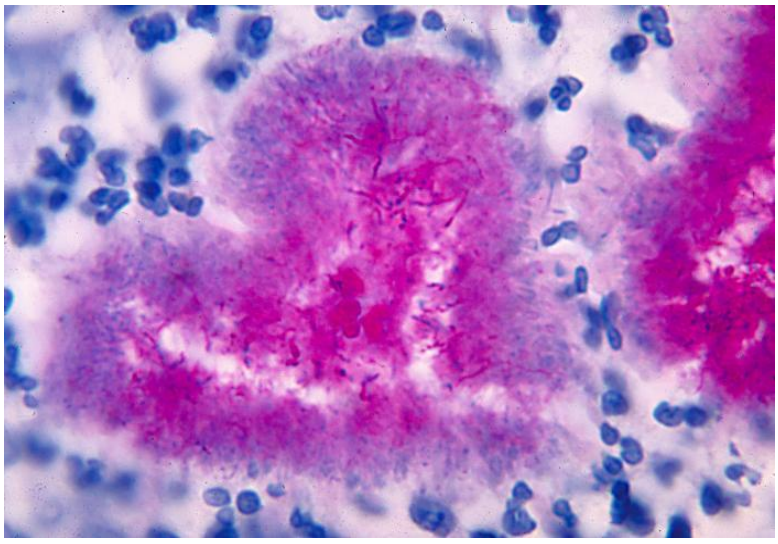

FIGURE 7.1.6B GRAM-POSITIVE ACID-FAST NOCARDIA BRASILIENSIS BACTERIA USING A MODIFIED FITE-FARACO STAIN. 80% OF CASES OF NOCARDIOSIS SHOW CLINICAL FEATURES OF INVASIVE PULMONARY INFECTION, DISSEMINATED DISEASE, OR BRAIN ABSCESS; 20% SHOW CELLULITIS.

COURTESY: DR ART DISALVO
EMERITUS DIRECTOR, NEVADA STATE LABORATORY
EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL

IN THE UNITED STATES AN ESTIMATED 500 - 1,000 NEW CASES OF NOCARDIOSIS INFECTION OCCUR ANNUALLY.

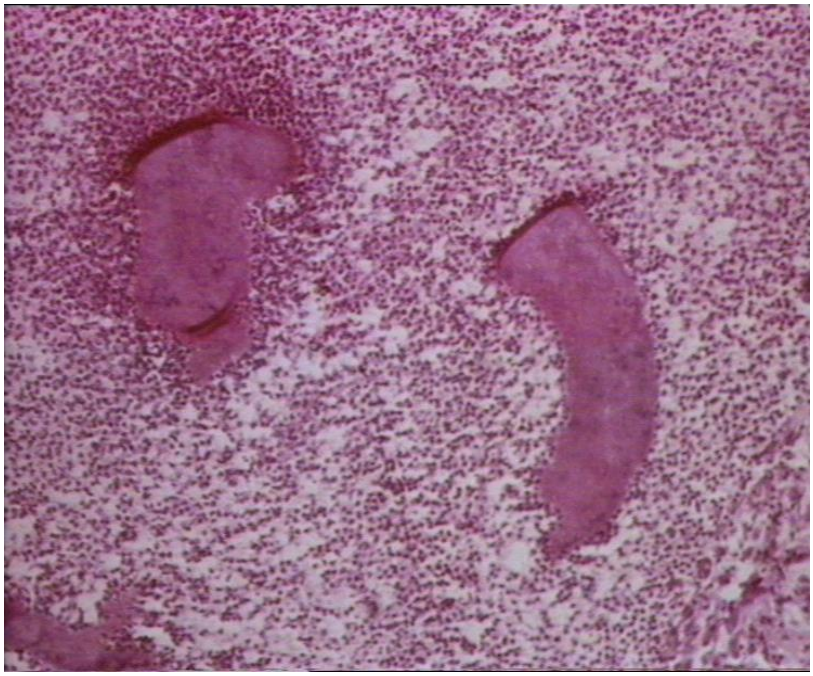

FIGURE 7.1.7 PLEURISY DUE TO NOCARDIOSIS

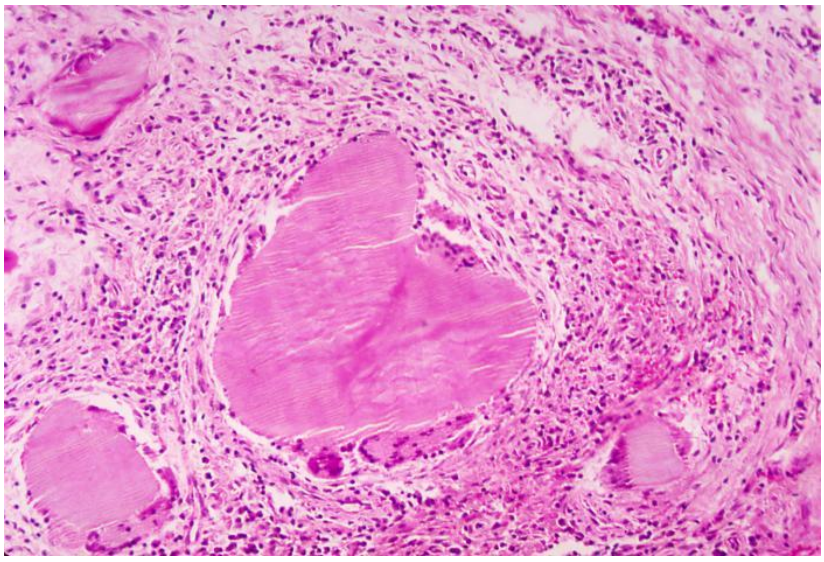

FIGURE 7.1.8 : ACTINOMYCOTIC MYCETOMATOUS GRANULE DUE TO THE BACTERIA STREPTOMYCES SOMALIENSIS. STREPTOMYCES SPP. ARE GRAM-POSITIVE AEROBIC ACTINOMYCETES KNOWN FOR THEIR PRODUCTION OF ANTIMICROBIAL SUBSTANCES. THOUGH THEY SELDOM CAUSE HUMAN DISEASE, INFECTIONS CAN MANIFEST AS LOCALIZED, CHRONIC SUPPURATIVE LESIONS OF THE SKIN.

Courtesy :

Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control

3.4 STREPTOMYCOSIS

The Streptomyces species usually cause the disease entity known as mycetoma (fungus tumour). These infections are usually subcutaneous, but they can penetrate deeper and invade the bone. Some species produce a protease which inhibits macrophages. Material sent to the lab is pus or skin biopsy. The streptomycetes are aerobic like Nocardia, and can grow on both bacterial and fungal (Sabouraud) media. They produce a chalky aerial mycelium with much branching. It is important to let the lab know the suspected organism because most bacterial pathogens will grow out overnight, but the actinomycetes take longer to be visible on the culture plates (48-72 hours). The various species of streptomyces produce granules of different size (figure 6.2.6), texture and colour. These granules, along with colonial growth and biochemical tests, allow the bacteriologist or mycologist to identify each species. The organisms are found world-wide. There are no serological tests, and the drugs of choice are the combination of sulfamethoxazole/trimethoprim or amphotericin B. In the tropics this disease may go undiagnosed or untreated for so long that surgical amputation may be the only effective treatment.

4.0 **CONCLUSION:** Three genera of actinomycetes: Actinomyces, Nocardia, and Streptomyces, have been shown to be higher bacteria, but they were thought to be fungi for many years because they have filamentous forms, 0.5 to 0.8 microns in diameter, which appear to branch. Some species form aerial mycelia in culture. The clinical manifestations of infection are similar to those of a systemic fungal infection. Actinomyces are anaerobic, while Nocardia and Streptomyces are aerobic. Nocardia stain partially acid-fast, Actinomyces and Streptomyces are not acid-fast. Actinomyces and Streptomyces produce granules. All genera may produce granules. They are Gram positive rods.

5.0: SUMMARY

In this unit we have learnt about the following:

- Introduction to the genera – "Actinomycetes"
- Key issues in Actinomycosis
- Key issues in Nocardiosis
- Key issues in Streptomycosis

6.0 Tutored Marked Assignments

1) Briefly discuss the genera –Actinomycetes

2) Write short notes on the following;

a) Actinomycosis

b) Nocardiosis

c) Streptomycosis

3) How would you carry out a quick diagnosis of the actinomycetes genera in the clinic?

7.0 References and Suggested further Readings

Benjamin E. Sunshine G. Leskowitz S: *Immunology*: a short course, ed 3, New York, 1996.Willey-Liss.

Constantine NT, Lana DP: Immunoassay for the diagnosis of infectious diseases. In Murray PR, Baron EJ Jorgensen JH, et al editors, *Manuals of clinical microbiology* ed. 8 Washington DC. 2003. ASM Press.

Detrick B. Hamilton RG Folds JD: *Manual of molecular and clinical laboratory immunology*, ed 7 , Washington , DC, 2006, ASM Press.

Dr Peter Darben, Queensland University of Technology clinical parasitology collection.

Dr Art DiSalvo
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Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control

UNIT 2:

1.0: Introduction to Yeasts

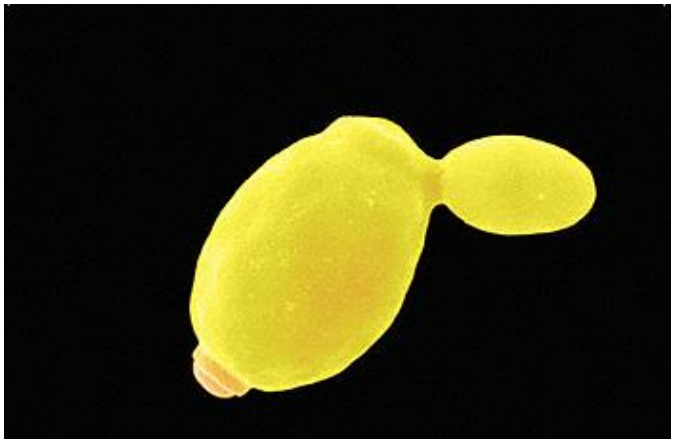

FIGURE 7.2.1 BREWER'S YEAST (ALSO KNOWN AS BAKER'S YEAST) WITH BUD AND BUD SCARS (SACCHAROMYCES CEREVISIAE).

COURTESY :DENNIS KUNKEL MICROSCOPY, INC.

Yeasts are single-celled budding organisms (figure 7.2.1). They do not produce mycelia. The colonies are usually visible on the plates in 24 to 48 hours. Their soft, moist colonies resemble bacterial cultures rather than moulds. There are many species of yeasts that can be pathogenic for humans. We shall only discuss the three most significant species:

- Candida albicans
- Cryptococcus neoformans
- Cryptococcus gattii

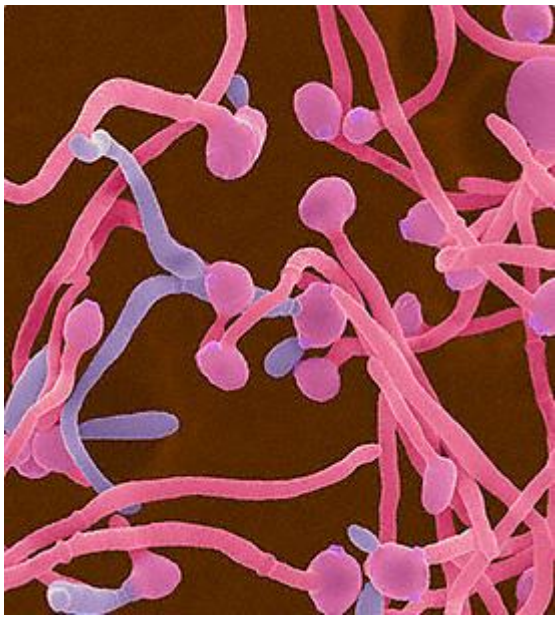

FIGURE 7.2.2 ***CANDIDA ALBICANS*** **- YEAST AND HYPHAE STAGES. A YEAST-LIKE FUNGUS COMMONLY OCCURRING ON HUMAN SKIN, IN THE UPPER RESPIRATORY, ALIMENTARY & FEMALE GENITAL TRACTS. THIS FUNGUS HAS A DIMORPHIC LIFE CYCLE WITH YEAST AND HYPHAL STAGES. THE YEAST PRODUCES HYPHAE (STRANDS) AND PSEUDOHYPHAE. THE PSEUDOHYPHAE CAN GIVE RISE TO YEAST CELLS BY APICAL OR LATERAL BUDDING. CAUSES CANDIDIASIS WHICH INCLUDES THRUSH (AN INFECTION OF THE MOUTH AND VAGINA) AND VULVO-VAGINITIS.**

Courtesy :Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

2.0 OBJECTIVES

At the end of this unit you should be able to freely discuss the following:

- Candidiasis
- Cryptococcosis
- Dermatophytes
- Chromoblastomycosis
- Mycetoma
- Mucormycosis
- Aspergillosis

3.0 CONTENTS

3.2 CANDIDIASIS (*Candida albicans*)

There are more than 20 species of the genus Candida that cause disease. The infections caused by all species of Candida are called candidiasis.

Candida albicans (figure 7.2.1 and 7.2.2) is an endogenous organism and the most common species in human infections. It can be found in 40 to 80% of normal human beings. It is present in the mouth (figure 7.2.4), gut, and vagina. It may be present as a commensal or a pathogenic organism.

- Candidiasis of the mouth (oropharyngeal candidiasis) is called thrush which is seen as white patches on the mucosa of the mouth including the tongue. The affected area can become inflamed and may cause difficulty in swallowing. Cracking and inflammation may occur around the mouth. This is referred to as oral cheilitis. Oral candidiasis may spread to the oesophagus (esophagitis). Although most people harbor Candida species, oral candidiasis is typically found in immuno-compromised individuals. These include people infected with HIV (9 to 31% of AIDS patients) or who have received immunosuppressive drugs for cancer chemotherapy (20%) and organ transplantation. Other factors that have been associated with oral candidiasis are diabetes, certain dentures and the use of corticosteroids. CDC estimates that between 5 and 7% of neonates develop oral candidiasis. Untreated oral candidiasis can lead to serious invasive disease. Treatment for the oral form consists of clotrimazole tablets or a nystatin suspension. If this is not effective,

especially in the case of oesophageal involvement, fluconazole or itraconazole. Some forms are resistant to these drugs in which case amphotericin B may be used.

- Genital or vulvovaginal candidiasis (yeast infection) cause genital itching, a burning sensation and vaginal discharge in females. In men, the penis may have an itching rash. This is rare in men but most women will have at least once episode of vulvovaginal candidiasis. Women are more at risk of the infection if they are:
    a. Pregnant
    b. Diabetic
    c. Use broad spectrum antibiotics
    d. Use corticosteroids

- Invasive candidiasis (candidemia) is a serious disease when Candida, which is normally on the skin or the gastro-intestinal tract, enters the bloodstream where it can disseminate to other organs. Symptoms include fever and chills that do not respond to anti-bacterial agents. These are often nosocomial infections of people who:
    a. have a central venous catheter
    b. are immunosuppressed
    c. take broad-spectrum antibiotics
    d. show neutropenia
    e. are on haemodialysis
    f. have diabetes

There are about 46,000 cases of candidemia each year in the United States and the disease shows high rates of morbidity and mortality. In hospital conditions with already sick people, mortality due to candidemia may be as high as 19-24%. Diagnosis of candidemia is by culture of the organism from blood.

Infections with Candida usually occur when a patient has some alteration in cellular immunity, normal flora or normal physiology. Patients with decreased cellular immunity have decreased resistance to fungal infections. Prolonged antibiotic or steroid therapy destroys the balance of normal flora in the intestine allowing the endogenous Candida to overcome the host. Invasive procedures, such as cardiac surgery and indwelling catheters, produce alterations in host physiology and some of these patients develop Candida infections. Although it most frequently infects the skin and mucosae, Candida

can cause pneumonia, septicaemia or endocarditis in the immuno-compromised patient.

The establishment of infection with Candida species appears to be a property of the host - not the organism. The more debilitated the host, the more invasive the disease.

The clinical material to be sent to the lab depends on the presentation of the disease: Such material may include blood cultures, vaginal discharge, urine, faeces, nail clippings or material from cutaneous or mucocutaneous lesions.

Candida is polymorphic yeast, i.e., yeast cells, hyphae and pseudohyphae are produced. It has been shown that Candida needs a transcription repressor to maintain the yeast form. This ability to assume various forms may be related to the pathogenicity of this organism. The yeast form is 10 to 12 microns in diameter, gram positive, and it grows overnight on most bacterial and fungal media. It also produces germ tubes (figure 7.2.9 and 7.2.10), and pseudohyphae (figure 7.2.6 and 7.2.7) may be formed from budding yeast cells that remain attached to each other. Spores may be formed on the pseudomycelium. These are called chlamydospores and they can be used to identify different species of Candida. Some mycologists think that the pseudomycelial form represents a more invasive form of the organism. The species are identified by biochemical reactions. The organism occurs world-wide.

The drugs of choice for systemic infection are itraconazole and fluconazole. If an artificial heart valve or in-dwelling catheter becomes infected, it must be replaced. Drug therapy alone will not suppress the organism if the foreign body remains in the host. This resistance is due to biofilms which we will discuss later.

CANDIDA SPECIES

Candida species (other than albicans) account for an increasing number of nosocomial infections. Speciation is important because there is significant antibiotic resistance among the different species.

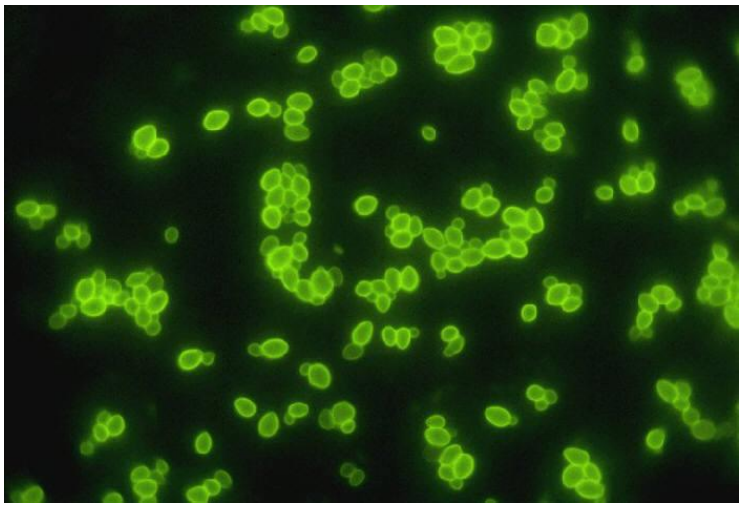

FIGURE 7.2.3 OVAL BUDDING YEAST CELLS OF CANDIDA ALBICANS. FLUORESCENT ANTIBODY STAIN.

Courtesy: **Dr Art DiSalvo**
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

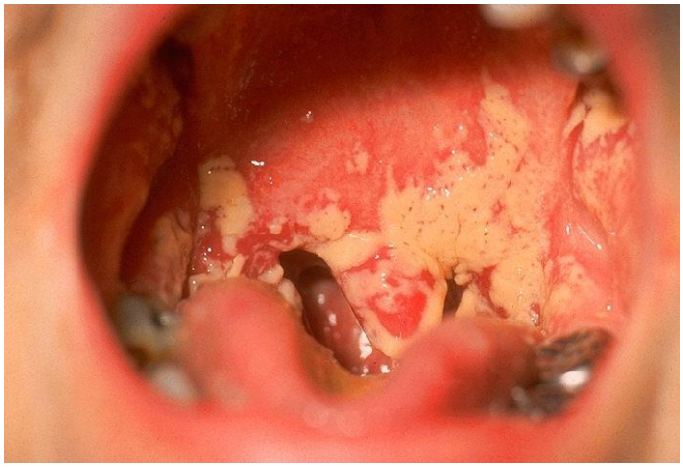

FIGURE 7.2.4 ORAL THRUSH.

Courtesy :DR ART DISALVO
EMERITUS DIRECTOR, NEVADA STATE LABORATORY, RENO
EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

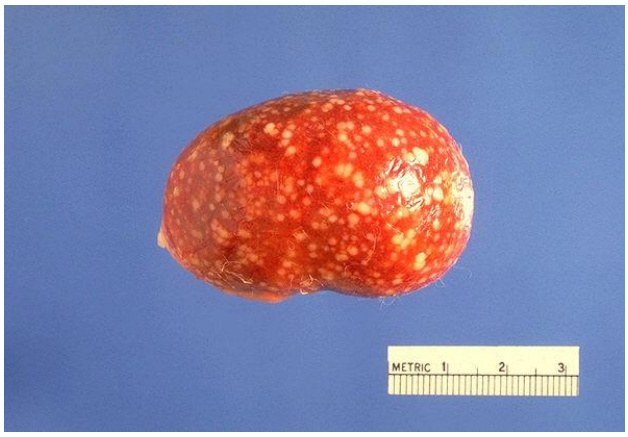

FIGURE 7.2.5 GROSS PATHOLOGY OF RABBIT KIDNEY LESIONS DUE TO EXPERIMENTAL CANDIDA ALBICANS INFECTION. RABBIT WAS CORTISONE-TREATED. CDC

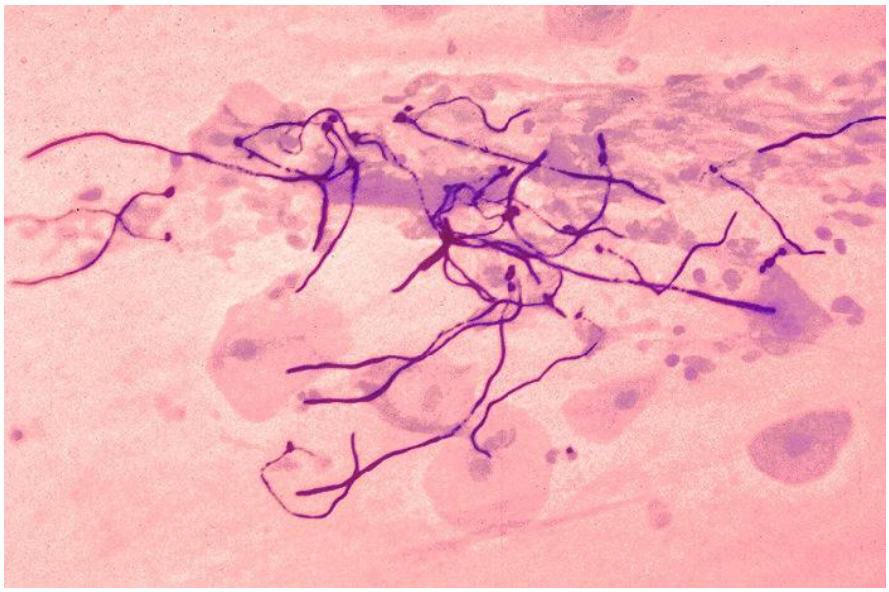

FIGURE 7.2.6 SPUTUM SMEAR FROM PATIENT WITH PULMONARY CANDIDIASIS. GRAM STAIN.

Courtesy: Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

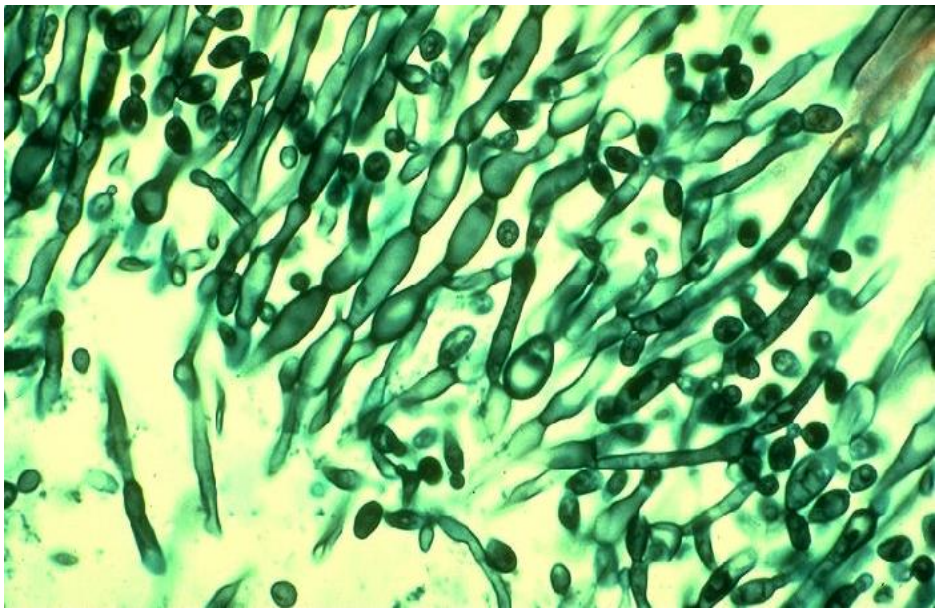

FIGURE 7.2.7 HISTOPATHOLOGY OF CANDIDA ALBICANS INFECTION. METHENAMINE SILVER AE AND TRUE HYPHAE. **DR ART DISALVO**
EMERITUS DIRECTOR, NEVADA STATE LABORATORY, RENO
EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

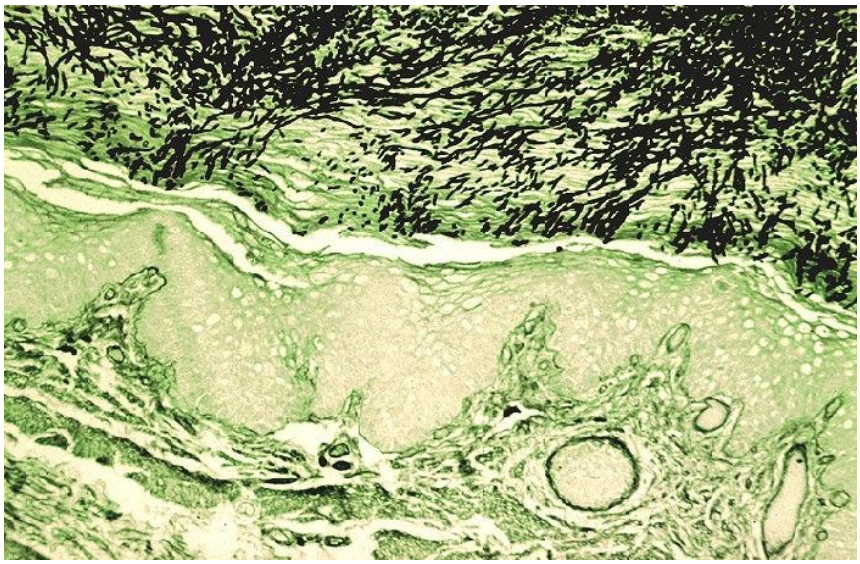

FIGURE 7.2.8 HISTOPATHOLOGY OF CANDIDA ESOPHAGITIS. METHENAMINE SILVER STAIN (DIGITALLY COLORIZED).

Courtesy **Dr Art DiSalvo**
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

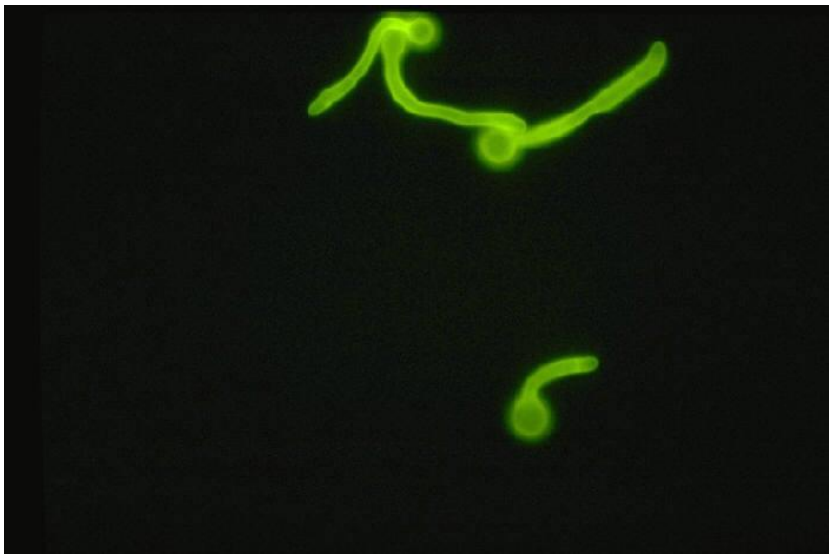

FIGURE 7.2.9 CANDIDA ALBICANS SHOWING GERM TUBES. CALCOFLUOR WHITE STAIN IN PEPTONE MEDIUM. GERM TUBE PRODUCTION IS A DIAGNOSTIC FEATURE OF C. ALBICANS.

Courtesy : DR ART DISALVO EMERITUS DIRECTOR, NEVADA STATE LABORATORY, RENO
EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

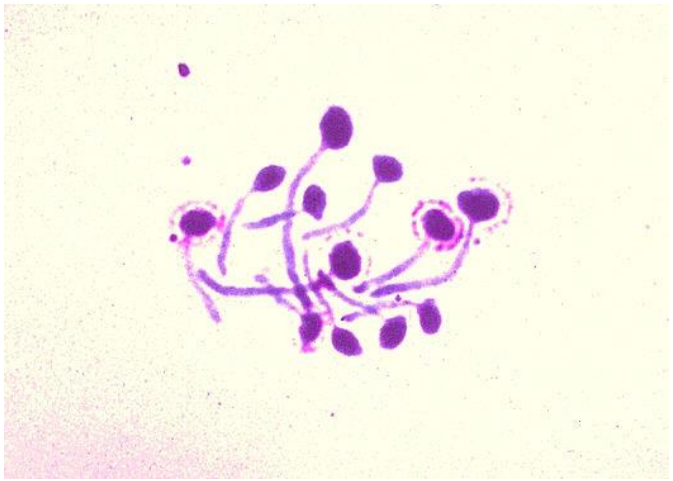

FIGURE 7.2.9 CANDIDA ALBICANS SHOWING GERM TUBE PRODUCTION IN SERUM. GRAM STAIN.

Courtesy: Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

FIGURE 7.2.10 GRAM-STAIN OF VAGINAL SMEAR SHOWING CANDIDA ALBICANS EPITHELIAL CELLS AND MANY GRAM-NEGATIVE RODS. (1,000X OIL)

DR ART DISALVO
EMERITUS DIRECTOR, NEVADA STATE LABORATORY, RENO
EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

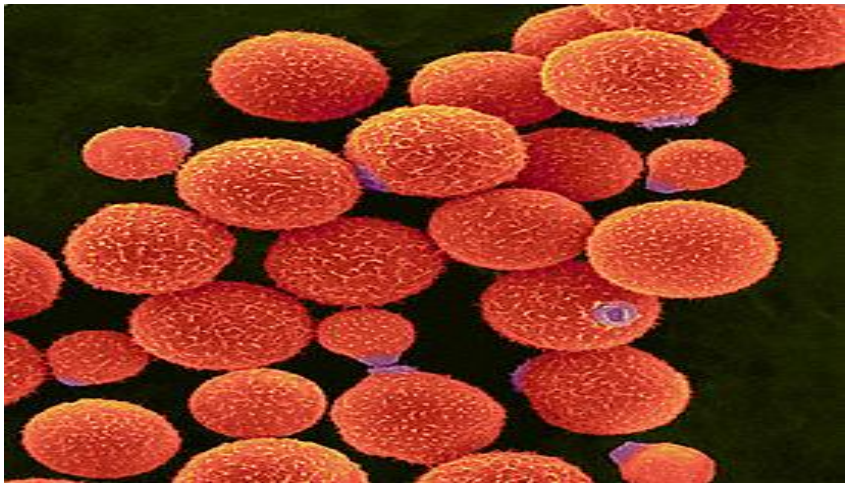

FIGURE 7.2.11 ENCAPSULATED PATHOGENIC YEAST FUNGUS (CRYPTOCOCCUS NEOFORMANS). A YEAST-LIKE FUNGUS THAT REPRODUCES BY BUDDING. A ACIDIC MUCOPOLYSACCHARIDE CAPSULE COMPLETELY ENCLOSES THE FUNGUS. IT CAN CAUSE THE DISEASE CALLED CRYPTOCOCCOSIS; ESPECIALLY IN IMMUNE DEFICIENT HUMANS, SUCH AS IN PATIENTS WITH HIV / AIDS. THE INFECTION MAY CAUSE MENINGITIS IN THE LUNGS, SKIN OR OTHER BODY REGIONS. THE MOST COMMON CLINICAL FORM IS MENINGOENCEPHALITIS. IT IS CAUSED BY INHALING THE FUNGUS FOUND IN SOIL THAT HAS BEEN CONTAMINATED BY PIGEON DROPPINGS.

Courtesy **Dr Art DiSalvo**
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

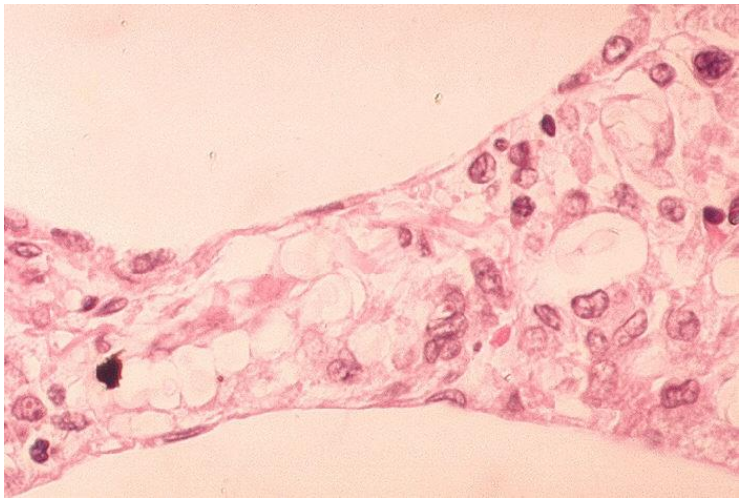

FIGURE 7.2.12 CRYPTOCOCCOSIS OF LUNG IN PATIENT WITH AIDS. HISTOPATHOLOGY OF LUNG SHOWS WIDENED ALVEOLAR SEPTUM CONTAINING A FEW INFLAMMATORY CELLS AND NUMEROUS YEASTS OF CRYPTOCOCCUS NEOFORMANS

Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

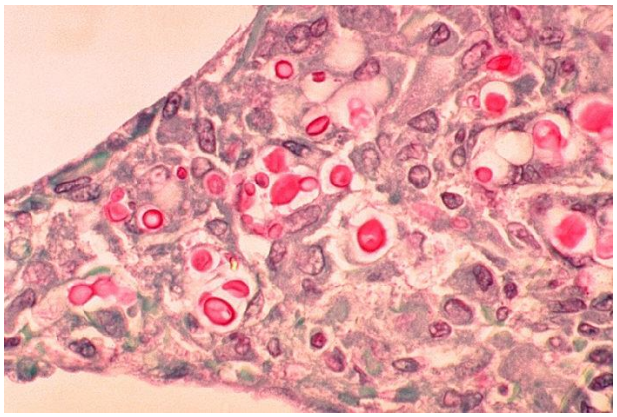

FIGURE 7.2.13 CRYPTOCOCCOSIS OF LUNG IN PATIENT WITH AIDS. MUCICARMINE STAIN. HISTOPATHOLOGY OF LUNG SHOWS WIDENED ALVEOLAR SEPTUM CONTAINING A FEW INFLAMMATORY CELLS AND NUMEROUS YEASTS OF CRYPTOCOCCUS NEOFORMANS. THE INNER LAYER OF THE YEAST CAPSULE STAINS RED.

Courtesy: **Dr Art DiSalvo**
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

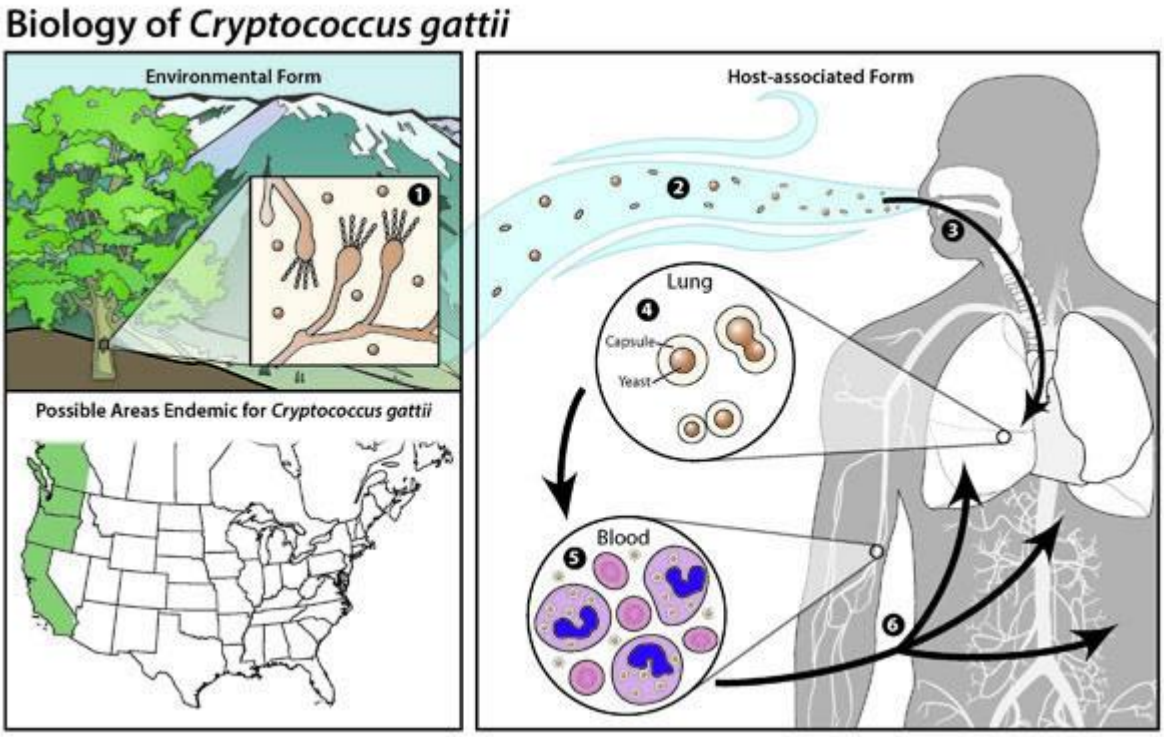

FIGURE 7.2.14A LIFE CYCLE OF CRYPTOCOCCUS GATTI.

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EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

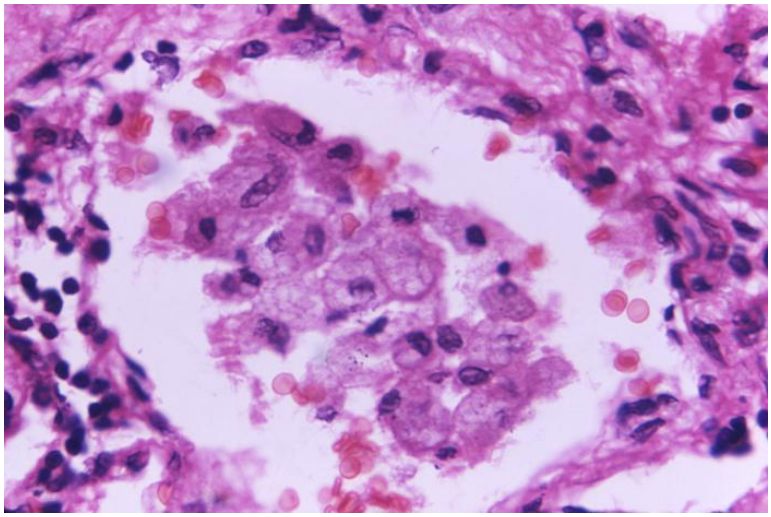

FIGURE 7.2.14B A LUNG LESION TISSUE SPECIMEN, WITH MORPHOLOGY ASSOCIATED WITH THE DISEASE CRYPTOCOCCOSIS DUE TO THE INFILTRATION OF CRYPTOCOCCUS. SP. FUNGAL ORGANISMS.

Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

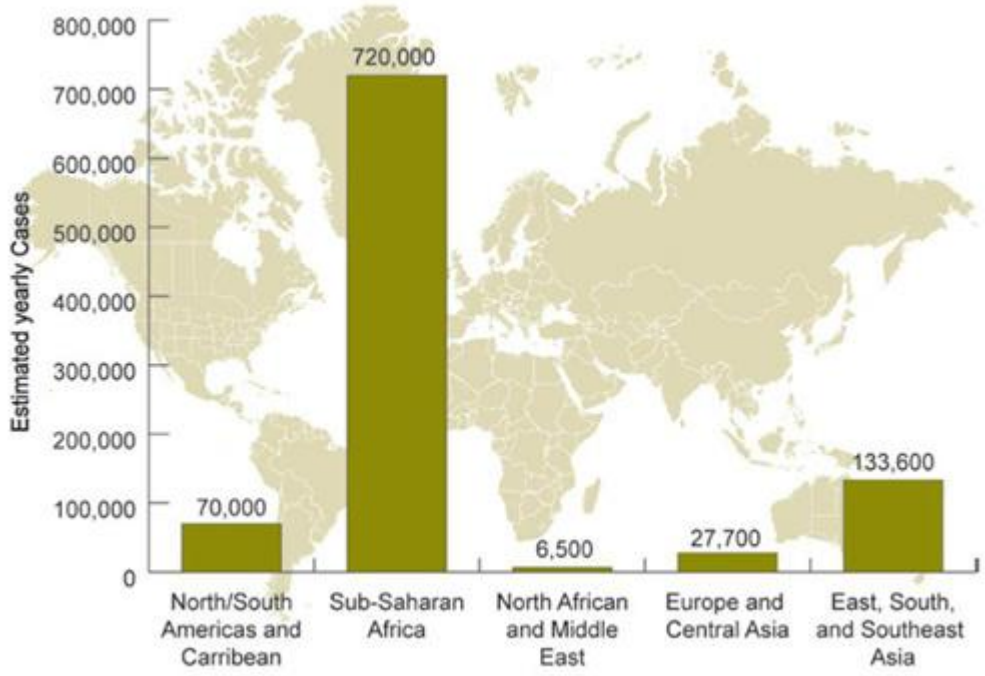

FIGURE 7.2.14C GLOBAL BURDEN OF HIV-RELATED CRYPTOCOCCAL MENINGITIS

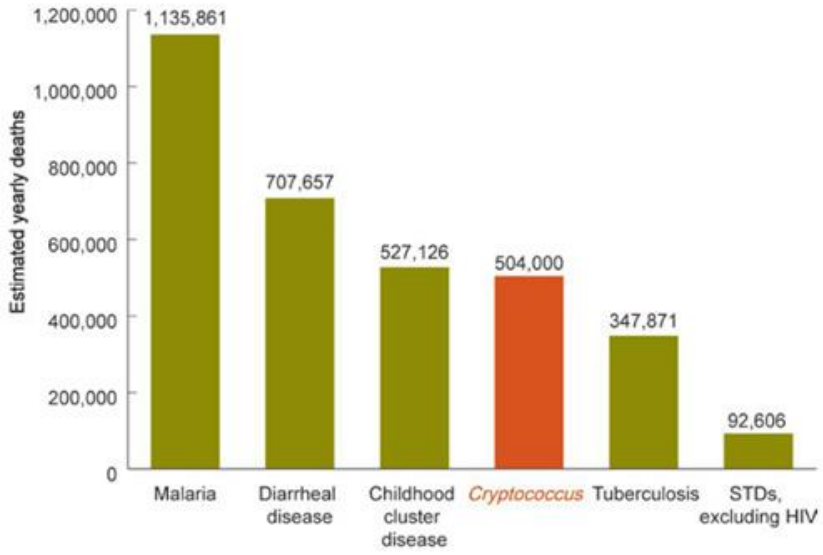

FIGURE 7.2.14D CAUSES OF DEATH IN SUB-SAHARAN AFRICA, EXCLUDING HIV/AIDS

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

3.3 : CRYPTOCOCCOSIS

Cryptococcus neoformans

Cryptococcosis manifests itself most commonly as meningitis but in recent years many cases of pulmonary disease have been recognized. Most infections are, however, asymptomatic.

C. neoformans is a very distinctive yeast. The cells, which are spherical and 3 to 7 microns in diameter (figure 7.3.12), produce buds that characteristically are narrow-based and the organism is surrounded by a polysaccharide capsule (figure 7.3.14).

There is evidence that the capsule may suppress T-cell function and can be considered a virulence factor. *C. neoformans* also produces an enzyme called phenol oxidase which appears to be another virulence factor.

The ecological niche of *C. neoformans* is pigeon and chicken droppings. However, although this organism can be easily recovered from pigeon droppings, a direct epidemiological link has yet to be established between exposure to pigeon droppings and a specific human infection. Infection and disease production is probably a property of the host -- not the organism. The source of human infection is not clear. This organism is ubiquitous, especially in areas such as abandoned buildings contaminated with pigeon droppings.

The portal of entry is the respiratory system. Evidence is developing which indicates that the initial exposure may be many years prior to the manifestation of disease. The organism can be sequestered for this time. Infection may be sub acute or chronic. The highly fatal meningoencephalitis caused by *C. neoformans* has a prolonged evolution of several months. The patient's symptoms may begin with vision problems and headache, which then progress to delirium, nuchal rigidity leading to coma and death unless the physician is thinking about Cryptococcus and does a spinal tap for diagnosis and institutes aggressive therapy. The CSF is examined for its characteristic chemistry (elevated protein and decreased glucose), cells (usually monocytes), and evidence of the organism. The latter is measured by the visual demonstration of the organism (India Ink preparation, figure 7.3.15) or by a serologic assay for the antigen of *C. neoformans*. The India Ink test, which demonstrates the capsule of this yeast, is supplemented by the latex agglutination test for antigen which is more sensitive and more specific. The Latex Agglutination test measures antigen, not antibody. A decreasing titer

indicates a good prognosis, while an increasing titer has a poor prognosis. When you consider Cryptococcosis, think of Capsules and CNS disease.

In addition to causing meningitis, C. neoformans may also infect lungs (figure 7.2.16) and skin. The disease in the lungs and skin is characterized by the formation of a granulomatous reaction with giant cells. As with other fungal diseases, there has been an increase in the recognition of pulmonary infection. The yeast may also form a mass in the mediastinum called a cryptococcoma.

The clinical material sent to the lab is CSF, biopsy material, and urine (for some unexplained reason the organism can be isolated from the urine in both the CNS and systemic infections). This organism will grow overnight on bacterial or fungal media at 37 C. but growth is a little slower at room temperature. In culture, the organism grows as creamy, white, mucoid (because of the capsule) colonies. Growth in culture is usually visible in 24 to 48 hours. As the culture ages, it turns brown due to a melanin produced by the phenol oxidase.

The organism is a round, single cell, yeast surrounded by a capsule. Identification is based on physiological reactions. Pathologists use a mucicarmine stain, which stains the capsule, to identify the organism in tissue sections (Figure 7.2.14). There is usually little or no inflammatory response. The Direct Fluorescent Antibody test identifies the organism in culture or tissue section specifically, by causing the yeast cell wall to stain green. To test the patient's serum there are three serologic tests: The Indirect Fluorescent Antibody test, the Tube Agglutination test for antibody, and the Latex Agglutination test for antigen. The latex agglutination test can be used as a prognostic test. As the patient improves, the serum antigen titer will also decrease.

The geographical distribution of this organism is world-wide and, like other yeast infections, is most commonly seen in immunocompromised patients. There are about 1 million cases of cryptococcal meningitis each year, mostly among HIV-infected AIDS patients, resulting in 625,000 deaths. Most cases are in sub-Saharan Africa (720,000) with 133,000 in southeast Asia and 70,000 in the Americas (figures 7.2.14 c and d).

The drugs of choice to treat Cryptococcus infection are amphotericin B and 5-Fluorocytosine (5-FC). 5-FC is an oral drug. If it is given as the only treatment, there are relapses so most physicians use both drugs simultaneously. These two drugs are synergistic, and thus, their association is advantageous.

Cryptococcus gattii

This is a newly recognized pathogenic species of Cryptococcus. It is found in soil and in association with several species of trees in tropical and sub-tropical regions of the world but has recently been found in the western United States and western Canada. The patient acquires the infection as a result of the inhalation of fungal spores which lodge in the lungs. Here, the spores transform into dividing yeast cells and disseminate to other parts of the body via the bloodstream, sometimes inside macrophages. There have been about 100 United States cases of Cryptococcus gattii infections in the period from 2004 to 2011 and almost all of them have been in the Pacific Northwest. In western Canada, 218 cases were reported between 1999 and 2007.The appearance of symptoms usually occurs several months (average six to seven) after breathing in the spores but in some cases, several years pass before symptoms are observed. The disease is not contagious.

Infection of the lungs is accompanied by

- malaise (fever and headache)
- a cough and shortness of breath
- chest pain

The fungus can spread to the nervous system, including the brain where is causes meningoencephalitis. According to the CDC, there is a long latent time (two to fourteen months) between exposure and the manifestation of symptoms. Cryptococcal meningitis symptoms include:

- Fever
- Headache
- Neck pain
- Nausea
- Light sensitivity
- Confusion

The presence of *Cryptococcus gatti* can lead to the growth of cryptococcomas in various parts of the body.

As with other fungal infections, people at most risk include those with compromised immune systems (AIDS and cancer patients and people on immunosuppressive therapy).

Identification of a Cryptococcus gattii infection is by microscopy (Figure7.2. 14b) after growth in the laboratory. To distinguish Cryptococcus gattii from Cryptococcus neoformans, the organisms are grown on canavanine-glycine-bromthymol blue agar. Only Cryptococcus gattii turns this blue.

The patient requires treatment by anti-fungal agents for up to six months. In severe cases, amphotericin B, often in combination with flucytosine, is recommended. In milder cases, fluconazole or itraconazole is used.

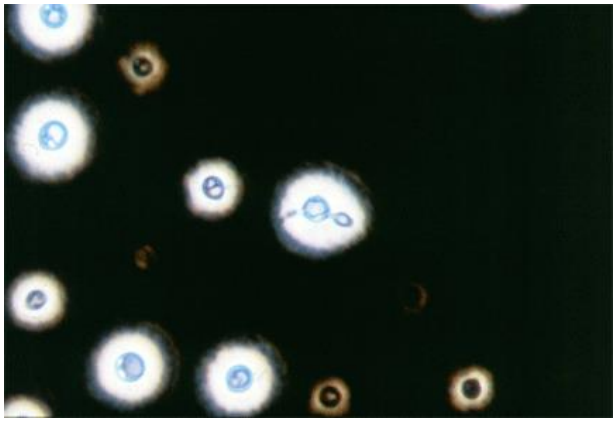

FIGURE 7.2.14 C. NEOFORMANS: INDIA INK PREPARATION

CDC: Adapted from BJ Park *et al*., AIDS 2009;23:525-530

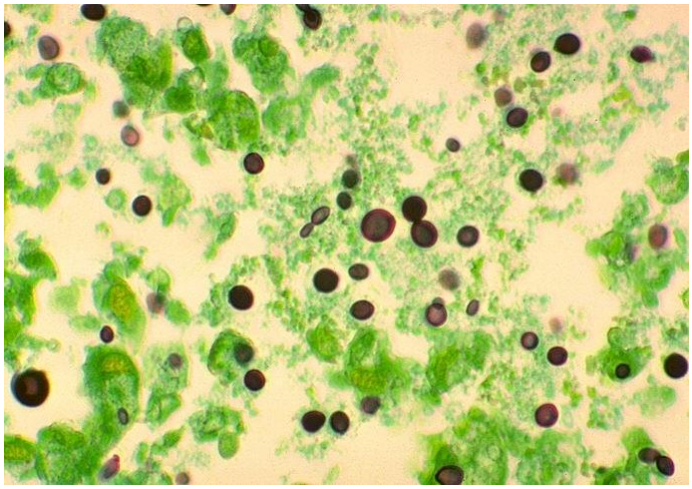

FIGURE 7.2.15 CRYPTOCOCCOSIS OF LUNG IN PATIENT WITH AIDS. METHENAMINE SILVER STAIN. HISTOPATHOLOGY OF LUNG SHOWS NUMEROUS EXTRACELLULAR YEASTS OF CRYPTOCOCCUS NEOFORMANS WITHIN ANALVEOLAR SPACE. YEASTS SHOW NARROW-BASE BUDDING AND CHARACTERISTIC VARIATION IN SIZE.

CDC: Adapted from BJ Park *et al*., AIDS 2009;23:525-530

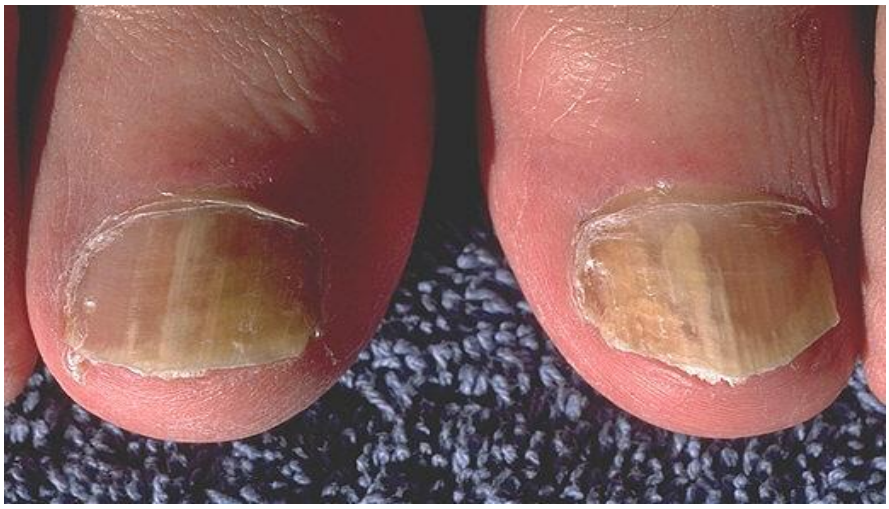

FIGURE 7.2.16 ONYCHOMYCOSIS DUE TO TRICHOPHYTON RUBRUM, RIGHT AND LEFT GREAT TOE. TINEA UNGUIUM.

CDC/Dr. Edwin P. Ewing, Jr. epe1@cdc.gov

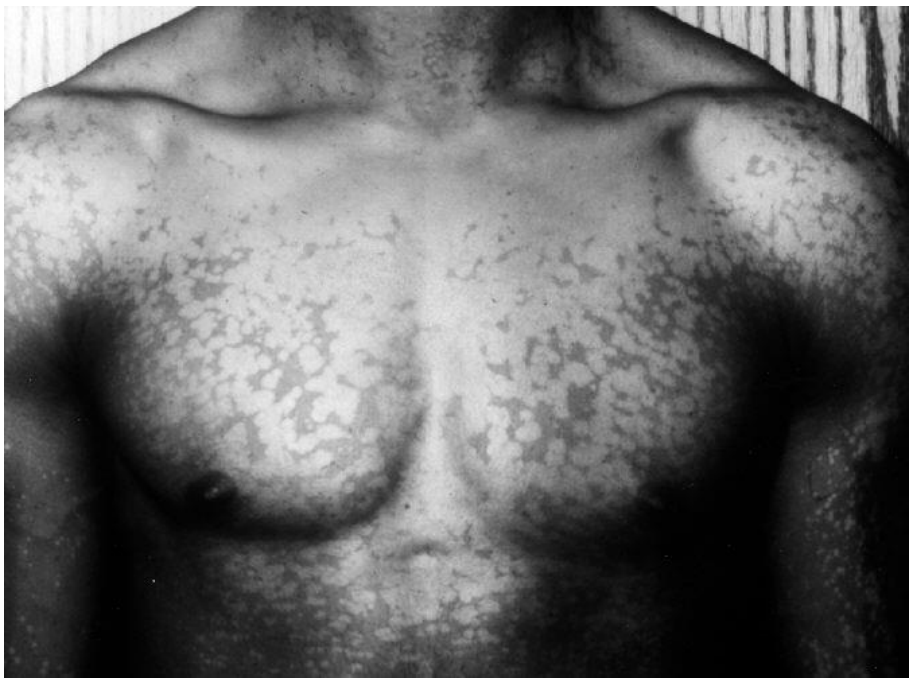

FIGURE 7.2.17 TINEA VERSICOLOR ON CHEST.

Courtesy CDC: Adapted CDC/Dr. Gavin Hart

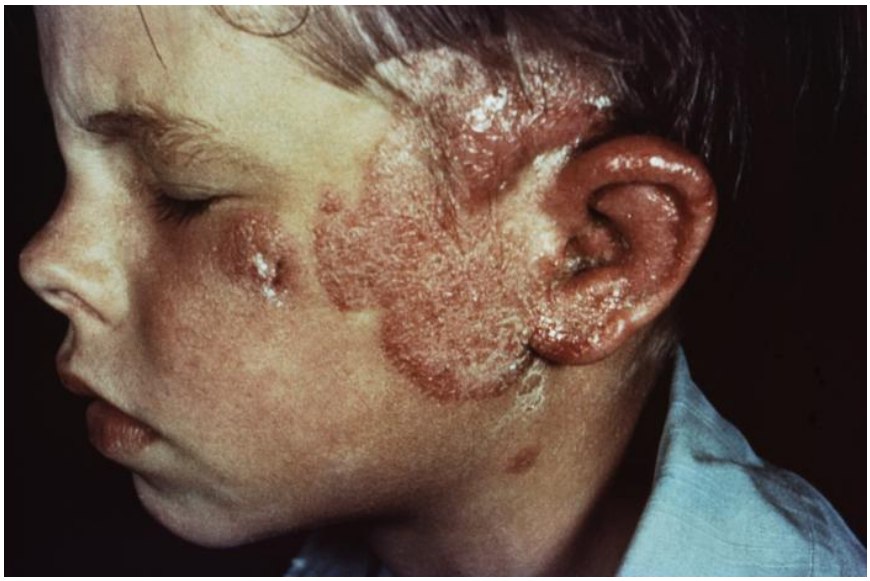

FIGURE 7.2.18 A CHILD WITH A RINGWORM (TINEA) FUNGAL INFECTION ON THE LEFT SIDE OF HIS FACE AND LEFT EAR. "TINEA FACIEI" OR "TINEA CAPITIS" IS THE NAME USED FOR INFECTIONS OF THE FACE CAUSED BY A DERMATOPHYTIC FUNGUS, BUT NOT INCLUDING INFECTION OF THE BEARDED AREAS, WHICH ARE CALLED "TINEA BARBAE". TINEA FACIEI INFECTIONS ARE UNCOMMON, AND ARE OFTEN INITIALLY MISDIAGNOSED.

Courtesy: CDC; Gene Mayer, Ph.D Emertius Professor of Pathology, Microbiology and Immunology University of South Carolina

CLINICAL MANIFESTATIONS OF SOME DERMATOPHYTES

Tinea means "ringworm" or "moth-like". Dermatologists use the term to refer to a variety of lesions of the skin or scalp.

- Tinea corporis - small lesions occurring anywhere on the body (figure 7.2.22, 7.2.23 and 7.2.24).
- Tinea pedis - "athlete's foot". Infection of toe webs and soles of feet.
- Tinea unguium (onychomycosis) - nails. Clipped and used for culture (figure 7.2.17).
- Tinea capitis - head. Frequently found in children (figure 7.2.19 and 7.2.20).
- Tinea cruris - "jock itch". Infection of the groin, perineum or perianal area.
- Tinea barbae - ringworm of the bearded areas of the face and neck (figure 7.2.21).
- Tinea versicolor - Characterized by a blotchy discoloration of skin which may itch. Up to 25% of the general population may have this lesion at any one time. Diagnosis is usually possible by direct microscopic examination of KOH-treated skin scrapings which show a typical aspect of mycelia and spores described as "spaghetti and meatballs." Tinea versicolor is caused by Malassezia furfur (figure 7.2.18).

ECOLOGY

The dermatophytes (which means skin plants) causing human infections may have different natural sources and modes of transmission:

- Anthropophagic - These are usually associated with humans only; transmission from man to man is by close contact or through contaminated objects.
- Zoophilic - These are usually associated with animals; transmission to man is by close contact with animals (cats, dogs, cows) or with contaminated products.
- Geophilic - These are usually found in the soil and are transmitted to man by direct exposure.

Knowledge of the species of dermatophyte and source of infection are important for proper treatment of the patient and control of the source. Invasion by zoophilic or geophilic organisms may cause inflammatory disease in man. Geographic distribution: Dermatophytes occur worldwide, but some species have geographically limited distribution.

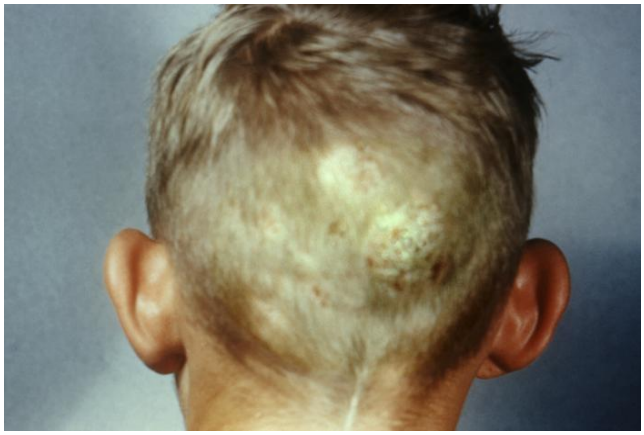

FIGURE 7.2.19 A CHILD WITH RINGWORM OF THE SCALP, CALLED "TINEA CAPITIS", CAUSED BY A *MICROSPORUM SP*.. TINEA CAPITIS IS AN INFECTION OF THE SCALP CAUSED BY MOLD-LIKE FUNGI CALLED DERMATOPHYTES, WHICH THRIVE IN WARM, MOIST AREAS. SUSCEPTIBILITY TO TINEA INFECTION IS INCREASED BY POOR HYGIENE, PROLONGED MOIST SKIN, AND MINOR SKIN OR SCALP INJURIES.

COURTESY : DR ART DISALVO **EMERITUS DIRECTOR, NEVADA STATE LABORATORY, RENO EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA**

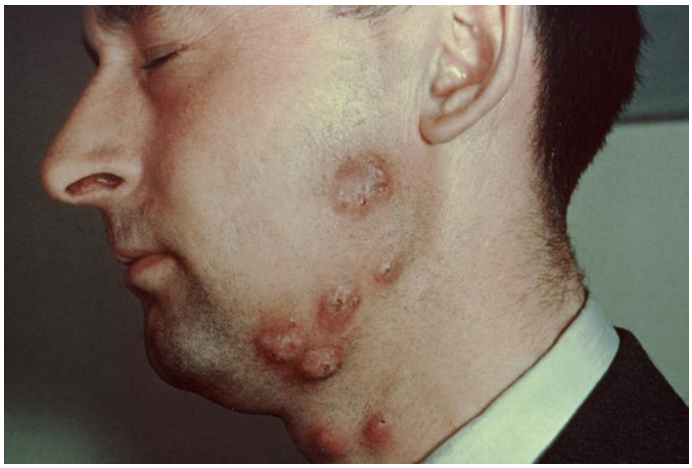

FIGURE 7.2.20 RINGWORM OF THE BEARDED AREAS OF THE FACE AND NECK, KNOWN AS "TINEA BARBAE", OR "BARBER'S ITCH". TINEA BARBAE IS DUE TO A DERMATOPHYTIC INFECTION AROUND THE BEARDED AREA OF MEN. GENERALLY, THE INFECTION OCCURS AS A FOLLICULAR INFLAMMATION, OR AS A CUTANEOUS GRANULOMATOUS LESION, I.E. A CHRONIC INFLAMMATORY REACTION.

COURTESY : CDC : DR ART DISALVO EMERITUS DIRECTOR, NEVADA STATE LABORATORY, RENO EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

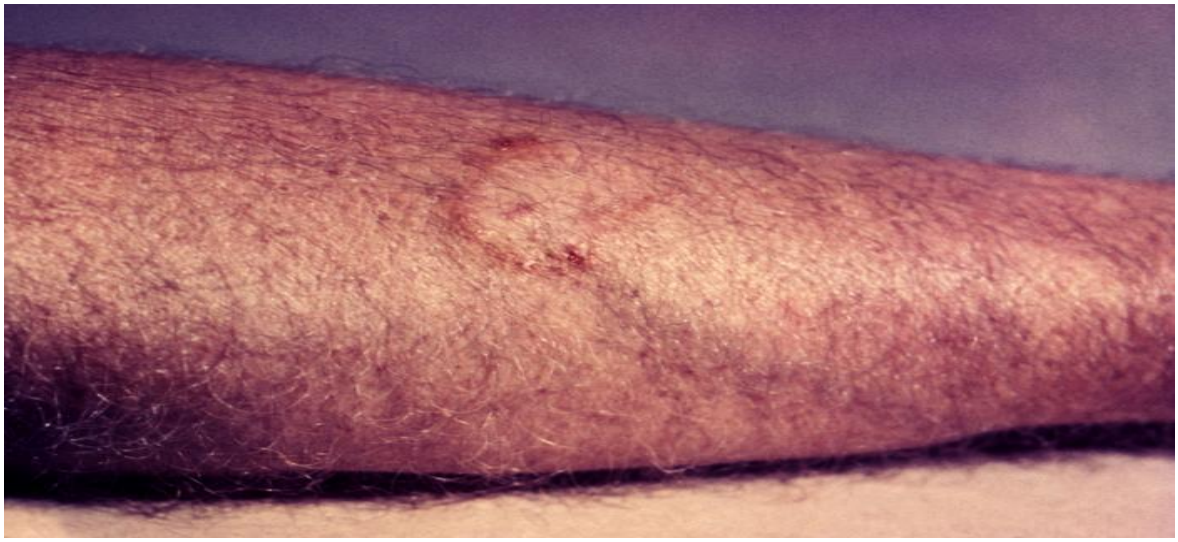

FIGURE 7.2.21 TINEA CORPORIS LESIONS, OR "RINGWORM" ON THIS PATIENT'S ARM DUE TO THE DERMATOPHYTIC FUNGUS TRICHOPHYTE RUBRUM. DERMATOPHYTIC MEMBERS OF THE GENUS TRICHOPHYTON INHABIT THE SOIL, HUMANS OR ANIMALS, AND ARE SOME OF THE LEADING CAUSES OF HAIR, SKIN AND NAIL INFECTIONS, OR DERMATOPHYTOSIS IN THEIR HUMAN HOSTS.

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Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

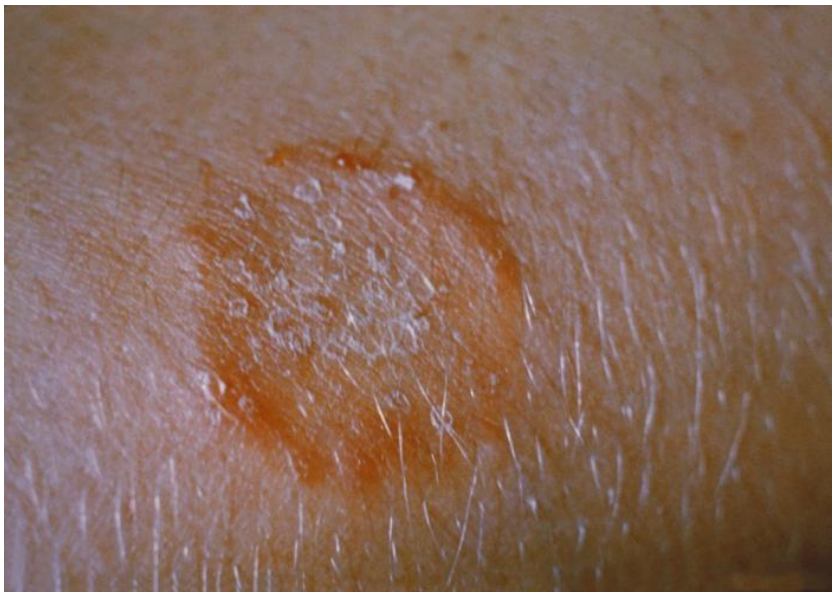

FIGURE 7.2.22 PATIENT WITH RINGWORM ON THE ARM, OR TINEA CORPORIS DUE TO TRICHOPHYTON MENTAGROPHYTES. THE GENUS TRICHOPHYTON INHABITS THE SOIL, HUMANS OR ANIMALS, AND IS ONE OF THE LEADING CAUSES OF HAIR, SKIN AND NAIL INFECTIONS, OR DERMATOPHYTOSIS IN HUMANS.

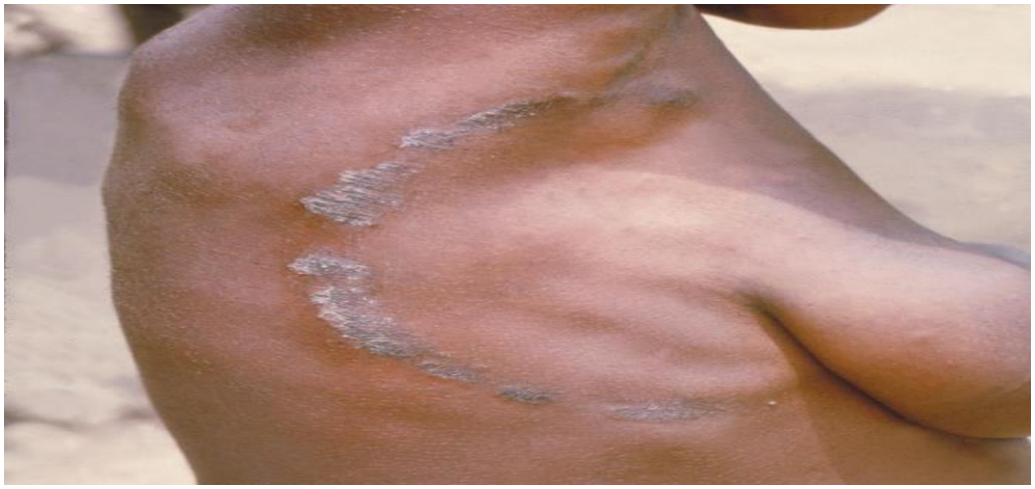

FIGURE 7.2.23 THIS PATIENT, A NATIVE OF NEW GUINEA, HAS RINGWORM ON THE SKIN OF THE RIGHT AXILLA AND FLANK DUE TO TRICHOPHYTON RUBRUM. USUALLY OCCURRING AS A SKIN PARASITE, OR DERMATOPHYTE ON MAN AND ANIMALS, THE GENUS TRICHOPHYTON IS CHARACTERIZED BY COLOURLESS SPORES THAT CAN CAUSE RINGWORM ON THE BODY. THIS CONDITION IS CALLED TINEA CORPORIS.

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Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

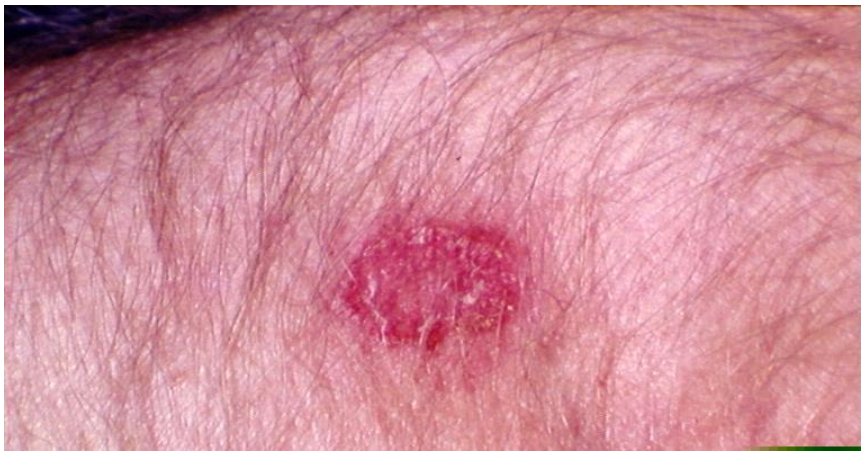

FIGURE 7.2.24 *TRICHOPHYTON MENTAGROPHYTES* CONTRACTED FROM A DOG

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Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

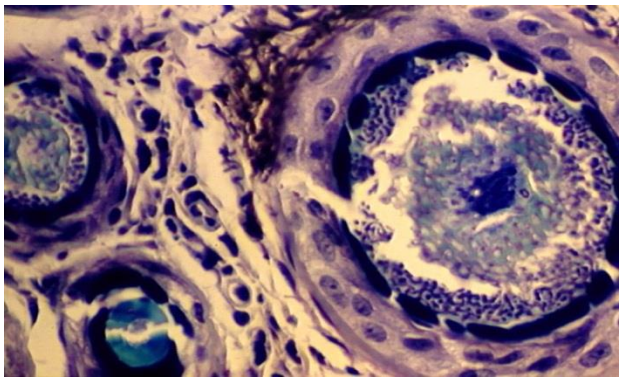

FIGURE 7.2.25 DERMATOMYCOSIS (RINGWORM) OF HAIR FOLLICLES

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

ETIOLOGIC AGENTS

There are three genera of dermatophytes:

- *Trichophyton species* (19 species) (figure 7.2.25).

These infect skin, hair and nails. They rarely cause subcutaneous infections, in immuno-compromised individuals. Trichophyton species take 2 to 3 weeks to grow in culture. The conidia are large (macroconidia), smooth, thin-wall, septate (0-10 septa), and pencil-shaped; colonies are a loose aerial mycelium that grow in a variety of colours. Identification requires special biochemical and morphological techniques (figure 7.2.26). Trichophyton rubrum is presently the most common cause of tinea in South Carolina. It can rarely

cause sub-cutaneous infections (kerion) in immunocompromised individuals, particularly patients with chronic myelogenous leukaemia

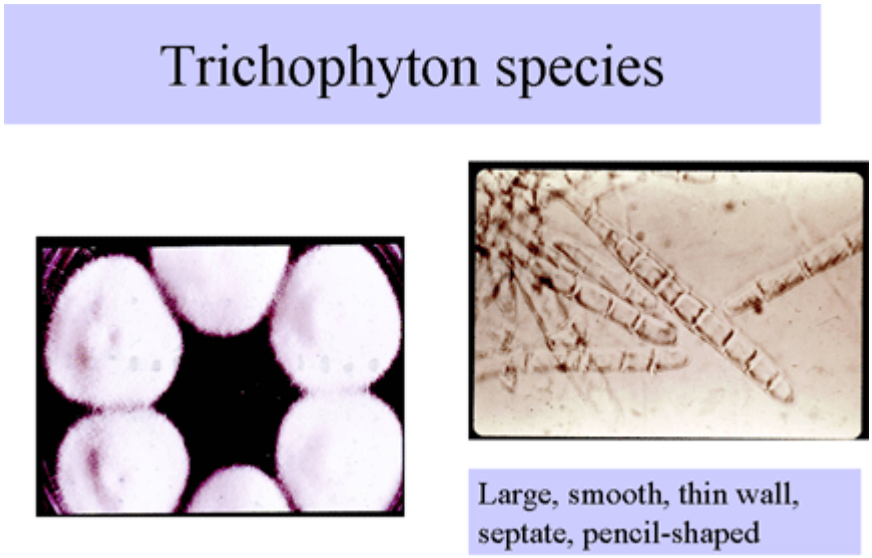

FIGURE 7.2.26A TRICHOPHYTON CONIDIA ARE LARGE, SMOOTH, THIN-WALLED, SEPTATE, AND PENCIL-SHAPED

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Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

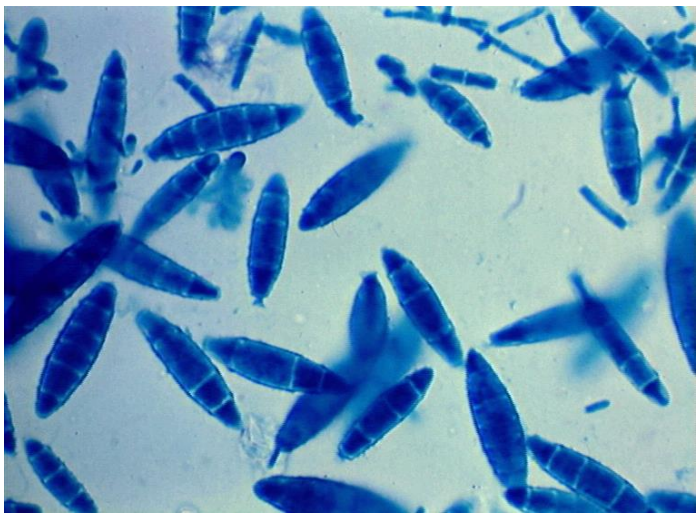

FIGURE7.2. 26A CATE STAIN USED AND PROVIDE SOURCE RINGWORM, STAINED PREPARATION, MACROCONIDIA OF *MICROSPORUM CANIS*

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EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBI

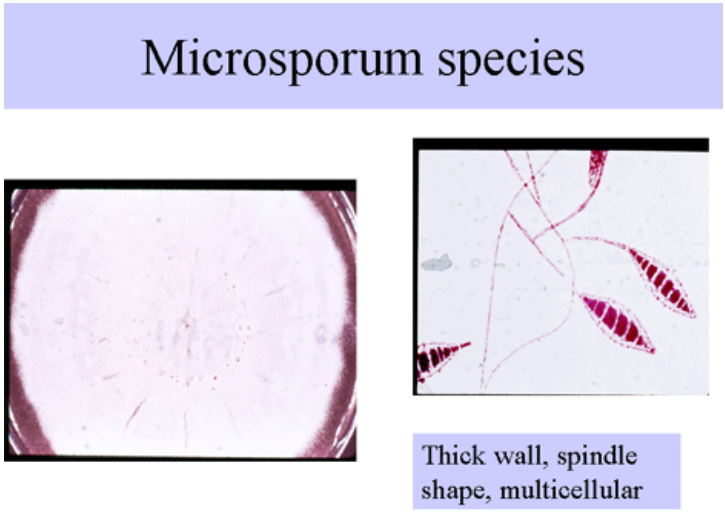

FIGURE 7.2.27B *MICROSPORUM SPECIES*: THICK WALL, SPINDLE SHAPE, MULTICELLULAR

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

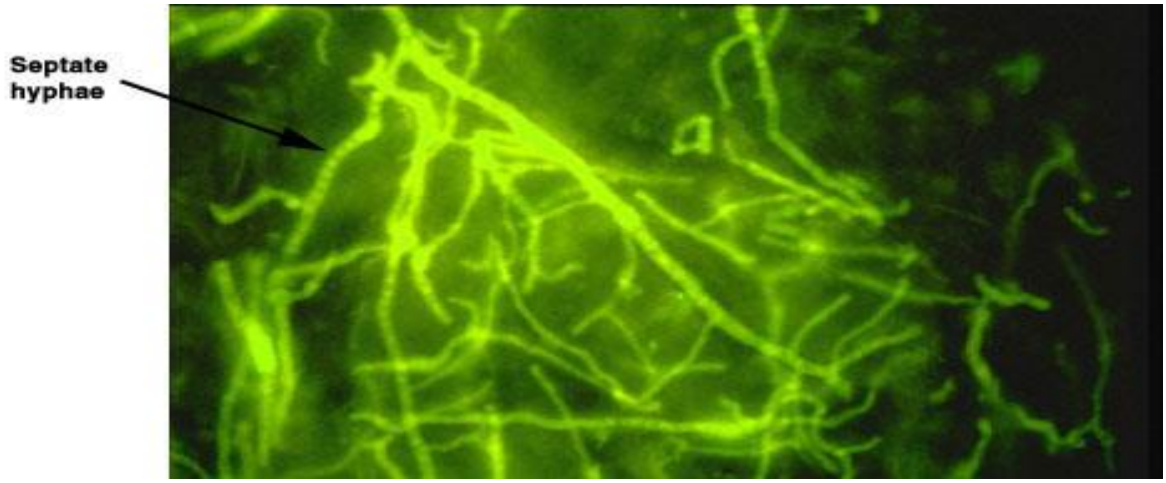

FIGURE 7.2.27 MICROSPORUM CANIS OBTAINED FROM A SKIN SCRAPING OF A PATIENT WITH RINGWORM ON THE NECK ACQUIRED FROM HER INFECTED CAT. THE FUNGUS IS IDENTIFIED AS A DERMATOPHYTE BY THIS CALCOFLUOR STAIN OF THE SKIN SCRAPINGS VIEWED AT 500X MAGNIFICATION. THE CALCOFLUOR DYE BINDS TO THE CHITIN IN THE FUNGUS AND FLUORESCES UNDER A FLUORESCENT LIGHT. COURTESY :

COURTESY : DR ART DISALVO
EMERITUS DIRECTOR, NEVADA STATE LABORATORY, RENO
EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

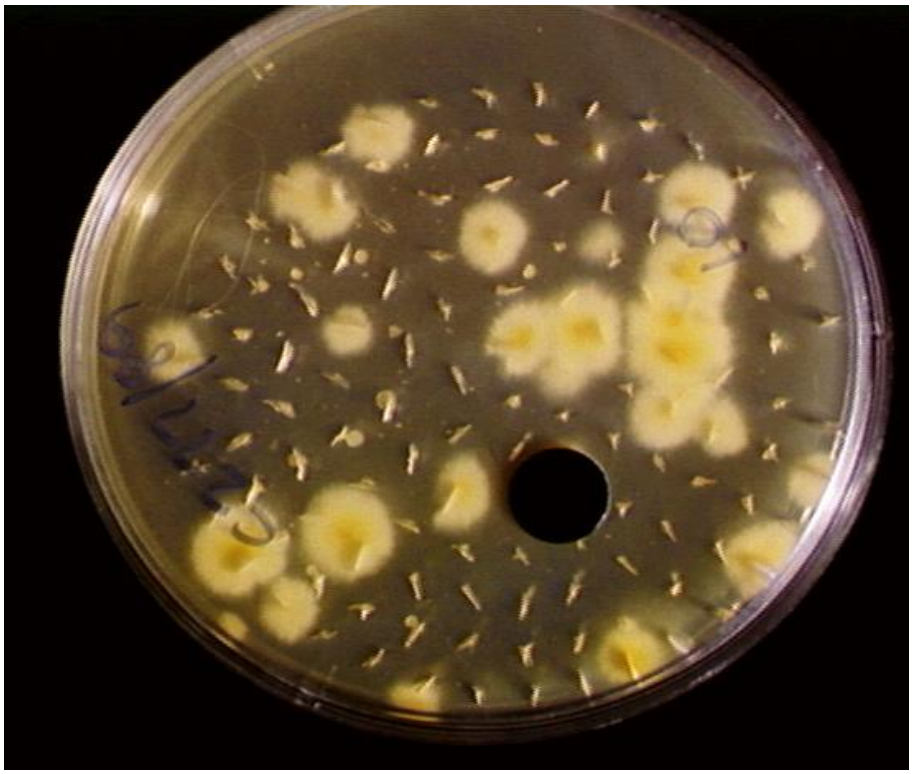

FIGURE 7.2.28 RINGWORM CAUSED BY MICROSPORUM GYPSEUM, CULTURE PLATE WITH SABOURAUD DEXTROSE AGAR

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Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

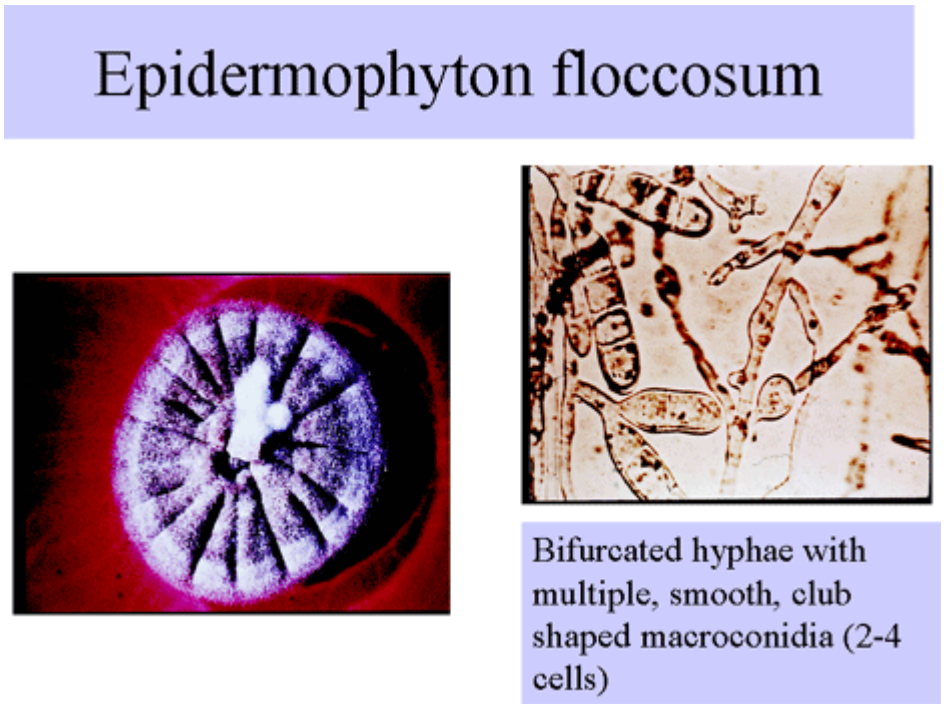

FIGURE 7.2.29 EPIDERMOPHYTON FLOCCOSUM :

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EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

- *Microsporum* species (13 species). These may infect skin and hair, rarely nails. The prevalence of infection has decreased significantly in recent years. When prevalent (15-20 years ago), this organism could be easily identified on the scalp because infected hairs fluoresce a bright green colour when illuminated with a UV-emitting Wood's light. The loose, cottony mycelia produce macroconidia (figure 7.2.27a and b) which are thick-walled, spindle-shaped, multicellular, and echinulate (spiny). Microsporum canis is one of the most common dermatophyte species infecting humans.
- *Epidermophyton floccosum.* These infect skin and nails and rarely hair. They form yellow-coloured, cottony cultures and are usually readily identified by the thick, bifurcated hyphae with multiple smooth, club-shaped macroconidia (figure 7.2.30).

THERAPY

Skin infections can be treated (more or less successfully) with a variety of drugs, such as:

- Tolfnatate (Tinactin) available over the counter - Topical
- Ketoconazole seems to be most effective for tinea versicolor and other dermatophytes.
- Itraconazole – oral
- Terbinifine (Lamisil) - oral, topical.
- Echinocandins (caspofungin)

For infections involving the scalp and particularly the nails, griseofulvin is commonly used. This antimycotic must be incorporated into the newly produced keratin layer to form a barrier against further invasion by the fungus. This is a very slow process requiring oral administration of the drug for long periods - up to 6 to 9 months for fingernail infections and 12 to18 months for toenail infections.

Itraconazole and terbinafine are the drugs of choice for onychomycoses.

THE DERMATOPHYTID REACTION

Patients infected with a dermatophyte may show a lesion, often on the hands, from which no fungi can be recovered or demonstrated. It is believed that these lesions, which often occur on the dominant hand (i.e. right-handed or left-handed), are secondary to immunological sensitization to a primary (and often unnoticed) infection located somewhere else (e.g. feet). These secondary lesions will not respond to topical treatment but will resolve if the primary infection is successfully treated.

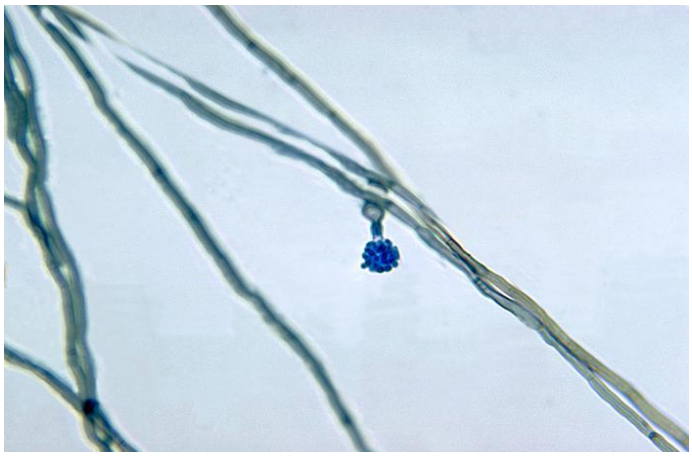

FIGURE 7.2.30 THIS SLIDE CULTURE OF THE FUNGUS FONSECAEA PEDROSOI, REVEALED THE PRESENCE OF A PHIALIDE WITH ACCOMPANYING PHIALOSPORES. FONSECAEA PEDROSOI IS ONE OF THE ETIOLOGIC PATHOGENS RESPONSIBLE FOR THE INFECTION KNOWN AS CHROMOBLASTOMYCOSIS, ESPECIALLY IN THE MORE HUMID REGIONS OF THE WORLD. NORMALLY IT IS FOUND AMONGST ROTTING WOODS AND SOIL DEBRIS.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

3.5 CHROMOBLASTOMYCOSIS

This is a chronic, localized infection of subcutaneous tissues caused by several species of dematiaceous fungi. The 3 most common agents are:

- Fonsecaea pedrosoi (figure 7.2.31)
- Cladosporium carrionii (figure 7.2.32 and 7.2.34)
- Phialophora verrucosa (figure 7.2.33)

These fungi, recognized by a variety of names, are saprobes located in soil and decaying vegetation. The route of entry is usually by trauma. The lesions are sub-cutaneous and the surface can be flat or verrucous (figure 7.2.34a). The lesions take several years to develop. These organisms are called dematiaceous fungi, because they have a black colour in the mycelium cell wall (in culture and in tissue). In tissue these fungi form sclerotic bodies which are the reproductive forms dividing by fission (figure 7.2.34b). These organisms induce a granulomatous reaction. The etiologic agents of chromoblastomycosis are septate, mould-like, branching, darkly pigmented which produce asexual fruits called conidia. We identify these fungi in culture by the shape and formation of the conidia. The fungi have a world-wide distribution especially in warmer climates like the tropics or the southern U.S. The melanin in the pigment may be a virulence factor. These organisms are distributed world-wide. There is no really successful therapy. Excision and local heat have been used with some

success. Flucytosine (5-FC) and itraconazole have also been used to treat (or control) this disease. Posaconazole is showing some promise as a therapeutic agent. There are no serological tests to aid in the diagnosis.

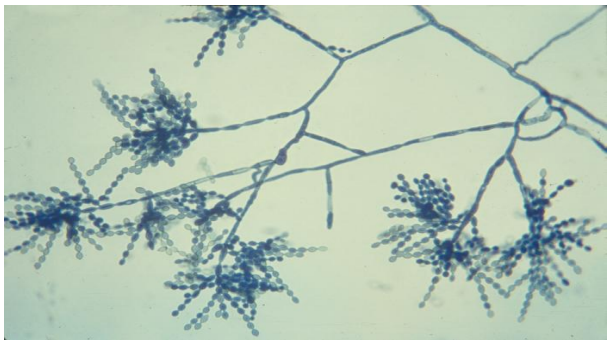

Figure 7.2.31 Cladosporium (Cladophialophora) carrionii, magnified 475X. The *C. carrionii* fungus is a common cause of chromoblastomycosis infections, and is particularly prevalent in arid and semi-arid areas, most often in tropical and subtropical zones.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

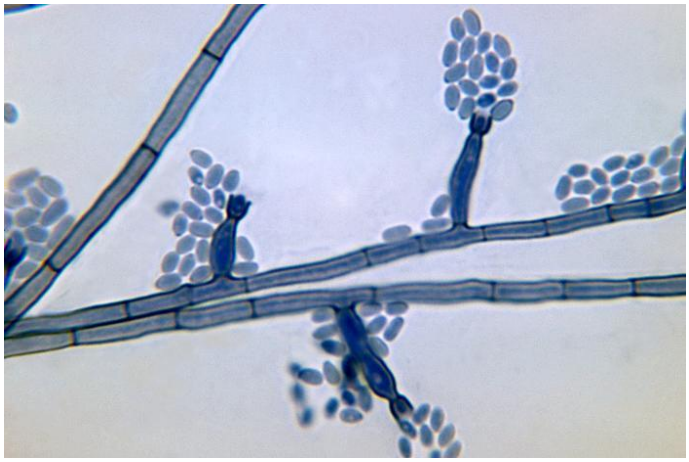

Figure 7.2.32 Conidia-laden conidiophores of a Phialophora verrucosa fungal organism from a slide culture. Note the flask-shaped phialides, each lipped by a collarette. Each phialide terminates in a bundle of round, to ovoid conidia. Phialophora spp. are known to be a cause of both chromoblastomycosis, and phaeohyphomycosis.
Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

FIGURE7.2. 33 PLATE CULTURE OF CLADOSPORIUM CARRIONII, AT FOUR WEEKS GROWTH. *C. CARRIONII* INFECTION IS A COMMON CAUSE OF CHROMOBLASTOMYCOSIS, AND IS PARTICULARLY PREVALENT IN ARID AND SEMI-ARID AREAS, MOST OFTEN IN TROPICAL AND SUBTROPICAL ZONES.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
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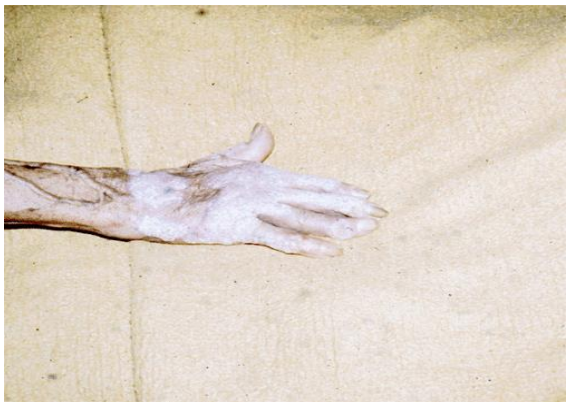

FIGURE 7.2.34A CHROMOBLASTOMYCOSIS LESIONS ARE SUB-CUTANEOUS AND THE SURFACE CAN BE FLAT OR VERRUCOUS COURTESY : DR ART DISALVO
EMERITUS DIRECTOR, NEVADA STATE LABORATORY, RENO
EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

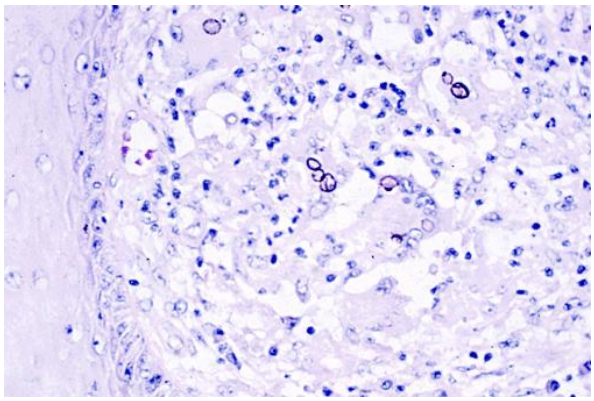

FIGURE 7.2.34B DEMATIACEOUS FUNGI: IN TISSUE THESE FUNGI FORM SCLEROTIC BODIES WHICH ARE THE REPRODUCTIVE FORMS DIVIDING BY FISSION

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

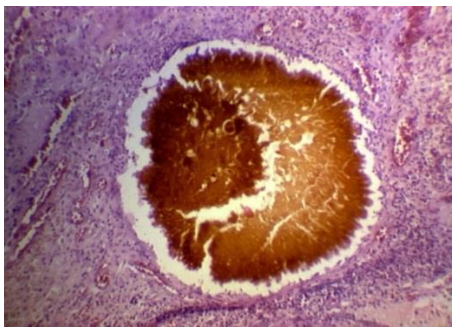

FIGURE 7.2.34 BLACK GRAIN MYCETOMA: SUBCUTANEOUS NODULE DUE TO MADURELLA MYCETOMATIS, MAGNIFIED X 100

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

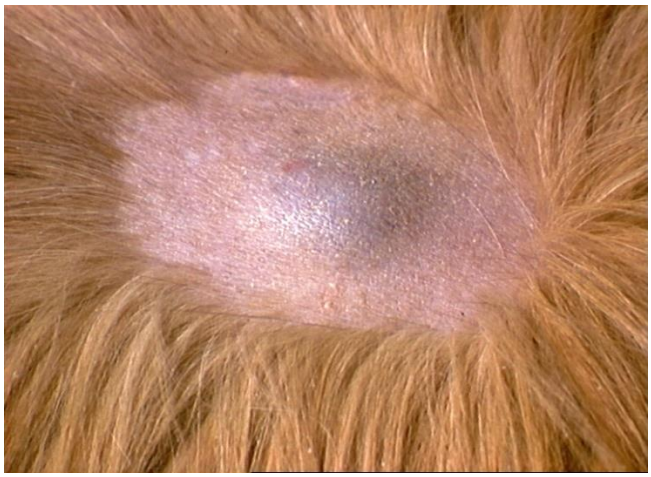

FIGURE 7.2.35 MYCETOMA WITH PRESENCE OF GEOTRICHUM

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

3.6 MYCETOMA (Maduromycosis)

Mycetoma (fungous tumors) are also chronic, subcutaneous infections (figure 7.2.35). These are called eumycotic mycetoma (tumors caused by the TRUE fungi as opposed to those caused by actinomycetes) (figure 7.2.36). These tumors frequently invade contiguous tissue, particularly the bone. A diagnosis of the etiologic agent is essential for patient management because the prognosis and therapy differs. Mycetoma characteristics:

1. tumefaction - swelling

2. granules - a variety of colours (white, brown, yellow, black)

3. draining sinus tracts

The three most common etiologic agents are:

1. Madurella Mycetomatis (figure 7.2.37 and 7.2.38)

2. *Exophiala jeanselmei (figure 7.2.39)

3. *Pseudallescheria boydii (figure7.2.40 and 7.2.41)

Clinical specimens for diagnosis:

1. pus - with granules

2. tissue - for histological examination

The colour, size and texture of the granules are an aid in the diagnosis of mycetoma. The agents of mycetoma are all filamentous fungi which require 7-10 days for visible growth on the culture media and then another several days for specific identification. These fungi are identified by the colonial morphology, conidia formation and biochemical reactions. The species of fungi cannot be distinguished in histopathological tissue sections. Treatment is very difficult, but terbinafine and itraconazole have been used with some success. Posaconazole seems to be efficacious.

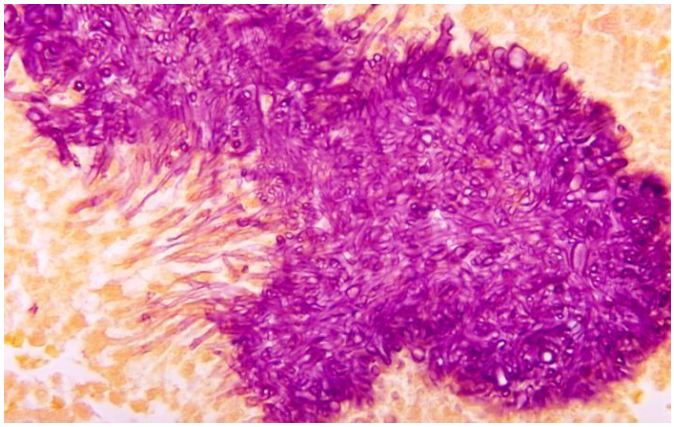

FIGURE 7.2.36 HISTOPATHOLOGIC APPEARANCE OF "BLACK GRAIN MYCETOMA" DUE TO MADURELLA MYCETOMATIS USING A GRIDLEY STAIN. "BLACK GRAIN MYCETOMA", THOUGH USUALLY A LOCALIZED INFECTION, CAN INVOLVE NOT ONLY THE SUPERFICIAL LAYERS OF SKIN, BUT UNDERLYING FASCIA AND BONES AS WELL, WITH THE FUNGAL PATHOGEN ENTERING THE BODY THROUGH A TRAUMATIC WOUND.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

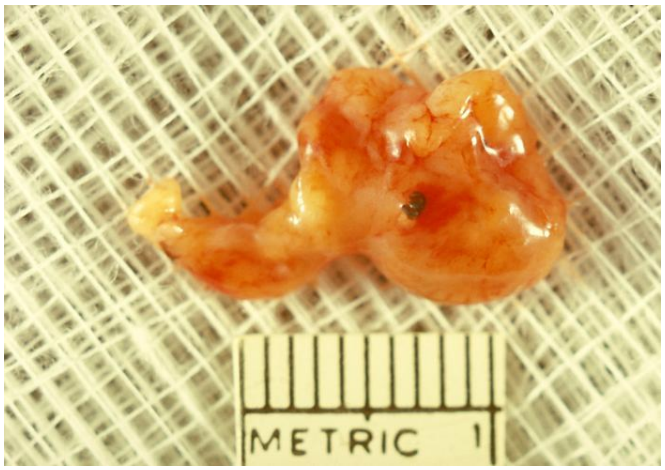

FIGURE 7.2.37 SPECIMEN OF FIBRO ADIPOSE TISSUE CONTAINING "BLACK GRAIN" MYCETOMA DUE TO THE FUNGUS MADURELLA GRISEA. SOME MADURELLA SPP. ARE A CAUSE OF MYCETOMA, A FUNGAL INFECTION CHARACTERIZED BY SCLEROTIA, OR LARGE BLACK MASSES OF HYPHAE. THE FUNGUS ENTERS THE HUMAN BODY VIA TRAUMA, WHICH USUALLY AFFECTS THE FOOT. THIS DISEASE PROCESS MAY TAKE SEVERAL YEARS.

Courtesy : Dr Art DiSalvo
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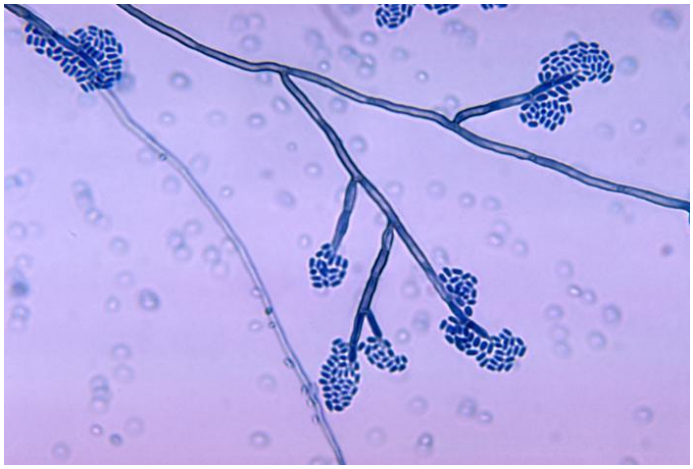

FIGURE 7.2.38 CONIDIOPHORES OF THE FUNGUS EXOPHIALA JEANSELMEI. EXOPHIALA JEANSELMEI, IS A WELL-DOCUMENTED HUMAN PATHOGEN. CLINICAL MANIFESTATIONS INCLUDE MYCETOMA, LOCALIZED CUTANEOUS INFECTIONS, SUBCUTANEOUS CYSTS, ENDOCARDITIS, CEREBRAL INVOLVEMENT, AND SYSTEMICALLY DISSEMINATED INFECTIONS.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

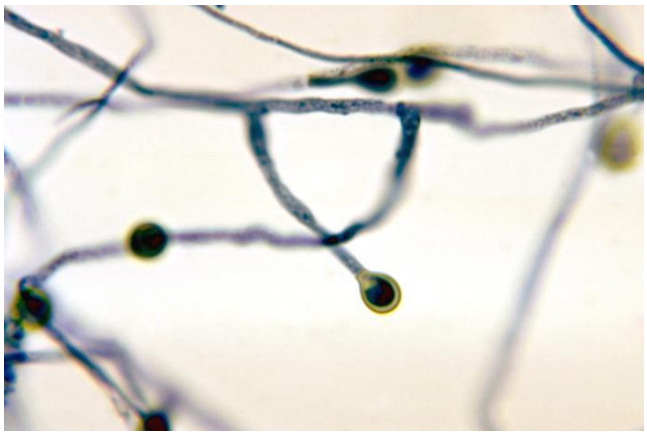

FIGURE 7.2.39 CONIDIOPHORES WITH CONIDIA OF THE FUNGUS PSEUDALLESCHERIA BOYDII FROM A SLIDE CULTURE. PSEUDALLESCHERIA BOYDII IS PATHOGENIC IN HUMANS, ESPECIALLY THOSE WHO ARE IMMUNOCOMPROMISED, CAUSING INFECTIONS IN ALMOST ALL BODY REGIONS, AND WHICH ARE CLASSIFIED UNDER THE BROAD HEADING OF "PSEUDALLESCHERIASIS".

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

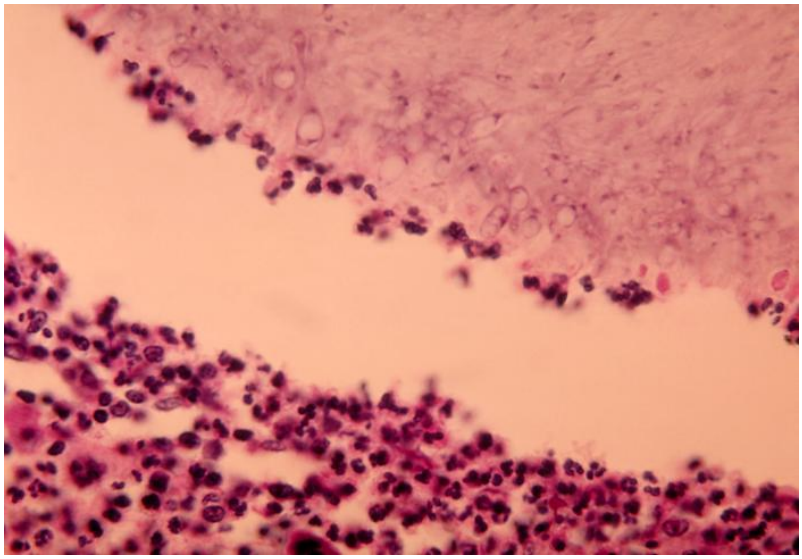

FIGURE 7.2.40 EUMYCOTIC MYCETOMA DUE TO THE FUNGUS PSEUDALLESCHERIA BOYDII. PSEUDALLESCHERIA BOYDII IS THE MOST COMMON ETIOLOGIC AGENT ASSOCIATED WITH EUMYCETOMA IN THE UNITED STATES. THE DISEASE IS A CHRONIC CUTANEOUS AND SUBCUTANEOUS INFECTION WITH THE FOOT BEING THE MOST COMMON SITE FOR LESIONS.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
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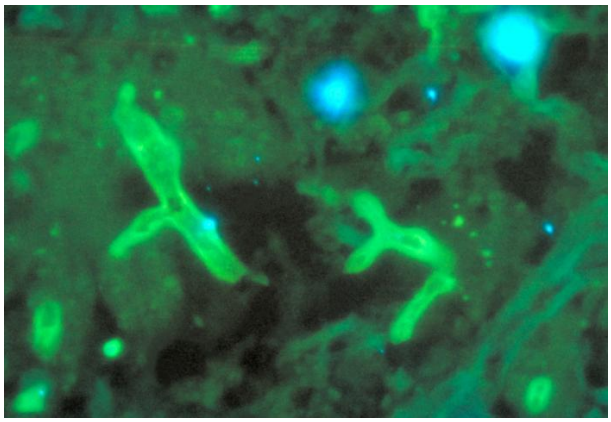

FIGURE 7.2.41 HISTOPATHOLOGIC CHANGES SEEN IN ZYGOMYCOSIS DUE TO RHIZOPUS ARRHIZUS USING FA STAIN TECHNIQUE. RHIZOPUS ARRHIZUS, THE MOST COMMON RHIZOPUS SPP., IS KNOWN TO BE THE CAUSE OF ZYGOMYCOSIS, AN ANGIOTROPIC DISEASE, WHICH MEANS THAT IT TENDS TO INVADE THE BLOOD VESSELS, THEREBY, FACILITATING ITS SYSTEMIC DISSEMINATION.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

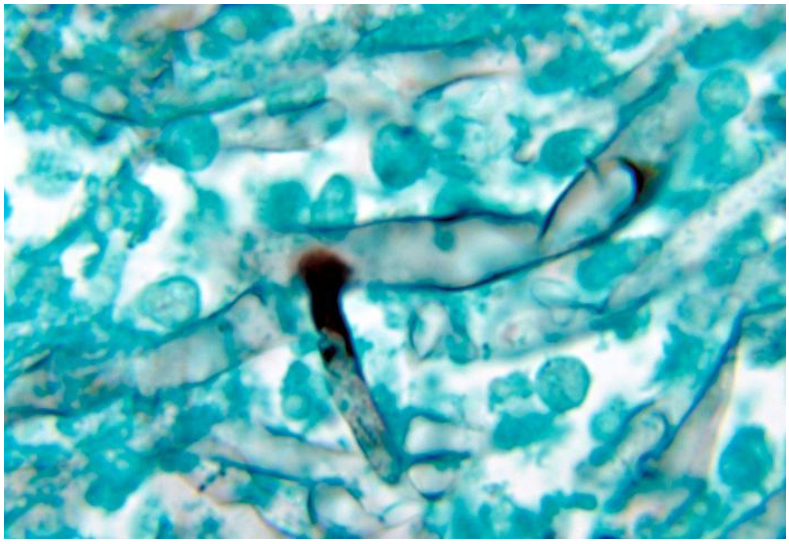

FIGURE 7.2.42 HISTOPATHOLOGIC CHANGES SEEN IN A HEART VALVE DUE TO ZYGOMYCOSIS CAUSED BY MUCOR PUSILLUS. USING METHENAMINE SILVER STAIN, ONE CAN DETECT THE PRESENCE OF FUNGAL ELEMENTS ASSOCIATED WITH ZYGOMYCOSIS, INCLUDING SPARSELY SEPTATE HYPHAE, AMONGST A MOSTLY ACUTE INFLAMMATORY PROCESS WITH SOME ISLAND OF CHRONIC GRANULOMATOUS INFLAMMATION.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

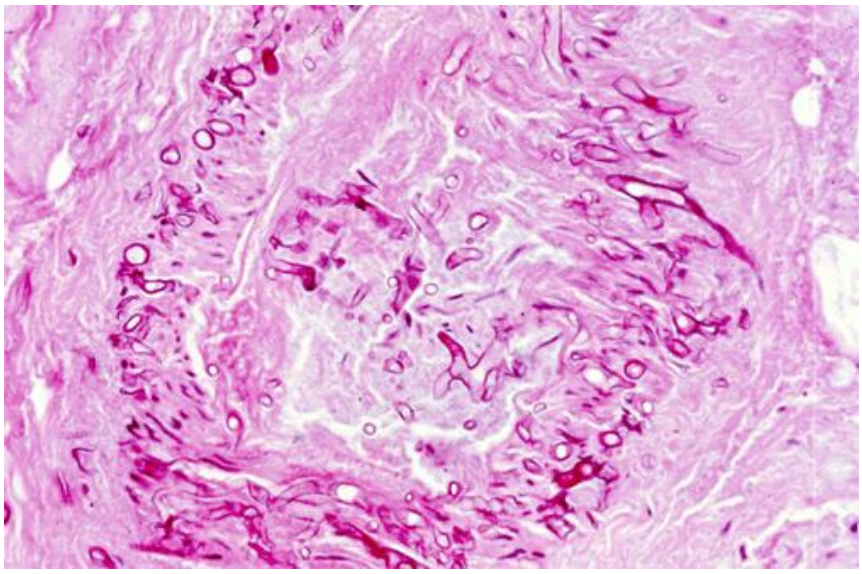

FIGURE 7.2.43 MUCOR SP. ENTER THE BRAIN VIA THE BLOOD VESSELS

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

3.7: MUCORMYCOSIS

Also known as zygomycosis and phycomycosis. Mucormycosis is an acute inflammation of soft tissue, usually with fungal invasion of the blood vessels. This rapidly fatal disease is caused by several different species in this class. The zygomycetes, like the Candida species, are ubiquitous and rarely cause disease in an immunocompetent host. Some characteristic underlying conditions which cause susceptibility are: diabetes, severe burns, immunosuppression or intravenous drug use.

The three most common genera causing this clinical entity are:

- Rhizopus species (figure 7.2.42)
- Mucor species (figure 7.2.43)
- Absidia species

Characteristics

These fungi are found world-wide, commonly in soil, food, organic debris etc. They are seen on decaying vegetables in the refrigerator and on mouldy bread.

Rhino cerebral infections are common. This disease is frequently seen in the uncontrolled diabetic patients.

Typical case

An uncontrolled diabetic patient comes to ER (may be comatose depending on the state of diabetes) and a cotton-like growth is observed on the roof of the mouth or in the nose. These are the hyphae of the organism. If untreated, the patient will die within a few hours or days. What do you do to help this patient first? Controlling the diabetic state is most important before administering amphotericin.

These fungi have a tendency to invade blood vessels (particularly arteries) and enter the brain via the blood vessels and by direct extension through the cribiform plate (figure 7.2.44). This is why they cause death so quickly.

Culture

A rapid growing, loose, white mould is visible within 24 to 48 hours. With age, and the formation of sporangia, the colony becomes dark gray. The sporangia contain the dark spores (figure 6.3.46). The mycelium is wide (10-15 microns), ribbon-like and non-septate (coenocytic). This same appearance is clear in tissue sections. The species are identified by the morphology in culture.

Treatment

Treatment consists of debridement and amphotericin

Identification

There is an immunodiffusion test available, but the physician cannot wait for these results before instituting rapid, vigorous intervention. The diagnosis and treatment must be immediate and based primarily on clinical observations.

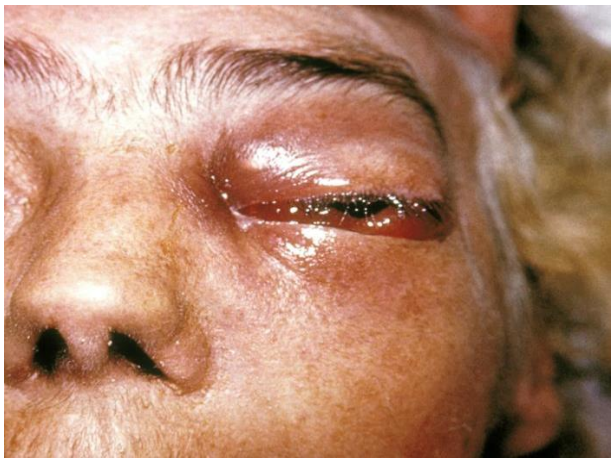

FIGURE 7.2.44 THIS PATIENT PRESENTED WITH A CASE OF A PERIORBITAL FUNGAL INFECTION KNOWN AS MUCORMYCOSIS, OR PHYCOMYCOSIS. MUCORMYCOSIS IS A DANGEROUS FUNGAL INFECTION USUALLY OCCURRING IN THE IMMUNOCOMPROMISED PATIENT, AFFECTING THE REGIONS OF THE EYE, NOSE, AND THROUGH ITS GROWTH AND DESTRUCTION OF THE PERIORBITAL TISSUES, IT WILL EVENTUALLY INVADE THE BRAIN CAVITY.

COURTESY : DR ART DISALVO
EMERITUS DIRECTOR, NEVADA STATE LABORATORY, RENO
EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

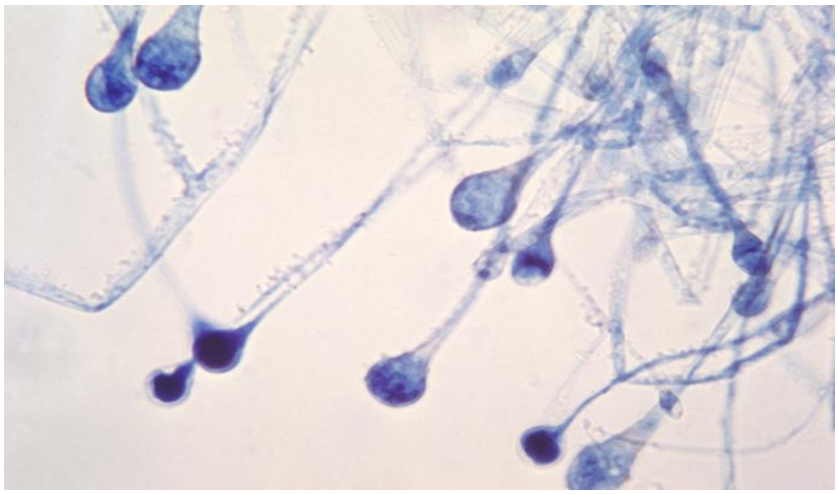

FIGURE 7.2.45A YOUNG SPORANGIA OF A MUCOR SPP. FUNGUS. MUCOR IS A COMMON INDOOR MOLD, AND IS AMONG THE FUNGI THAT CAUSE THE GROUP OF INFECTIONS KNOWN AS ZYGOMYCOSIS. THE INFECTION TYPICALLY INVOLVES THE RHINO-FACIAL-CRANIAL AREA, LUNGS, GI TRACT, SKIN, OR LESS COMMONLY OTHER ORGAN SYSTEMS.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

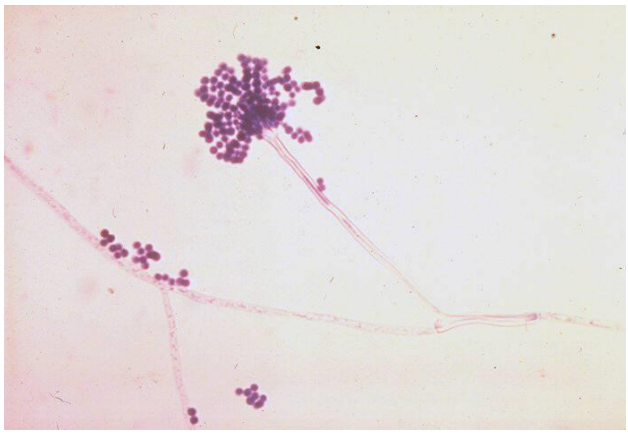

FIGURE 7.2.46B CONIDIA: PHIALOCONIDIA OF ASPERGILLUS FUMIGATUS

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

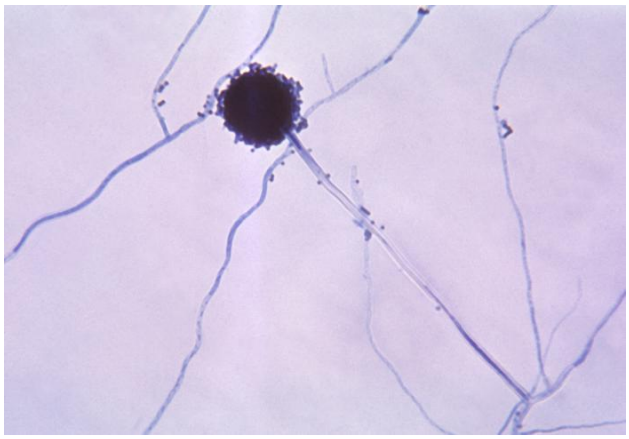

FIGURE 7.2.46 THIS PHOTOMICROGRAPH SHOWS THE CONIDIAL HEAD OF AN ASPERGILLUS NIGER FUNGUS. CONIDIAL HEADS OF ASPERGILLUS NIGER ARE LARGE, GLOBOSE, AND DARK BROWN, AND CONTAIN THE FUNGAL SPORES, FACILITATING PROPAGATION OF THE ORGANISM. THIS IS ONE OF THE MOST COMMON SPECIES ASSOCIATED WITH INVASIVE "PULMONARY ASPERGILLOSIS".

COURTESY : DR ART DISALVO
EMERITUS DIRECTOR, NEVADA STATE LABORATORY, RENO
EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

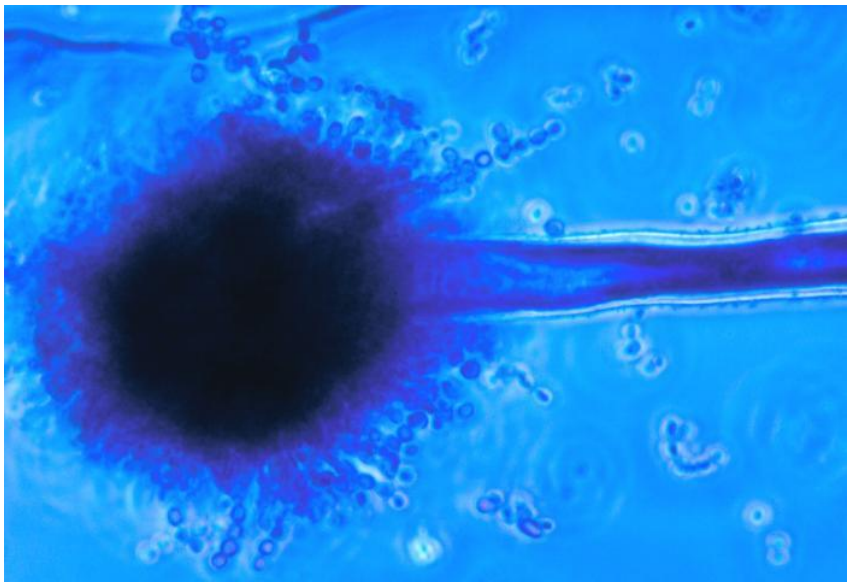

FIGURE 7.2.47 THIS PHOTOMICROGRAPH DEPICTS THE APPEARANCE OF A CONIDIOPHORE OF THE FUNGUS ASPERGILLUS FLAVUS. ASPERGILLUS SPP. ARE FILAMENTOUS, COSMOPOLITAN AND UBIQUITOUS FUNGI FOUND IN NATURE, ARE COMMONLY ISOLATED FROM SOIL, PLANT DEBRIS, AND INDOOR AIR ENVIRONMENTS, AND ARE THE MOST COMMONLY ISOLATED FILAMENTOUS FUNGI IN INVASIVE INFECTIONS.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

3.8: ASPERGILLOSIS

Aspergilli produce a wide variety of diseases. Like the zygomycetes, they are ubiquitous in nature and play a significant role in the degradation of plant material as in composting. Similar to Candida and the Zygomycetes, they rarely infect a normal host. The organism is distributed world-wide and is commonly found in soil, food, paint, air vents. They can even grow in disinfectant. There are more than one hundred species of aspergilli The most common etiologic agents of aspergillosis in the United States:

Aspergillus fumigatus (figure 7.2.46)

Aspergillus niger (figure 7.2.47)

Aspergillus flavus (figure 7.2.48)

There are three clinical types of pulmonary aspergillosis:

Allergic hypersensitivity to the organism. Symptoms may vary from mild respiratory distress to alveolar fibrosis.

Aggressive tissue invasion. Aspergillosis is primarily a pulmonary disease, but the aspergilli may disseminate to any organ. They may cause endocarditis, osteomyelitis, otomycosis and cutaneous lesions.

Fungus ball which is characteristically seen in the old cavities of TB patients. This is easily recognized by x-ray (figure 7.3.49), because the lesion (actually a colony of mould growing in the cavity) is shaped like a half-moon (semi-lunar growth). The patients may cough up the fungus elements because the organism frequently invades the bronchus. Chains of conidia can sometimes be seen in the sputum.

Culture

Aspergilli require 1-3 weeks for growth. the colony begins as a dense white mycelium which later assumes a variety of colours, according to species, based on the colour of the conidia. The hyphae are branching and septate. Species differentiation is based on the formation of spores as well as their colour, shape and texture.

Histopathology

The septate hyphae are wide and form dichotomous branching, i.e., a single hypha branches into two even hyphae, and then the mycelium continues branching in this fashion (figure 7.2.50).

Serology

There is an excellent serological test for aspergillosis which is an Immunodiffusion test. There may be 1 to 5 precipitin bands. Three or more bands usually indicate increasingly severity of the disease. i.e., tissue invasion.

Treatment

Voriconazole and Amphotericin B.

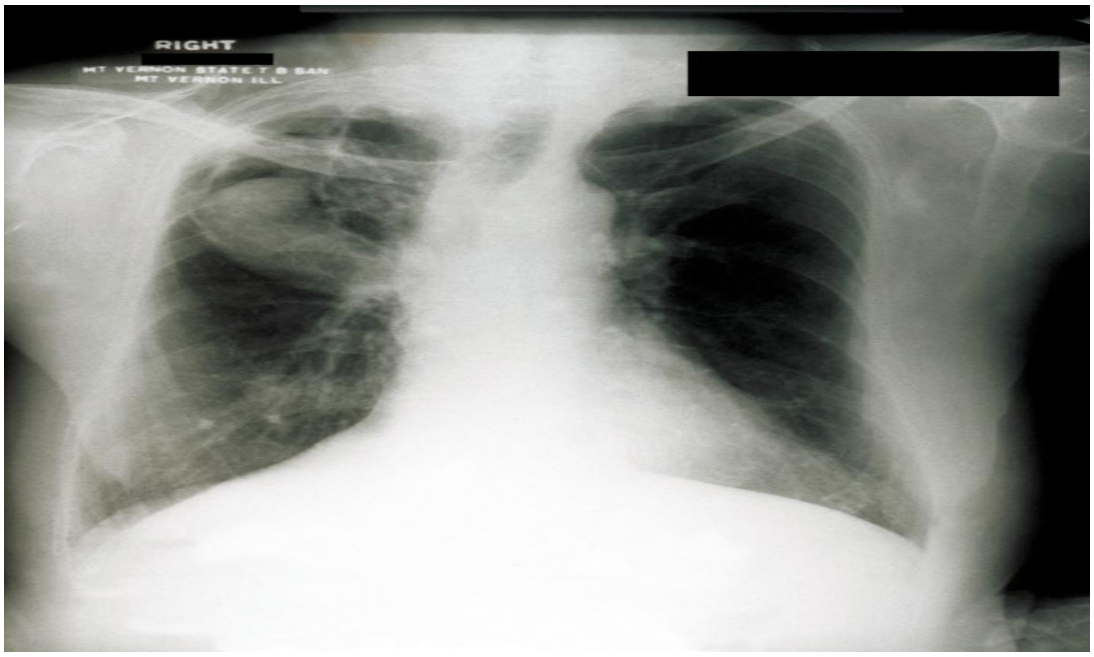

FIGURE 7.2.48 THIS CHEST RADIOGRAPH SHOWS PROBABLE ASPERGILLOSIS WITH AN ASPERGILLOMA, OR FUNGUS BALL IN THE UPPER LOBE OF THE RIGHT LUNG. LUNG DISEASES THAT DAMAGE A LUNG CAN CAUSE CAVITIES THAT CAN LEAVE A PERSON MORE SUSCEPTIBLE TO DEVELOPING AN ASPERGILLOMA, OR FUNGUS BALL. THE FUNGUS CAN THEN BEGIN SECRETING TOXIC AND ALLERGIC PRODUCTS, WHICH MAY MAKE THE PERSON FEEL ILL.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

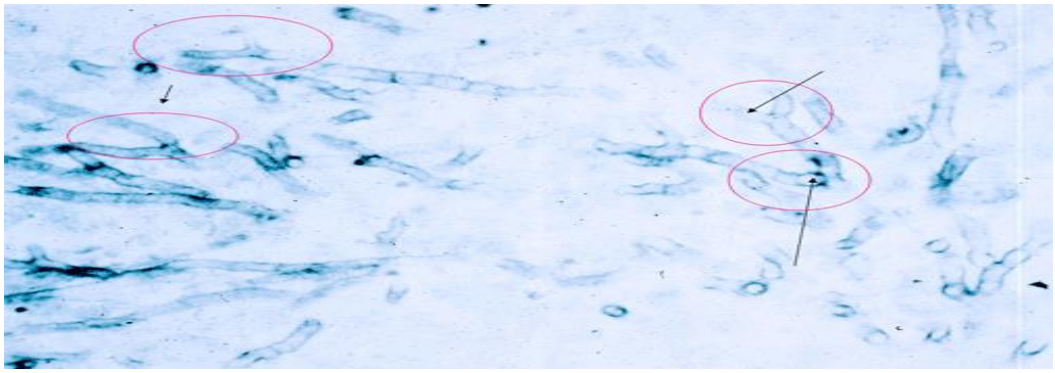

FIGURE 7.2.49 BRANCHING OF ASPERGILLUS HYPHAE COURTESY :

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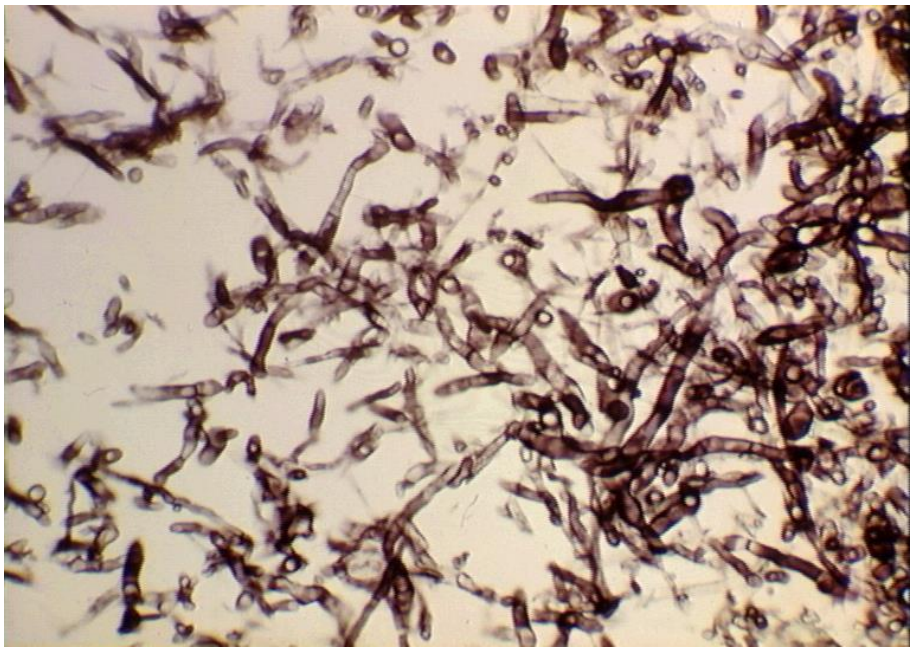

FIGURE 7.2.50 ASPERGILLOSIS. HUMAN MOUTH. GOMORI'S SILVER METHENAMINE STAIN

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

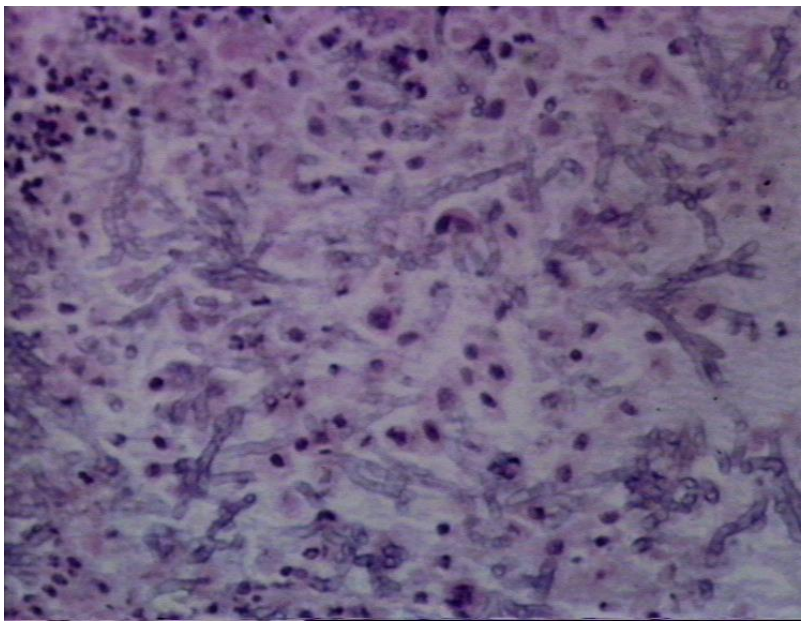

FIGURE 7.2.51 LUNG: ASPERGILLUS HYPHAE IN FUNGAL PNEUMONIA

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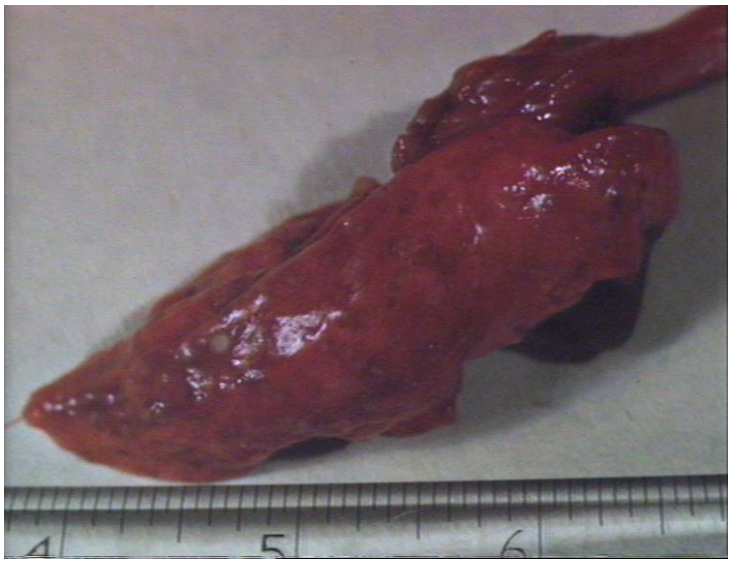

FIGURE 7.2.52 FUNGAL GRANULOMAS IN LUNG CAUSED BY ASPERGILLUS FUMIGATUS

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

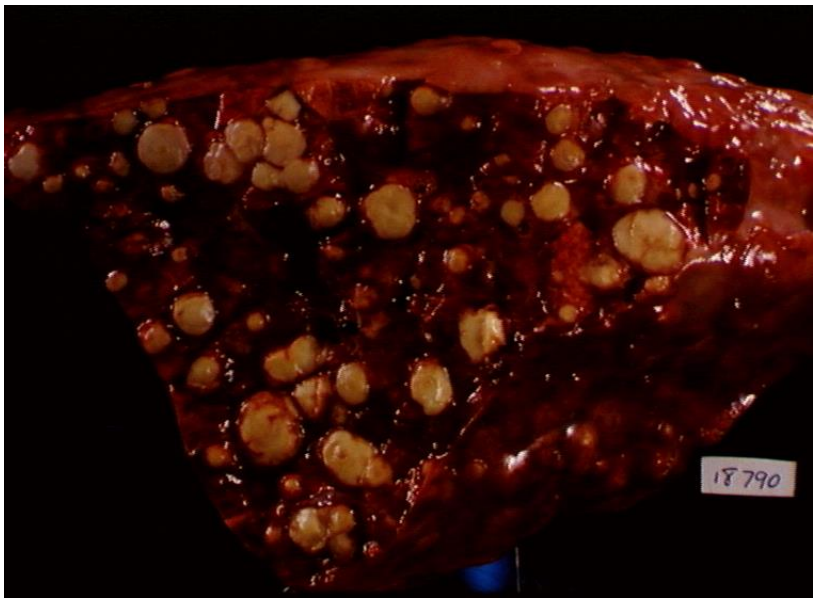

FIGURE 7 .2.53 ASPERGILLUS PNEUMONIA IN LUNG OF DEER

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
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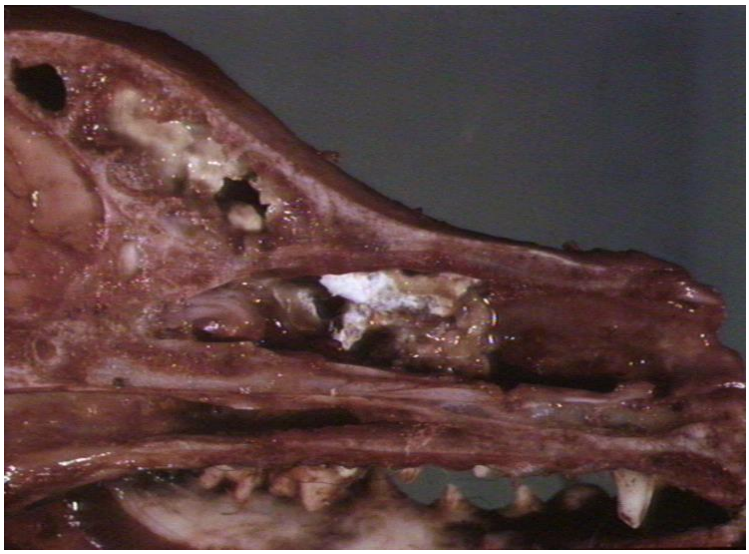

FIGURE 7.2.54 NASAL ASPERGILLOSIS

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EMERITUS DIRECTOR, NEVADA STATE LABORATORY, RENO
EMERITUS DIRECTOR OF LABORATORIES, SOUTH CAROLINA DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL, COLUMBIA

4.0: CONCLUSION

Yeasts are single-celled budding organisms. They do not produce mycelia. Their soft, moist colonies resemble bacterial cultures rather than moulds. There are many species of yeasts that can be pathogenic for humans. Cryptococcosis manifests itself most commonly as meningitis but in recent years many cases of pulmonary disease have been recognized. Most infections are, however, asymptomatic. ***Cryptococcus gattii*** is a newly recognized pathogenic species of Cryptococcus. It is found in soil and in association with several species of trees in tropical and sub-tropical regions of the world but has recently been found in the western United States and western Canada. The patient acquires the infection as a result of the inhalation of fungal spores which lodge in the lungs. Some clinical manifestations of some dermatophytes are given as follows: Tinea corporis - small lesions occurring anywhere on the body Tinea pedis - "athlete's foot". Infection of toe webs and soles of feet. Tinea unguium (onychomycosis) - nails. Tinea capitis - head. Frequently found in children. Tinea cruris - "jock itch". Infection of the groin, perineum or perianal area. Tinea barbae - ringworm of the bearded areas of the face and neck. Tinea versicolor - Characterized by a blotchy discoloration of skin which may itch. Diagnosis is usually possible by direct microscopic examination of KOH-treated skin scrapings which show a typical aspect of mycelia and spores

described as "spaghetti and meatballs." Tinea versicolor is caused by Malassezia furfur

5.0: SUMMARY

In this unit we have learnt about the following

- Yeasts
- Candidiasis
- Cryptococcosis
- Dermatophytes
- Chromoblastomycosis
- Mycetoma
- Mucormycosis
- Aspergillosis

6.0 TUTORED MARKED ASSIGNMENT

a) How would you carry out a quick diagnosis of yeast cells in a clinical.

b) Write briefly on the following:

i) Candidiasis

ii) Chromoblastomycosis

iii) Mucormycosis

iv) Aspergillosis

c) What is Mycetoma?

7.0 REFERENCES AND SUGGESTED FURTHER READING

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Dr Peter Darben, Queensland University of Technology clinical parasitology collection.

Courtesy : Dr Art DiSalvo
Emeritus Director, Nevada State Laboratory, Reno
Emeritus Director of Laboratories, South Carolina Department of Health and Environmental Control, Columbia

Onychomycosis due to *Trychophyton rubrum*, right and left great toe. Tinea unguium. CDC/Dr. Edwin P. Ewing, Jr.
epe1@cdc.gov