DIPLOMA IN CLINICAL MEDICINE & SURGERY

MEDICAL MICROBIOLOGY 1

MEM

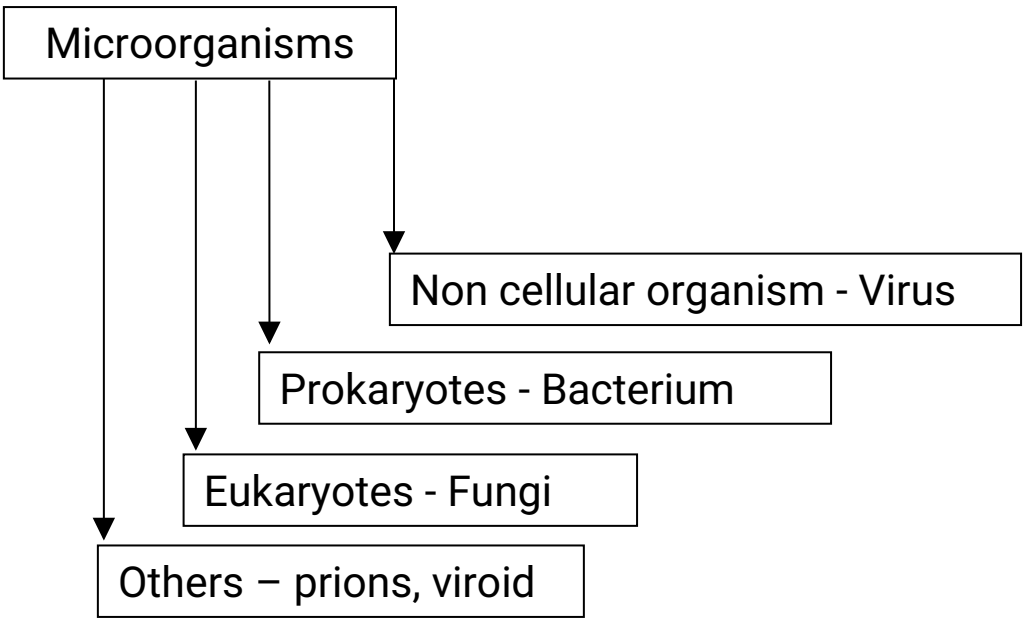
INTRODUCTION TO MICROBIOLOGY

COURSE OBJECTIVES

1. To provide the students with basic knowledge of microorganisms in general.
2. To apply principles, concepts and terminologies used in microbiology.
3. To study the main characteristics of microbes of medical importance.
4. To classify the cocci.
5. To classify bacilli.

What is microbiology?

- Microbes or microorganisms are minute living things that are usually unable to be viewed with the naked eye.
  Examples include bacteria, fungi, protozoa, algae, viruses.
  Some are pathogenic while many are beneficial.
- "Germ" – rapidly multiplying cells.
- Microbiology is the study of organisms that are too small to be seen with naked eye. Microbiologists are concerned with characteristics and functions such as morphology, cytology, physiology, ecology, taxonomy, genetics and molecular biology.



- Organisms included in study of microbiology include;
    - Bacteria – Bacteriology
    - Protozoans – Protozoology
    - Algae – Phycology
    - Parasites – Parasitology
    - Fungi – Mycology
    - Viruses – Virology

Fundamentals of microbiology (Bacteriology, virology, mycology)

- Biological properties;
    - Morphology, identification.
    - Antigenic structure.
- Pathogenesis and pathology
    - Clinical finding
- Diagnostic laboratory tests
- Immunity
- Epidemiology, treatment, prevention and control.

History of microbiology

i. **Antony von Leeuwenhoek** (1675)

He described the animalcules (little animals) which he found in water when examining rain-water under his home-made microscope. Making microscope and magnifying lenses was his hobby.

ii. **Marc Antony von Penciz** (1762)

He was a Viennese physician, published a thesis in which he stated that it was his belief that disease was caused by living organism and that each disease had its own organism. He not only believed that there was an organism for each disease but also thought that the organism multiplied within the body and suggested that they might be transferred from person to person through that air as Girolamo Francastaro had suggested.

iii. **John Needhan** (1748)

He was probably the greatest supporter of the theory of spontaneous degeneration. He published material advocating for that theory. (Spontaneous means occurring

without external stimulation).

iv. **Edward Jenner** (1796)

He was an English physician. He introduced the modern method of vaccination to prevent small-pox. May 14th, 1796 marked epical contribution to the world preventive medicine. Through careful observation, Jenner noted that milkmaids who contracted cow-pox while milking were subsequently immune to small-pox. He performed a vaccination against small-pox by transferring materials from cow-pox pustule on the hand of a milkmaid to the arm of a small boy named James Phipps. Six weeks later the boy was inoculated with small-pox and failed to develop the disease.

v. **Theodor Schwann** (1861)

He proved that yeasts were living things. He disapproved the theory of spontaneous degeneration and he is regarded as the founder of the germ theory of putrefaction and Fermentation. He gave an accurate account of yeasts and their mode of reproduction by budding.

vi. **Louis Pasteur (1861)**

He was a French scientist (chemist) who disapproved the theory of spontaneous degeneration in his work of fermentation. He introduced the concept of anaerobic bacteria and his work on fermentation proved that the breakdown of sugar to alcohol was the result of the action of micro-organism.

He devised the process of destroying bacteria known as pasteurization. He proved that “disease of wine” could be prevented without altering the flavor by heating the wine for a short time to a temperature (55 to 60 degrees centigrade) a little more than half-way between freezing and boiling point.

This process known as pasteurization is employed throughout the civilized world today to preserve milk and other perishable foods. Pasteur discovered a method of breeding bacteria by putting them in “sterile soup” which is now known as “bacteria broth”. He also developed anthrax vaccine. The initial demonstration of this vaccine is one of the most dramatic episodes in the medical history. He worked in the treatment of rabies which is most widely known and developed a vaccine against it.

He discovered that rabies infection attached the brain. He got the brain of infected dog and kept it in a sterile container in order to reduce the virulence of the organisms in it. From several experiments, he produced a dosage that could prevent a health dog from contacting rabies and later, he was able to immunize rabies a child with this preparation from the dog’s brain. Louis Pasteur died in 1895 and his body lies in the Pasteur Institute of France today.

vii. **Joseph Lord Lister (1865)**

An English surgeon, applied antiseptic treatment to the prevention and cure of wound infection. From his observations in surgery, he concluded that wound infections were due to microbes. With this in mind, he protected wounds with dressings saturated with solutions of carbolic acid (phenol) and devised operating rooms procedures calculated to destroy micro-organisms. These methods were far reaching in effects that he will be always be known as the "father of antiseptic surgery".

viii. **William Budd (1873)**

He made a very accurate study of typhoid fever and believed that the disease was infectious and the causative organism was excreted in the feaces of the patient. He suspected that contamination of milk and water played an important part in the spread of the disease.

ix. **Robert Koch (1876)**

He was a German bacteriologist. He is second only to Louis Pasteur on his contribution to the science of bacteriology. He isolated anthrax bacilli and proved it as a sole agent that is responsible for anthrax disease. He perfected his technique of isolating bacteria in pure culture. This method is used up to today. He introduced the use of solid culture media. He was the first to stain bacterial smears in accordance with the methods used today. Likewise, the hanging drop method for studying bacterial motility as used today is a product of his genius. He discovered mycobacterium tuberculosis also known as Koch's bacilli. He also discovered tuberculin known today as Koch's postulate which remains to this day the basis of experimental investigation of infectious disease.

x. **Han Christian Gram (1884)**

He was a Danish physician working in Berlin. He introduced a very important method of differential staining known as Grams' method. Gram utilized method earlier used by Paul Ehrlichs' basic procedure in staining bacteria. He noted that after staining bacteria with methyl violet while other do not (Later take Counter-stain Neutral Red) Gram method remain essentially unaltered today and is used widely.

xi. **Ziehl Ziehl and Friederich Neelsen (1892)**

Ziehl and Neelsen developed the modern method of staining mycobacterium tuberculus. The merit of staining tubercle bacilli belong to Paul Ehrlichs whose method slightly modified by the two scientists is used today and their names are attached to the staining method i.e. Ziehl-Neelsen Staining.

xii. **William Henry Welch (1892)**

Welch is well known for his original research in microbiology and pathology, especially for his studies of clostridium perfrigens and its relation to gas-gangrene. This organism is often referred as to Welch bacillus (in old books- clostridium Welch).

xiii. **Paul Ehrlich (1896)**

He was a German scientist of extra-ordinary genius. He contributed a great mass of knowledge of medical science and a large number of technical methods he proposed are now in daily use in microbiology and chemical laboratories. He applied stains to cells and tissue for purposes of revealing their functions. He founded the science of haematology. He did important research in immunology. He found that the specific effect of immune serum could be demonstrated in vivo and vitro and introduced methods of standardizing toxins and antibodies.

xiv. **Howard T. Ricketts (1909)**

Ricketts, an American scientist demonstrated that Rocky Mountain spotted fever was transmitted by the wood tick. Several years later he showed that tarbardillo (American typhus) was transmitted by the body louse and in the investigation, he contracted the disease and died of it, thus becoming a martyr to the disease process he was studying. His name has been given to the whole group of related organisms known as the Rickettsia.

xv. **Sir Alexander Fleming (1928)**

Fleming, a Scottish bacteriologist found a patch of green mould which had fallen haphazardly upon one of his cultures and he became interested in its antibacterial action. He reported that this mould- penicillium notatum elaborated during its growth a substance which inhibited the development of certain bacteria. He called the substance "penicillin". Fleming own life at one time was saved from an overwhelming pneumonia by the very agent he had discovered. To his great delight, he made a speed and dramatic recovery.

NOTE

Following this great discovery, many types of antibiotics and chemotherapeutic substances have been discovered and produced synthetically.

Gender equality should be embraced because of its significance to the society and this is confirmed with the few women who also made significant contributions to the area of Microbiology, they include:

i. **Edna Steinhardt (1913):** She was the first person to use culture technique to grow virus.

ii. **Alice C. Evans (1917-1923):** She investigated undulant fever (Brucellosis).

iii. **Gladys Dick (1921-1927):** She assisted her husband George Dick in his work on bacteriological and serological study of "scarlet fever".

iv. **Alice Woodruff (1931):** She grew virus in a fertile egg.

v. **Rebecca Lancefield (1928-1933):** She published the methods of classification of streptococci.

vi. **Sarah Steward (1953):** She carried out a significant research in tumors in animal induced by virus.

Definition of terminologies

1. **Aerobe** – organisms that grow in the presence of atmospheric oxygen.
2. **Anaerobe** – grows in absence of free oxygen.
3. **Aerotolerant** anaerobes – microbes that grow equally well whether or not oxygen is present.
4. **Facultative anaerobes** – microbes that don't require oxygen for growth but do grow better in its presence.
5. **Microaerophile** – requires low level of oxygen for growth around 2 to 10 % but is damaged by normal atmospheric oxygen level.
6. **Monotrichous** – having a single flagellum.
7. **Amphitrichous** – having a single flagellum at each end.
8. **Lophotrichous** – cluster of flagella at one end or both ends.
9. **Peritrichous** – flagella all over the body.
10. **Attenuation** – reduced virulence but can provoke the immune system.
11. **Pathogen** – any virus, bacteria or other agents that cause disease.
12. **Pathogenicity** – ability to cause disease.
13. **Virulence** – degree or intensity of pathogenicity of an organism.
14. **Disinfection** – killing, inhibition or removal of microorganisms that may cause disease. It usually refers to the treatment of inanimate.
15. **Sterilization** – process by which all living cells, viable spores, viruses, and viroids are either destroyed or removed from an object or habitat.
16. **Sanitization** – reduction of the microbial population on an inanimate object to levels judged safe by public health standards.
17. **Bacteremia** – presence of viable bacteria in the blood.
18. **Septicemia** – severe condition of bacteremia which includes rapid multiplication

of bacteria and toxins in the blood.

19. **Viremia** – presence of the virus in the blood.
20. **Pyrogenic** – inducing fever
21. **Antipyretic** – which reduces temperature, given in case of fever.
22. **Hemolysis** – disruption of red blood cells and release of their haemoglobin.
23. **α-hemolysis** – partial hemolysis, the greenish zone of incomplete hemolysis forms around the colony.
24. **β-hemolysis** – clear zone of complete hemolysis.
25. **γ-hemolysis** – no hemolysis.
26. **Antibiotic** – substance of microbial origin or its derivative that kills susceptible microorganisms or inhibits their growth.
27. **Antibody** – also known as immunoglobulin, a glycoprotein produced in response to the antigen.
28. **Antigen** – substance, when introduced into the body, stimulates the production of antibodies.
29. **Bacteriostatic** – inhibiting the growth of bacteria without killing them.
30. **Bactericide** - an agent that kills bacteria.
31. **Saprophytes** - These microorganisms are nonpathogenic; their natural habitat is dead organic matter
32. **Commensals** - Normal inhabitants of skin and mucosa; the normal flora is thus the total commensal population
33. **Pathogenic microorganisms** - Classic disease-causing pathogens
34. **Opportunists or facultatively pathogenic microorganisms** - Can cause disease in immunocompromised individuals given an “opportune” situation; these are frequently germs of the normal flora or occasionally from the surrounding environment, animals, or other germ carriers
35. **Incubation period** - Time between infection and manifestation of disease symptoms; this specific disease characteristic can be measured in hours, days, weeks, or even years
36. **Prepatency** - A parasitological term: time between infection and first appearance of products of sexual reproduction of the pathogen.

37. **Infection spectrum** - The totality of host species “susceptible” to infection by a given pathogen
38. **Minimum infective dose** - Smallest number of pathogens sufficient to cause an infection
39. **Mode of infection** - Method or pathway used by pathogen to invade.
40. **Contamination** - Microbiological presence of microorganisms on objects, in the environment, or in samples for analysis
41. **Colonization** - Presence of microorganisms on skin or mucosa; no penetration into tissues; typical of normal flora; pathogenic microorganisms occasionally also show colonization behavior
42. **Infection** - Invasion of a host organism by microorganisms, proliferation of the invading organisms, and host reaction
43. **In-apparent (or subclinical) infection** - Infection without outbreak of clinical symptoms
44. **Infectious disease (or clinical infection)** - Infection with outbreak of clinical symptoms
45. **Probability of manifestation** - Frequency of clinical manifestation of an infection in disposed individuals (%)
46. **Endogenous infection** - Infection arising from the colonizing flora
47. **Exogenous infection** - Infection arising from invasion of host by microorganisms from sources external to it
48. **Nosocomial infection** - Infection acquired during hospitalization (urinary tract infections, infections of the respiratory organs, wound infection, sepsis)
49. **Local infection** - Infection that remains restricted to the portal of entry and surrounding area
50. **Generalized infection** - Lymphogenous and/or hematogenous spread of invading pathogen starting from the portal of entry; infection of organs to which pathogen shows a specific affinity (organotropism); three stages: incubation, generalization, organ manifestation
51. **Sepsis** - Systemic disease caused by microorganisms and/or their toxic products; there is often a localized focus of infection from which pathogens or toxic

products enter the bloodstream continuously or in intermittent phases

52. **Superinfection** - Occurrence of a second infection in the course of a first infection
53. **Relapses** - Series of infections by the same pathogen
54. **Reinfection** - Series of infections by different pathogens

CLASSIFICATION OF MICROORGANISMS

The agents of human infectious diseases are bacteria, fungi (yeasts and molds), protozoa, helminths (worms), and viruses.

Bacterial cells have a prokaryotic nucleus, whereas fungal, protozoan, and helminth cells have a eukaryotic nucleus.

Viruses are not cells and do not have a nucleus.

All cells contain both DNA and RNA, whereas viruses contain either DNA or RNA, but not both.

Bacterial and fungal cells are surrounded by a rigid cell wall, whereas, protozoan, and helminth cells have a flexible cell membrane.

The bacterial cell wall contains peptidoglycan, whereas the fungal cell wall contains chitin.

Prokaryotic and eukaryotic cells

All microorganisms that are capable of self-multiplication can be differentiated by their cell type into one two groups;

- The prokaryotic group – with very simple cell structure, and includes bacteria, rickettsiae, chlamydiae, and mycoplasma.
- The eukaryotic group – with complex cell structure and includes protozoa and fungi.

The differences between prokaryote and eukaryote cells

Prokaryotic cells	Eukaryotic cells
Small cells (< 5μm)	Larger cells (> 10μm)
Lack nucleus	Have nucleus
Membrane-bound organelles are absent	Membrane-bound organelles are present
Unicellular	Mostly multicellular
Circular DNA form	Linear DNA form
Simple cell structure	Complex call structure
Multiply through binary fission	Multiply through mitosis

Classification of bacteria

The classification of bacteria is based on various criteria, such as the morphology, staining characteristics, ability to grow in the presence or absence of oxygen, and ability to form spores.

The criterion currently used is the base sequence of the genome DNA. Several bacteria have been reclassified on the basis of this information.

Bacteria are further broken down into taxonomic groups (taxa) based on relationships best elucidated by knowledge of the evolutionary facts. In formal terms, the prokaryotes are classified in phyla, classes, orders, families, genera, and species, plus subtaxa if any:

Family: Enterobacteriaceae

Genus: Escherichia

Species: E. coli

1. **Morphology of bacteria**

   Several species of bacteria are able to change their form, especially after being grown on natural media. Organisms which show variation in form are described as **pleomorphic**.

   Morphologically bacteria can resemble;

   - Cocci – round (singular coccus), they may form pairs (diplococci, e.g.

mengicocci and gonococci), chains (streptococci, e.g. *Streptococcus pyogens*) or irregular groups (staphylococci, e.g. *Staphylococcus aureus*)

- Bacilli – rods (singular bacillus), they may form:
    - Chains e.g. *Streptobacillus* species
    - Branching chains e.g. *lactobacilli*.
    - Mass together e.g. *Mycobacterium* leprae.
    - Remain attached at various angles resembling Chinese letters, e.g. *Corynebacterium diptheriae*.
- Vibrios – curved rods (singular vibrio) e.g. *Vibrio cholera*
- Spirilla – coiled (singular spirillum) e.g. *Spirillum minus*
- Spirochaetes – coiled (singular spirochaete). Are devided into three; treponemes (*T. pallidum* and *T. pertenue*), borreliae (*B. duttoni* and *B. vincenti*), leptospires (*Leptospira interrogans*).

2. **Staining characteristics (Gram stain)**

It remains an important and useful technique to this day as it allows a large proportion of clinically important bacteria to be classified as either Gram positive or negative based on their morphology and differential staining properties. Slides are sequentially stained with crystal violet, iodine, then destained with alcohol and counter-stained with safranin. Gram positive bacteria stain blue-purple and Gram negative bacteria stain red. The difference between the two groups is believed to be due to a much larger peptidoglycan (cell wall) in Gram positives. As a result the iodine and crystal violet precipitate in the thickened cell wall and are not eluted by alcohol in contrast with the Gram negatives where the crystal violet is readily eluted from the bacteria.

Some bacteria such as mycobacteria (the cause of tuberculosis) are not reliably stained due to the large lipid content of the peptidoglycan. Alternative staining techniques (Kinyoun or acid fast stain) are therefore used that take advantage of the resistance to destaining after lengthier initial staining.

3. **Growth Requirements**

Microorganisms can be grouped on the basis of their need for oxygen to grow.

Facultative anaerobic bacteria can grow in high oxygen or low oxygen content and are among the more versatile bacteria.

In contrast, **strict anaerobic** bacteria grow only in conditions where there is minimal or no oxygen present in the environment. Bacteria such as bacteroides found in the large bowel are examples of anaerobes.

Strict aerobes only grow in the presence of significant quantities of oxygen e.g. *Pseudomonas aeruginosa*,

Microaerophilic bacteria grow under conditions of reduced oxygen and sometimes also require increased levels of carbon dioxide e.g. *Neisseria species*

4. **Biochemical reactions**

Clinical microbiology laboratories typically will identify a pathogen in a clinical sample, purify the microorganism by plating a single colony of the microorganism on a separate plate, and then perform a series of biochemical studies that will identify the bacterial species. For example lactose fermentation test.

5. **Serologic systems:**

Selected antisera can be used to classify different bacterial species. This may be based on either carbohydrate or protein antigens from the bacterial cell wall or the capsular polysaccharide. (Group A streptococcal M proteins or O and H polysaccharide antigens of salmonella).

6. **Environmental Reservoirs:**

When considering likely pathogens it is also important to know which of the different species are found in different locations. Environmental reservoirs are generally divided into those that are endogenous (*i.e.,* on or within the human body) and exogenous (somewhere in the environment).

Assignment

Draw a chart classifying bacteria based on Gram staining characteristics, morphology, growth requirements and biochemical tests with examples.

PROPERTIES OF MICROORGANISMS

These include the structure, reproduction, Physiology of Metabolism and Growth in Bacteria, pathogenesis and antigenicity, and genetic properties.

i. **Bacterial structure**

The bacterial cell consist of:

- Cytoplasm containing the bacterial chromosome (genome), ribosomes, stored energy inclusions and plasmids (extra chromosomal fragments).
- Cytoplasmic membrane and mesosomes.
- Cell wall except bacteria with deficient cell walls.
- External structures including (depending on the species) a capsule, fimbrae (pili), and flagella.
- Spores are formed by Bacillus and Clostridium species of bacteria.

i. **Genome**: organized into chromosomes consist of a single circular chromosomes of double-stranded DNA coiled in the cytoplasm.

ii. **Plasmids**: double-stranded circular DNA materials enabling genetic material exchange.

iii. **Ribosomes**: site of protein production composed of RNA and

proteins.

iv. **Inclusion granules**: composed of volutin, lipid and polysaccharide and are source of energy.

v. **Cytoplasmic membrane and mesosomes**: cytoplasmic membrane controls the movement of water, nutrients, and excretory substances in and out of the cell and also secretes extracellular enzymes and toxins. Mesosomes are sites for respiratory enzymes and assist with cell reproduction.

vi. **Cell wall**: protects against osmotic damage. Based on the composition of the bacterial cell wall, most bacteria when stained by Gram staining technique can be divided into those that are Gram positive i.e. retain the stain crystal violet and those that are Gram negative i.e. are decolorized and take up the colour of the red counterstain.

vii. **External structure:** flagella (for motility e.g. Salmonella species), fimbriae/pili (enable adherence to host cells and to one another and sex pili enable conjugation), capsule (increases virulence of the organism).

viii. **Spores**: change in the enzyme activity and morphology. Formation of resistant endospores.

Assignment

Draw and label the structure of a bacteria

ii. Reproduction of bacteria

- Bacteria by simple cell division known as binary fission (splitting into two).
- The single-stranded DNA reproduces itself exactly.
- The information required to make the cell's protein is encoded in the bacterial genome.
- Messenger (m) RNA is transcribed from the DNA chromosome and the proteins translated from the mRNA are assembled by the ribosomes. Several enzymes are involved in DNA replication and protein production.
- Bacterial mutation may occur in response to environmental changes.

- Gene transfer occurs when fragments of chromosomal DNA from one bacterium are transferred into another bacterial cell by phage (virus that infects a bacterium).
- When a bacterial species produces several forms each with its own characteristic, the variations are called strains.

iii. The Physiology of Metabolism and Growth in Bacteria

- Human pathogenic bacteria are chemosynthetic and organotrophic (chemo-organotrophic). They derive energy from the breakdown of organic nutrients and use this chemical energy both for resynthesis and secondary activities.
- Bacteria oxidize nutrient substrates by means of either respiration or fermentation. In respiration, O_2 is the electron and proton acceptor, in fermentation an organic molecule performs this function. Human pathogenic bacteria are classified in terms of their O_2 requirements and tolerance as facultative anaerobes, obligate aerobes, obligate anaerobes, or aerotolerant anaerobes.
- Nutrient broth or agar is used to cultivate bacteria. Nutrient agar contains the inert substrate agarose.
- Selective and indicator mediums are used frequently in diagnostic bacteriology.
- Bacteria reproduce by means of simple transverse binary fission. The time required for complete cell division is called generation time. The in-vitro generation time of rapidly proliferating species is 15–30 minutes. This time is much longer in vivo. The growth curve for proliferation in nutrient broth is normally characterized by the phases lag, log (or exponential) growth, stationary growth, and death.

Assignment

Draw and describe the bacterial growth curve.

iv. Pathogenesis

The term **pathogen** refers to those microbes capable of causing disease, especially if they cause disease in immunocompetent people. The term

opportunistic pathogen refers to microbes that are capable of causing disease only in immunocompromised people. **Virulence** is a measure of a microbe's ability to cause disease (i.e., a highly virulent microbe requires fewer organisms to cause disease than a less virulent one). The virulence of a microbe is determined by **virulence factors,** such as capsules, possession of pili, production of extracellular enzymes, exotoxins, or endotoxins production. Whether a person gets an infectious disease or not is determined by the balance between the number and virulence of the microbes and the competency of that person's host defenses.

Many infections are **asymptomatic** or **in apparent** because our host defenses have eliminated the microorganism before it could multiply to sufficient numbers to cause the symptoms of disease.

The term **infection** has two meanings:

- The **presence of microbes** in the body.
- The **symptoms of disease**. The presence of microbes in the body does not always result in symptoms of disease

Bacteria cause the symptoms of disease by two main mechanisms:

- Production of toxins (both exotoxins and endotoxins).
- Induction of inflammation.

Most bacterial infections are communicable (i.e., capable of spreading from person to person), but some are not (e.g., botulism and Legionella pneumonia).

Three epidemiologic terms are often used to describe infections:

- **Endemic** infections are those that occur at a persistent, usually low level in a certain geographic area (constantly present in a community or region.). Sporadic infection is one which breaks out only occasionally.
- **Epidemics** are those infections that occur at a much higher rate than usual (an acute outbreak.)
- **Pandemics** are those infections that spread rapidly over large areas of the globe.

Determinants of Bacterial Pathogenesis

- **Transmission**
    - The modes of transmission of microbes include both human-to-human and nonhuman-to-human processes. Nonhuman sources include animals, soil, water, and food.
    - Human-to-human transmission can occur either by direct contact or indirectly via a vector such as an insect, notably ticks or mosquitoes. Animal-to-human transmission can also occur either by direct contact with the animal or indirectly via a vector.
    - The main “portals of entry” into the body are the respiratory tract, gastrointestinal tract, skin, and genital tract.
    - Human diseases for which animals are the reservoir are called zoonoses.
- **Adherence to Cell Surfaces**
    - Pili are the main mechanism by which bacteria adhere to human cells. They are fibers that extend from the surface of bacteria that mediate attachment to specific receptors on cells.
    - Glycocalyx is a polysaccharide “slime layer” secreted by some strains of bacteria that mediates strong adherence to certain structures such as heart valves, prosthetic implants, and catheters.
- **Invasion, Inflammation, & Intracellular Survival**
    - Invasion of tissue is enhanced by enzymes secreted by bacteria. For example, hyaluronidase produced by Streptococcus pyogenes degrades hyaluronic acid in the subcutaneous tissue, allowing the organism to spread rapidly.
      The capsule surrounding bacteria is antiphagocytic (i.e., it retards the phagocyte from ingesting the organism).
    - Inflammation is an important host defense induced by the presence of bacteria in the body. There are two types of inflammation, **pyogenic** (pus-producing) and **granulomatous,** (granuloma-producing bacteria) and bacteria typically elicit one type or the other.

- Bacteria can evade our host defenses by a process called intracellular survival (i.e., bacteria that can live within cells are protected from attack by macrophages and neutrophils).

- **Exotoxins**
  - Exotoxins are polypeptides secreted by certain bacteria that alter specific cell functions resulting in the symptoms of disease. They are produced by both gram-positive and gram-negative bacteria, whereas endotoxin is found only in gram-negative bacteria.
  - Exotoxins are antigenic and induce antibodies called antitoxins. Exotoxins can be modified to form toxoids, which are antigenic but not toxic. Toxoids, such as tetanus toxoid, are used to immunize against disease.
- **Endotoxins**
  - Endotoxins are lipopolysaccharides (LPS) located in the outer membrane only of gram-negative bacteria. They are not secreted by bacteria.
  - Endotoxins are poorly antigenic, do not induce antitoxins, and do not form toxoids.

Typical Stages of an Infectious Disease

There are often four discrete stages;

- The **incubation period** is the time between the moment the person is exposed to the microbe (or toxin) and the appearance of symptoms.
- The **prodrome period** is the time during which nonspecific symptoms occur.
- The **specific-illness period** is the time during which the characteristic features of the disease occur.
- The **recovery period** is the time during which symptoms resolve and health is restored.

After the recovery period, some people become **chronic carriers** of the organism and in others **latent** infections develop.

Some people have **subclinical** infections during which they remain asymptomatic. The presence of antibodies reveals that a prior infection has occurred.

v. **Genetic properties**

- Bacteria have only one copy of their genome DNA (i.e., they are ***haploid***). In contrast, eukaryotic cells have two copies of their genome DNA (i.e., they are ***diploid***). Bacterial DNA is circular; human nuclear DNA is linear.
- The transfer of DNA within bacterial cells occurs by two processes: movement of transposons and programmed rearrangements.
    - ***Transposons*** are small pieces of DNA that move readily from one site on the bacterial chromosome to another or from the bacterial chromosome to a plasmid. Medically, transposons are important because they commonly ***carry antibiotic resistance genes***. The transfer of transposons on plasmids to other bacteria by conjugation contributes significantly to antibiotic resistance.
    - ***Programmed rearrangements*** are the movement of genes from inactive (storage) sites into active sites, where they are expressed as new proteins. Medically, this is important because bacteria can acquire new proteins (antigens) on their surface and evade the immune system. Two important organisms in which this occurs are *Neisseria gonorrhoeae*, the cause of gonorrhea, and *Trypanosoma brucei*, a protozoan that causes African sleeping sickness.
- The transfer of DNA between bacterial cells occurs mainly by two processes: **conjugation** and **transduction**.
    - **Conjugation** is the process by which DNA, either plasmid or chromosomal, is transferred directly from one bacterium to another. For conjugation to occur, the donor bacterium must have the ***sex pilus***. Plasmids carrying antibiotic resistance genes are commonly transferred by conjugation.
    - **Transduction** is the process by which DNA, either plasmid or chromosomal, is transferred from one bacterium to another by a

virus. The transferred DNA integrates into the chromosomal DNA of the recipient, and new proteins, such as exotoxins, are made - a process called ***lysogenic conversion***.

- **Transformation** is the process by which DNA itself, either DNA released from dying cells or DNA purified in the laboratory, enters a recipient bacterium. Medically, this process appears to be less important than conjugation and transduction.

CLINICAL IMPORTANCE OF MICROORGANISMS

The clinical importance include;

- Diagnosis, treatment, prevention and control of diseases.
- Production of antibiotics.
- Production of vaccines.
- Digestion of food in human.
- Used as food supplements such as yeast.

SOURCES OF MICROORGANISMS

Every infection has a source. The **primary** source of infection is defined as the location at which the pathogen is present and reproduces. **Secondary** sources of infection are inanimate objects, materials, or third persons contributing to transmission of pathogens from the primary source to disposed persons.

Primary Sources of Infection

- **Infected person** - The most important source; as a rule, pathogens are excreted by the organ system through which the infection entered; there are some exceptions
- **Carriers during incubation** - Excretion during incubation period; typical of many viral diseases
- **Carriers in convalescence** - Excretion after the disease has been overcome; typical of enteric salmonellosis
- **Chronic carriers** - Continued excretion for three or more months (even years) after disease has been overcome; typical of typhoid fever
- **Asymptomatic carriers** - They carry pathogenic germs on skin or mucosa without developing “infection”
- **Animal carriers** - Diseased or healthy animals that excrete pathogenic germs
- **Environment** - Soil, plants, water; primary source of microorganisms with natural habitat in these biotopes

NOTE

Read and make notes on Normal Flora of the Skin, the Respiratory Tract, the Intestinal Tract and the Genitourinary Tract.

MODES OF TRANSMISSION

- An understanding of the mode of transmission of bacteria and other infectious agents is extremely important from a public health perspective, because interrupting the chain of transmission is an excellent way to prevent infectious diseases.
- Pathogens can be transmitted from a source of infection by direct contact or indirectly.
- Person-to-person transmission constitutes a **homologous** chain of infection. The infections involved are called **anthroponoses**.
- In cases in which the pathogen is transmitted to humans from other vertebrates (and occasionally the other way around) we have a **heterologous** chain of infection and the infections are known as **zoonoses.**
- **Fomites** are inanimate objects, such as towels, that serve as a source of microorganisms that can cause infectious diseases.
- There are four important portals of entry: respiratory tract, gastrointestinal tract, genital tract, and skin
- **Modes of transmission include;**
    - Ingestion of pathogens in contaminated food or water. E.g. cholera, typhoid.
    - Inhaling pathogens in airborne droplets. E.g. tuberculosis, influenza.
    - Direct contact from one person to another e.g. HIV, syphilis
    - Bite of an arthropod vector e.g. bubonic plague, dengue, and rift valley fever.
    - Through open wounds or cuts as in infections of the skin such as boils and abscesses and tetanus.
    - Blood and blood products such as HIV, HBV and HCV.
    - Vertical/ congenital transmission from mother to child during pregnancy or childbirth such as HIV

Factors influencing transmission of microorganisms.

- Inadequate surveillance, preventive and control measures.

- Socioeconomic factors including poverty, malnutrition.
- Inadequate and contaminated water supplies.
- Climatic factors including extreme rainfall and flooding.
- Geographical factors including the difficulties to access to remote areas to provide vaccination.
- Unavailability of drugs and non-compliance by patients.
- Ineffective health education or lack of access to health education.

LABORATORY INVESTIGATION OF MICROBIAL INFECTIONS

This investigation involves:

- Examining specimens to detect, isolate, and identify pathogens or their products using;
  - Microscopy – motility, morphology, and staining reactions
  - Culture techniques – growth of pure colonies, isolation, identification and antimicrobial sensitivity testing.
  - Biochemical methods – identification of pathogens by their enzymatic and fermentation reactions.
  - Immunological (antigen) tests – involves use of specific antibodies (antisera or labelled antibodies)

INFECTION PREVENTION AND CONTROL

An effective countermeasure aimed at interrupting the chain of infection takes the form of prophylactic measures that is the exposure prophylaxis and immunization prophylaxis.

Exposure Prophylaxis

- Exposure prophylaxis begins with isolation of the source of infection, in particular of infected persons, as required for the disease at hand.
- Quarantine healthy persons who have been in contact with a source of infection.
- Further measures of exposure prophylaxis include disinfection and sterilization, use of insecticides and pesticides, and eradication of animal carriers.

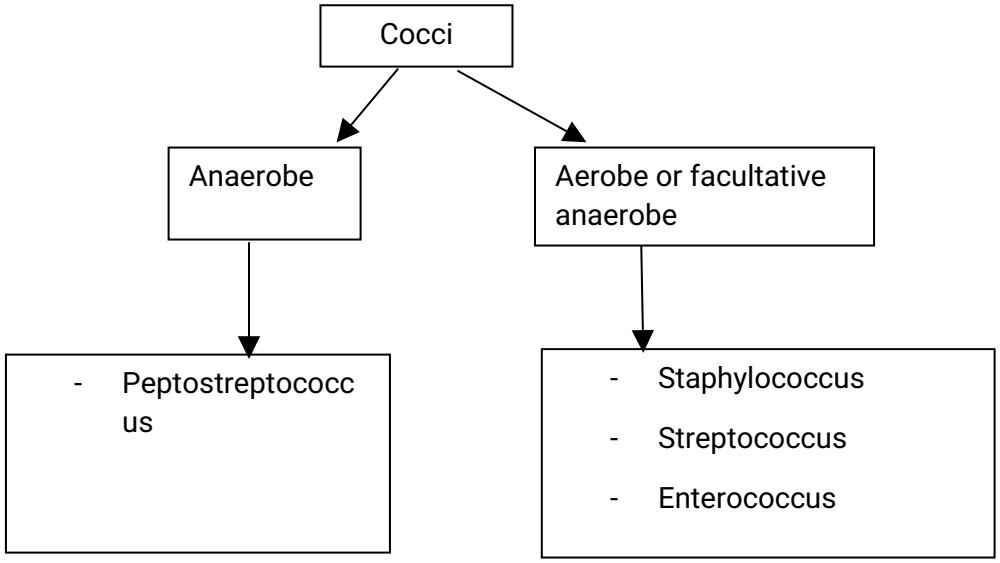
Immunization Prophylaxis

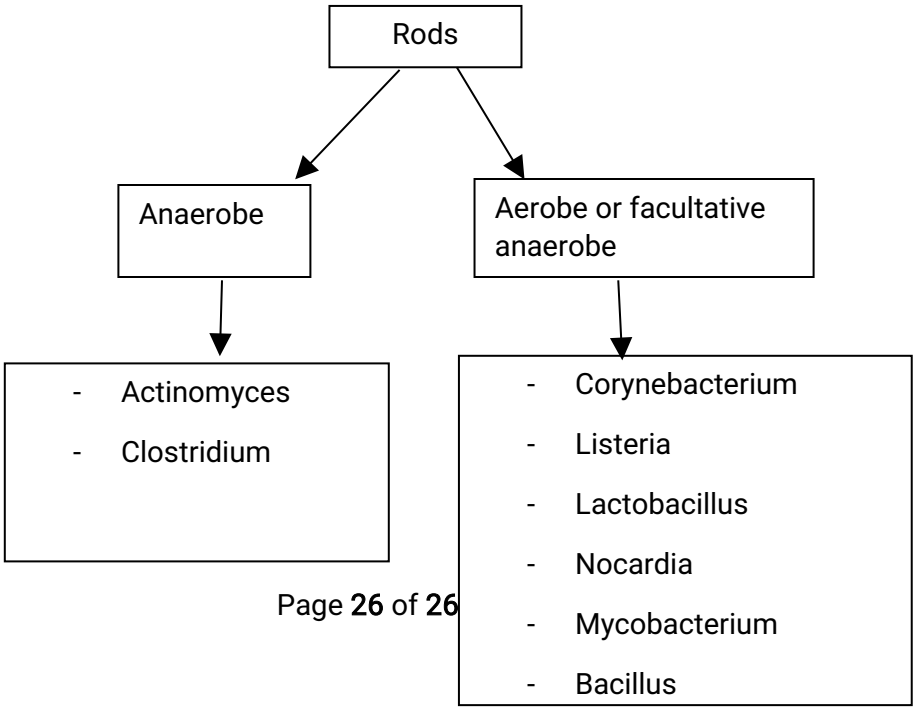
Active immunization. In active immunization, the immune system is stimulated by administration of vaccines to develop a disease-specific immunity.

Passive immunization. This vaccination method involves administration of antibodies produced in a different host. The passive immunity obtained by this method is limited to a few weeks (or months at most).

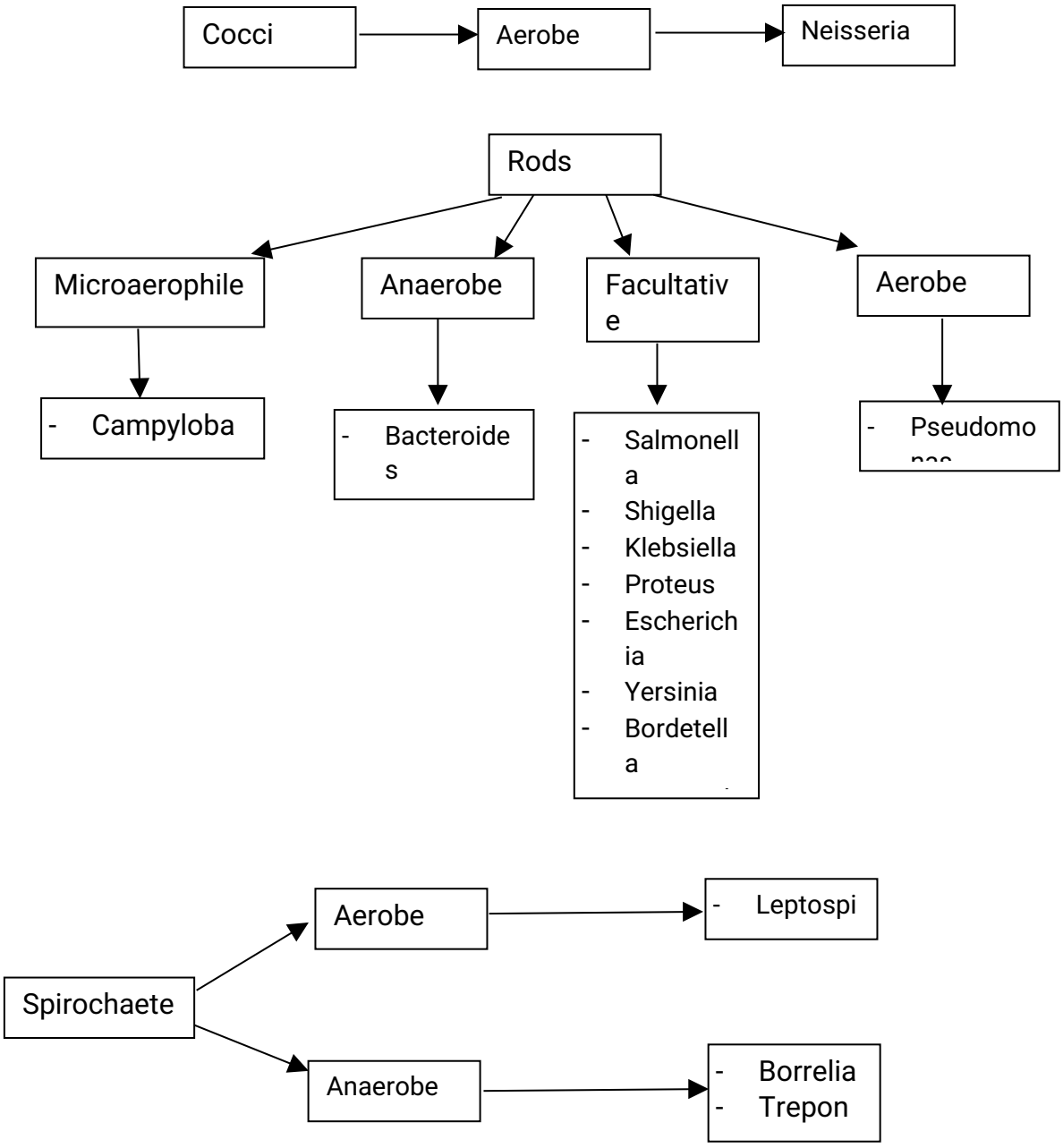
NOTE

Read and make notes on host's defenses.





GRAM NEGATIVE BACTERIA



GRAM-POSITIVE COCCI

There are two medically important genera of gram-positive cocci: *Staphylococcus* and *Streptococcus*. Two of the most important human pathogens are *Staphylococcus aureus* and *Streptococcus pyogenes*. Staphylococci and streptococci are non-motile and do not form spores.

Both staphylococci and streptococci are gram-positive cocci, but they are distinguished by two main criteria:

(1) Microscopically, staphylococci appear in grapelike clusters, whereas streptococci are in chains.

(2) Biochemically, staphylococci produce catalase (i.e., they degrade hydrogen peroxide), whereas streptococci do not.

STAPHYLOCOCCUS

Diseases

Staphylococcus aureus causes abscesses, various pyogenic infections (e.g., endocarditis, septic arthritis, and osteomyelitis), food poisoning, scalded skin syndrome, and toxic shock syndrome. It is one of the most common causes of hospital-acquired pneumonia, septicemia, and surgical-wound infections. It is an important cause of skin infections, such as folliculitis, cellulitis, and impetigo. It is the most common cause of bacterial conjunctivitis.

Staphylococcus epidermidis can cause endocarditis and prosthetic joint infections. *Staphylococcus saprophyticus* causes urinary tract infections. Kawasaki syndrome is a disease of unknown etiology that may be caused by certain strains of *S. aureus*.

Important Properties

Staphylococci are spherical gram-positive cocci arranged in irregular grapelike clusters. All staphylococci produce **catalase,** whereas no streptococci do (catalase degrades H_2O_2 into O_2 and H_2O). Catalase is an important virulence factor. Bacteria that make catalase can survive the killing effect of H_2O_2 within neutrophils.

Three species of staphylococci are human pathogens: *S. aureus, S. epidermidis*, and *S. saprophyticus*. Of the three, *S. aureus* is by far the most important. *S. aureus* is distinguished from the others primarily by;

- Coagulase production. Coagulase is an enzyme that causes plasma to clot by activating prothrombin to form thrombin. Thrombin then catalyzes the activation of fibrinogen to form the fibrin clot. *S. epidermidis* and *S. saprophyticus* are often referred to as coagulase-negative staphylococci.
- *S. aureus* produces a carotenoid pigment called **staphyloxanthin,** which imparts

a golden color to its colonies. This pigment enhances the pathogenicity of the organism by inactivating the microbicidal effect of superoxides and other reactive oxygen species within neutrophils. *S. epidermidis* does not synthesize this pigment and produces white colonies. The virulence of *S. epidermidis* is significantly less than that of *S. aureus*.

- *S. aureus* usually ferments mannitol and hemolyzes red blood cells, whereas *S. epidermidis* and *S. saprophyticus* do not. Hemolysis of red cells by hemolysins produced by *S.aureus* is the source of iron required for growth of the organism. The iron in hemoglobin is recovered by the bacteria and utilized in the synthesis of cytochrome enzymes used to produce energy.
- More than 90% of *S. aureus* strains contain plasmids that encode β-lactamase, the enzyme that degrades many, but not all, penicillins. Some strains of *S. aureus* are resistant to the β-lactamase–resistant penicillins, such as methicillin and nafcillin, by virtue of changes in the penicillin-binding protein (PBP) in their cell membrane.

S. aureus has several important cell wall components and antigens:

i. **Protein A** is the major protein in the cell wall. It is an important virulence factor because it binds to IgG at the complement-binding site, thereby preventing the activation of complement. As a consequence the opsonization and phagocytosis of the organisms are greatly reduced. Protein A is used in certain tests in the clinical laboratory because it binds to IgG and forms a “coagglutinate” with antigen–antibody complexes. The coagulase-negative staphylococci do not produce protein A.

ii. **Teichoic acids** are polymers of ribitol phosphate. They mediate adherence of the staphylococci to mucosal cells. **Lipoteichoic acids** play a role in the induction of septic shock by inducing cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) from macrophages.

iii. Polysaccharide capsule is also an important virulence factor. There are 11 serotypes based on the antigenicity of the capsular polysaccharide, but types 5 and 8 cause 85% of infections. Some strains of *S. aureus* are coated with a small amount of polysaccharide capsule, called a microcapsule. The capsule is poorly immunogenic, which has made producing an effective vaccine difficult.

iv. Surface receptors for specific staphylococcal bacteriophages permit the “phage typing” of strains for epidemiologic purposes. Teichoic acids make up part of these receptors.

v. The peptidoglycan of *S. aureus* has endotoxin-like properties (i.e., it can stimulate macrophages to produce cytokines and can activate the

complement and coagulation cascades). This explains the ability of *S. aureus* to cause the clinical findings of septic shock yet not possess endotoxin.

Transmission

Humans are the reservoir for staphylococci. The **nose is the main site of colonization of *S. aureus***, and approximately 30% of people are colonized at any one time. People who are chronic carriers of *S. aureus* in their nose have an increased risk of skin infections caused by *S. aureus*.
The skin, especially of hospital personnel and patients, is also a common site of *S. aureus* colonization. Hand contact is an important mode of transmission, and hand washing decreases transmission.
S. aureus is also found in the vagina of approximately 5% of women, which predisposes them to toxic shock syndrome. Additional sources of staphylococcal infection are shedding from human lesions and fomites such as towels and clothing contaminated by these lesions.
Disease caused by *S. aureus* is favored by a heavily contaminated environment (e.g., family members with boils) and a compromised immune system.

S. epidermidis is found primarily on the human skin and can enter the bloodstream at the site of intravenous catheters that penetrate through the skin.

S. saprophyticus is found primarily on the mucosa of the genital tract in young women and from that site can ascend into the urinary bladder to cause urinary tract infections.

Pathogenesis

Staphylococcus aureus

It causes disease both by producing toxins and by inducing pyogenic inflammation. The typical lesion of S. aureus infection is an **abscess**. Abscesses undergo central necrosis and usually drain to the outside (e.g. boils), but organisms may disseminate via the bloodstream as well. **Foreign bodies,** such as sutures and intravenous catheters, are important predisposing factors to infection by S. *aureus*.

Several important toxins and enzymes are produced by S. aureus. The three clinically important exotoxins are enterotoxin, toxic shock syndrome toxin, and exfoliatin.

i. **Enterotoxin** causes food poisoning characterized by prominent vomiting and watery, non-bloody diarrhea.

ii. **Toxic shock syndrome toxin** (TSST) causes toxic shock, especially in tampon-using menstruating women or in individuals with wound infections. Toxic shock also occurs in patients with nasal packing used to stop bleeding from the nose. TSST is produced locally by S. aureus in the

vagina, nose, or other infected site. The toxin enters the bloodstream, causing a toxemia. Blood cultures typically do not grow S. aureus.

iii. **Exfoliatin** causes “scalded skin” syndrome in young children

iv. Several exotoxins can kill leukocytes (leukocidins) and cause necrosis of tissues in vivo. Of these, the two most important are alpha toxin and P-V leukocidin.

v. The enzymes include **coagulase,** fibrinolysin, hyaluronidase, proteases, nucleases, and lipases.

Staphylococcus epidermidis & Staphylococcus saprophyticus

Unlike S. *aureus*, these two coagulase-negative staphylococci do not produce exotoxins. Thus, they do not cause food poisoning or toxic shock syndrome. They do, however, cause pyogenic infections. For example, S. *epidermidis* is a prominent cause of pyogenic infections on prosthetic implants such as heart valves and hip joints, and S. *saprophyticus* causes urinary tract infections, especially cystitis.

Clinical Findings

The important clinical manifestations caused by S. aureus can be divided into two groups: pyogenic (pus-producing) and toxin-mediated. S. aureus is a major cause of skin, soft tissue, bone, joint, lung, heart, and kidney infections.

Laboratory Diagnosis

Smears from staphylococcal lesions reveal gram-positive cocci in grapelike clusters.

Cultures of *S. aureus* typically yield golden-yellow colonies that are usually β-hemolytic. *S. aureus* is **coagulase-positive**. Mannitol-salt agar is a commonly used screening device for *S. aureus*. Cultures of coagulase negative staphylococci typically yield white colonies that are non-hemolytic. The two coagulase-negative staphylococci are distinguished by their reaction to the antibiotic novobiocin: *S. epidermidis* is sensitive, whereas *S. saprophyticus* is resistant.

There are no serologic or skin tests used for the diagnosis of any acute staphylococcal infection.

In toxic shock syndrome, isolation of *S. aureus* is not required to make a diagnosis as long as the clinical criteria are met.

Treatment

- 90% or more of *S. aureus* strains are resistant to penicillin. Most of the strains produce β-**lactamase**. Such organisms can be treated with β-lactamase–resistant penicillins (e.g., nafcillin or cloxacillin), some cephalosporins, or vancomycin. Treatment with a combination of a β-lactamase–sensitive penicillin

(e.g., amoxicillin) and a β-lactamase inhibitor (e.g., clavulanic acid) is also useful.

- Approximately 20% of *S. aureus* strains are **methicillin-resistant** or nafcillin-resistant by virtue of altered penicillin-binding proteins. These resistant strains of *S. aureus* are often abbreviated **MRSA** or NRSA, respectively. Such organisms can produce sizable outbreaks of disease, especially in hospitals. The drug of choice for these staphylococci is vancomycin, to which gentamicin is sometimes added.
- Daptomycin is also useful. Trimethoprim-sulfamethoxazole or clindamycin can be used to treat non–life-threatening infections caused by these organisms. Note that
- MRSA strains are resistant to almost all β-lactam drugs, including both penicillins and cephalosporins. Ceftaroline fosamil is the first β-lactam drug useful for the treatment of MRSA infections.
- Strains of *S. aureus* with intermediate resistance to vancomycin (VISA strains) and with complete resistance to vancomycin (VRSA strains) have been isolated from patients. These strains are typically methicillin-/nafcillin-resistant as well, which makes them very difficult to treat. Daptomycin (Cubicin) can be used to treat infections by these organisms. Quinupristin-dalfopristin (Synercid) is another useful choice.
- The treatment of toxic shock syndrome involves correction of the shock by using fluids, pressor drugs, and inotropic drugs; administration of a β-lactamase–resistant penicillin such as nafcillin; and removal of the tampon or debridement of the infected site as needed. Pooled serum globulins, which contain antibodies against TSST, may be useful.
- Mupirocin is very effective as a topical antibiotic in skin infections caused by *S. aureus*. It has also been used to reduce nasal carriage of the organism in hospital personnel and in patients with recurrent staphylococcal infections. A topical skin antiseptic, such as chlorhexidine, can be added to mupirocin.
- Some strains of staphylococci exhibit **tolerance** (i.e., they can be inhibited by antibiotics but are not killed). (That is, the ratio of minimum bactericidal concentration [MBC] to minimum inhibitory concentration [MIC] is very high.)
- Tolerance may result from failure of the drugs to inactivate inhibitors of autolytic enzymes that degrade the organism. Tolerant organisms should be treated with drug combinations.
- Drainage (spontaneous or surgical) is the cornerstone of abscess treatment. **Incision and drainage (I&D)** is often sufficient treatment for a skin abscess (e.g. furuncle [boil]); antibiotics are not necessary in most cases. Previous infection

provides only partial immunity to reinfection.

- *S. epidermidis* is highly antibiotic resistant. Most strains produce β-lactamase but are sensitive to β-lactamase–resistant drugs such as nafcillin. These are called methicillin-sensitive strains (MSSE). Some strains are methicillin/nafcillin resistant (MRSE) due to altered penicillin-binding proteins. The drug of choice is vancomycin, to which either rifampin or an aminoglycoside can be added. Removal of the catheter or other device is often necessary. *S. saprophyticus* urinary tract infections can be treated with trimethoprim-sulfamethoxazole or a quinolone, such as ciprofloxacin.

Prevention

- There is no vaccine against staphylococci. Cleanliness, frequent handwashing, and aseptic management of lesions help to control spread of *S. aureus*.
- Persistent colonization of the nose by *S. aureus* can be reduced by intranasal mupirocin or by oral antibiotics, such as ciprofloxacin or trimethoprim-sulfamethoxazole, but is difficult to eliminate completely.
- Shedders may have to be removed from high-risk areas (e.g., operating rooms and newborn nurseries).
- Cefazolin is often used perioperative to prevent staphylococcal surgical-wound infections.

STREPTOCOCCI

- The streptococci, enterococci, and related organisms are gram-positive spherical bacteria that characteristically form pairs or chains during growth.
- They are widely distributed in nature. Some are members of the normal human microorganism; others are associated with important human diseases attributable to the direct effects of infection or in other cases to an immunologic response to them.
- Streptococci elaborate a variety of extracellular substances and enzymes.
- The streptococci are a large and heterogeneous group of bacteria, and no one system suffices to classify them.

CLASSIFICATION OF STREPTOCOCCI

The classification of streptococci into major categories is based on:

1. Colony morphology and hemolytic reactions on blood agar,
2. Serologic specificity of the cell wall group-specific substance (Lancefield antigens) and other cell wall or capsular antigens,
3. Biochemical reactions and resistance to physical and chemical factors,
4. Molecular genetics

A. Hemolysis

Many streptococci are able to hemolyse red blood cells in vitro in varying degrees.

- β-**hemolysis** - Complete disruption of erythrocytes with clearing of the blood around the bacterial growth e.g. Streptococcus *pyogenes* and Streptococcus *agalactiae*.
- α-**hemolysis** -Incomplete lysis of erythrocytes with reduction of hemoglobin and the formation of green pigment e.g. Streptococcus *pneumoniae*.
- γ- [gamma-] **hemolysis** – non hemolytic e.g. Streptococcus *bovis*.

B. Group-Specific Substance (Lancefield Classification)

This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping into **Lancefield groups A–H** and **K–U**. The serologic specificity of the group specific carbohydrate is determined by an amino sugar.

Group A- Streptococcus *pyogenes*, group B – Streptococcus *agalactiae*, group D –

Streptococcus *bovis* and group C,G – Streptococcus *dysagalactiae*.

C. Capsular Polysaccharides

The antigenic specificity of the capsular polysaccharides is used to classify *Streptococcus pneumoniae* into more than 90 types and to type the group B streptococci (*Streptococcus agalactiae*).

D. Biochemical Reactions

Biochemical tests include sugar fermentation reactions, tests for the presence of enzymes, and tests for susceptibility or resistance to certain chemical agents.

Biochemical tests are most often used to classify streptococci after the colony growth and hemolytic characteristics have been observed.

Biochemical tests are used for species that typically do not react with the commonly used antibody preparations for the group-specific substances, groups A, B, C, F, and G. For example, the viridans streptococci are α-hemolytic or non-hemolytic and do not react with the antibodies commonly used for the Lancefield classification.

STREPTOCOCCUS PYOGENES

- Most streptococci that contain the group A antigen are *S pyogenes*.
- *S pyogenes* is the main human pathogen associated with local or systemic invasion and poststreptococcal immunologic disorders.
- It produces large (1 cm in diameter) zones of β-hemolysis around colonies greater than 0.5 mm in diameter.
- They are PYR-positive (hydrolysis of l-pyrrolidonyl-β-naphthylamide) are susceptible to bacitracin.

Morphology and Identification

A. Typical Organisms

- Individual cocci are spherical or ovoid and are arranged in chains.
- The cocci divide in a plane perpendicular to the long axis of the chain.
- The members of the chain often have a striking diplococcal appearance, and rod-like forms are occasionally seen.
- The lengths of the chains vary widely and are conditioned by environmental factors.
- Streptococci are gram positive; however, as a culture ages and the bacteria die, they lose their gram positivity and can appear to be gram negative; for some streptococci, this can occur after overnight incubation.
- Most group A strains produce capsules composed of hyaluronic acid. The

capsules are most noticeable in very young cultures. They impede phagocytosis. The hyaluronic acid capsule likely plays a greater role in virulence. The capsule binds to hyaluronic-acid-binding protein, CD44, present on human epithelial cells. Binding induces disruption of intercellular junctions allowing microorganisms to remain extracellular as they penetrate the epithelium.

- Hair-like pili project through the capsule of group A streptococci. The pili consist partly of M protein and are covered with **lipoteichoic acid** which is important in the attachment of streptococci to epithelial cells.

B. Culture

- Most streptococci grow in solid media as discoid colonies, usually 1–2 mm in diameter. *S pyogenes* is β-hemolytic.

C. Growth Characteristics

- Energy is obtained principally from the utilization of glucose with lactic acid as the end product. The solid media or broth is enriched with blood or tissue fluids to enhance growth.
- Growth and hemolysis are aided by incubation in 10% CO2 at 37°C.
- Most streptococci are facultative anaerobes and grow under aerobic and anaerobic conditions.

D. Variation

- Variants of the same *Streptococcus* strain may show different colony forms. This is particularly marked among *S pyogenes* strains, giving rise to either matte or glossy colonies. Matte colonies consist of organisms that produce much M protein and generally are virulent. The *S pyogenes* in glossy colonies tend to produce little M protein and are often not virulent.

Antigenic Structure

A. M Protein

- This substance is a major virulence factor of *S pyogenes*.
- M protein is a filamentous structure anchored to the cell membrane that penetrates and projects from the streptococcal cell wall. When M protein is present, the streptococci are virulent, and in the absence of M type-specific antibodies, they are able to resist phagocytosis by polymorphonuclear leukocytes by inhibiting activation of the alternate complement pathway.
- There are two major structural classes of M protein, classes I and II.
- M protein and other streptococcal cell wall antigens have an important role in the

pathogenesis of rheumatic fever.

- Conserved antigenic domains on the class I M protein cross-react with human cardiac muscle, and the class I M protein may be a virulence determinant for rheumatic fever.

Toxins and Enzymes

More than 20 extracellular products that are antigenic are elaborated by *S pyogenes*, including the following.

A. Streptokinase (Fibrinolysin)

- Streptokinase is produced by many strains of group A β-hemolytic streptococci. It transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins, allowing the bacteria to escape from blood clots.
- This process of digestion may be interfered with by nonspecific serum inhibitors and by a specific antibody, antistreptokinase.
- Streptokinase has been given intravenously for treatment of pulmonary emboli, coronary artery, and venous thromboses.

B. Deoxyribonucleases

- Streptococcal deoxyribonucleases A, B, C, and D degrade DNA (DNases) and similar to streptokinase facilitate the spread of streptococci in tissue by liquefying pus.
- The enzymatic activity can be measured by the decrease in viscosity of known DNA solutions. Purulent exudates owe their viscosity largely to deoxyribonucleoprotein.
- Mixtures of streptokinase and DNases are used in “enzymatic debridement.” They help to liquefy exudates and facilitate removal of pus and necrotic tissue; antimicrobial drugs thus gain better access, and infected surfaces recover more quickly.

C. Hyaluronidase

- Hyaluronidase splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor).
- Hyaluronidases are antigenic and specific for each bacterial or tissue source. After infection with hyaluronidase-producing organisms, specific antibodies are found in the serum.

D. **Pyrogenic Exotoxins (Erythrogenic Toxin)**

- Pyrogenic exotoxins are elaborated by *S pyogenes*.
- There are three antigenically distinct **streptococcal pyrogenic exotoxins (Spe): A, B, and C**. SpeA has been most widely studied. It is produced by group A streptococci that carry a lysogenic phage.
- The streptococcal pyrogenic exotoxins have been associated with **streptococcal toxic shock syndrome** and **scarlet fever**.
- Most strains of group A streptococci isolated from patients with streptococcal toxic shock syndrome either produce Spe A or have the gene that codes for it;
- The pyrogenic exotoxins act as super antigens, which stimulate T cells by binding to the class II major histocompatibility complex in the Vβ region of the T-cell receptor. The activated T cells release cytokines that mediate shock and tissue injury.

E. **Hemolysins**

- The β-hemolytic group A *S. pyogenes* elaborates two hemolysins (streptolysins) that not only lyse the membranes of erythrocytes but also damage a variety of other cell types.
- **Streptolysin O** is a protein that is hemolytically active in the reduced state but rapidly inactivated in the presence of oxygen. Streptolysin O is responsible for some of the hemolysis seen when growth occurs in cuts made deep into the medium in blood agar plates.
- It combines quantitatively with **antistreptolysin O (ASO)**, an antibody that appears in humans after infection with any streptococci that produce streptolysin O. This antibody blocks hemolysis by streptolysin O. This phenomenon forms the basis of a quantitative test for the antibody.
- **Streptolysin S** is the agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates. It is elaborated in the presence of serum—hence the name Streptolysin S. It is not antigenic.

Pathogenesis and Clinical Findings

A variety of distinct disease processes are associated with *S pyogenes* infections. The infections can be divided into several categories.

A. Diseases Attributable to Invasion by *S pyogenes*, β-Hemolytic Group A Streptococci

The portal of entry determines the principal clinical picture. In each case, however, there

is a diffuse and rapidly spreading infection that involves the tissues and extends along lymphatic pathways with only minimal local suppuration. From the lymphatics, the infection can extend to the bloodstream.

1. **Erysipelas—** If the portal of entry is the skin, erysipelas results. Lesions are raised and characteristically red. There is massive brawny edema and a rapidly advancing, sharply demarcated margin of infection.
2. **Cellulitis**—Streptococcal cellulitis is an acute, rapidly spreading infection of the skin and subcutaneous tissues. It follows infection associated with mild trauma, burns, wounds, or surgical incisions. Pain, tenderness, swelling, and erythema occur. Cellulitis is differentiated from erysipelas by two clinical findings: In cellulitis, the lesion is not raised, and the line between the involved and uninvolved tissue is indistinct.
3. **Necrotizing fasciitis (streptococcal gangrene)—** There is extensive and very rapidly spreading necrosis of the skin, tissues, and fascia. Bacteria other than *S pyogenes* can also cause necrotizing fasciitis. The group A streptococci that cause necrotizing fasciitis have sometimes been termed *flesh-eating bacteria*.
4. **Puerperal fever**—If the streptococci enter the uterus after delivery, puerperal fever develops, which is essentially a septicemia originating in the infected wound (endometritis).
5. **Bacteremia or sepsis**—Infection of traumatic or surgical wounds with streptococci results in bacteremia, which can rapidly be fatal. *S pyogenes* bacteremia can also occur with skin infections, such as cellulitis and rarely pharyngitis.

B. **Diseases Attributable to Local Infection With *S pyogenes* and Their Byproducts**

1. **Streptococcal sore throat**—The most common infection caused by β-hemolytic *S pyogenes* is streptococcal sore throat or pharyngitis. *S pyogenes* adheres to the pharyngeal epithelium by means of lipoteichoic acid–covered surface pili and by means of hyaluronic acid in encapsulated strains. In infants and small children, the sore throat occurs as a subacute nasopharyngitis with a thin serous discharge and little fever but with a tendency of the infection to extend to the middle ear and the mastoid. The cervical lymph nodes are usually enlarged. The illness may persist for weeks. In older children and adults, the disease is more acute and is characterized by intense nasopharyngitis, tonsillitis, and intense redness and edema of the mucous membranes, with purulent exudate; enlarged, tender cervical lymph nodes; and (usually) a high fever. Twenty percent of infections are asymptomatic. *S pyogenes* infection of the upper respiratory tract does not usually involve the lungs.
2. **Streptococcal pyoderma**—Local infection of superficial layers of skin, especially

in children, is called **impetigo**. It consists of superficial vesicles that break down and eroded areas whose denuded surface is covered with pus and later is encrusted. It spreads by continuity and is highly communicable, especially in hot, humid climates. More widespread infection occurs in eczematous or wounded skin or in burns and may progress to cellulitis.

C. Invasive Group A Streptococcal Infections, Streptococcal Toxic Shock Syndrome, and Scarlet Fever

- Invasive *S pyogenes* infections with **streptococcal toxic shock syndrome** are characterized by shock, bacteremia, respiratory failure, and multiorgan failure. Death occurs in about 30% of patients. The infections tend to occur after minor trauma in otherwise healthy persons with several presentations of soft tissue infection. These include necrotizing fasciitis, myositis, and infections at other soft tissue sites; bacteremia occurs frequently. In some patients, the disease presents with focal soft tissue infection accompanied by fever and rapidly progressive shock with multiorgan failure. Erythema and desquamation may occur.

- Pyrogenic exotoxins A–C also cause **scarlet fever** in association with *S pyogenes* pharyngitis or with skin or soft tissue infection. The pharyngitis may be severe. The rash appears on the trunk after 24 hours of illness and spreads to involve the extremities. Streptococcal toxic shock syndrome and scarlet fever are clinically overlapping diseases.

D. Poststreptococcal Diseases (Rheumatic Fever, Glomerulonephritis)

After an acute *S pyogenes* infection, there is a latent period of 1–4 weeks (mean 7 days), after which nephritis or rheumatic fever occasionally develops. The latent period suggests that these poststreptococcal diseases are not attributable to the direct effect of disseminated bacteria but instead represent a hypersensitivity response. Nephritis is more commonly preceded by infection of the skin; rheumatic fever is more commonly preceded by infection of the respiratory tract.

Diagnostic Laboratory Tests

A. Specimens

- Specimens to be obtained depend on the nature of the streptococcal infection. A throat swab, pus, cerebrospinal fluid or other sterile body fluid, or blood is obtained for culture.

- Serum is obtained for antibody determinations.

B. Smears

- Smears from pus often show single cocci or pairs rather than definite chains. Cocci are sometimes gram negative because the organisms are no longer viable

and have lost their ability to retain the blue dye (crystal violet) and be gram positive.

- If smears of pus show streptococci but cultures fail to grow, anaerobic organisms must be suspected. Smears of throat swabs are rarely contributory because viridans streptococci are always present and have the same appearance as group A streptococci on stained smears.

C. Culture

- Specimens suspected of containing streptococci are cultured on blood agar plates. If anaerobes are suspected, suitable anaerobic media must also be inoculated. Incubation in
- 10% CO2 often speeds hemolysis. Slicing the inoculum into the blood agar has a similar effect because oxygen does not readily diffuse through the medium to the deeply embedded organisms, and it is oxygen that inactivates streptolysin O.

- Blood cultures will grow hemolytic group A streptococci (eg, in sepsis) within hours or a few days.
- *S pyogenes* can be identified by rapid tests specific for the presence of the group A-specific antigen and by the PYR test.
- Streptococci belonging to group A may be presumptively identified by inhibition of growth by bacitracin, but this should be used only when more definitive tests are not available.

D. Antigen Detection Tests

- Several commercial kits are available for rapid detection of group A streptococcal antigen from throat swabs. These kits use enzymatic or chemical methods to extract the antigen from the swab, then use enzyme immunoassay (EIA) or agglutination tests to demonstrate the presence of the antigen.
- The tests can be completed in minutes to hours after the specimen is obtained. They are 60–90% sensitive, depending on the prevalence of the disease in the population, and
- 98–99% specific compared with culture methods.
- More sensitive assays that use DNA probes or nucleic acid amplification techniques are now available and are beginning to replace the earlier antigen detection tests, although they remain more costly.

E. Serologic Tests

- A rise in the titer of antibodies to many group A streptococcal antigens can be estimated. Such antibodies include ASO, particularly in respiratory disease; anti-DNase B and antihyaluronidase, particularly in skin infections; antistreptokinase; anti-M type-specific antibodies; and others. Of these, the anti-ASO titer is most widely used.

Treatment

- All *S pyogenes* are susceptible to penicillin G. Macrolides, such as erythromycin and clindamycin, have often been recommended for penicillin-allergic patients and for patients with necrotizing fasciitis. However, resistance to macrolide antibiotics has been increasing and some are resistant to tetracyclines. Antimicrobial drugs have no effect on established glomerulonephritis and rheumatic fever.

Epidemiology, Prevention, and Control

Although humans can be asymptomatic nasopharyngeal or perineal carriers of *S pyogenes*, the organism should be considered significant if it is detected by culture or other means.

The ultimate source of group A streptococci is a person harboring these organisms. The individual may have a clinical or subclinical infection or may be a carrier distributing streptococci directly to other persons via droplets from the respiratory tract or skin. The nasal discharges of a person harboring *S pyogenes* are the most dangerous source for spread of these organisms.

Control procedures are directed mainly at the human source:

1. Detection and early antimicrobial therapy of respiratory and skin infections with group A streptococci. Prompt eradication of streptococci from early infections can effectively prevent the development of poststreptococcal disease.
2. Antistreptococcal chemoprophylaxis in persons who have suffered an attack of rheumatic fever. This involves giving one injection of benzathine penicillin G intramuscularly every 3–4 weeks or daily oral penicillin or oral sulfonamide. The first attack of rheumatic fever infrequently causes major heart damage; however, such persons are particularly susceptible to reinfections with streptococci that precipitate relapses of rheumatic activity and give rise to cardiac damage. Chemoprophylaxis in such individuals, especially children, must be continued for years. Chemoprophylaxis is not used in glomerulonephritis because of the small number of nephritogenic types of streptococci. An exception may be family groups with a high rate of poststreptococcal nephritis.
3. Eradication of *S pyogenes* from carriers. This is especially important when

carriers are in areas such as obstetric delivery rooms, operating rooms, classrooms, or nurseries. Unfortunately, it is often difficult to eradicate β-hemolytic streptococci from permanent carriers, and individuals may occasionally have to be shifted away from “sensitive” areas for some time.

STREPTOCOCCUS AGALACTIAE

- These are the **group B streptococci**. They typically are β-hemolytic and produce zones of hemolysis that are only slightly larger than the colonies (1–2 mm in diameter). The group B streptococci hydrolyze sodium hippurate and give a positive response in the so-called CAMP test.
- Group B streptococci are part of the normal vaginal flora and lower gastrointestinal tract in 5–30% of women. Group B streptococcal infection during the first month of life may present as fulminant sepsis, meningitis, or respiratory distress syndrome.
- Group B streptococcal infections are increasing among non-pregnant adults. The elderly adults and immunocompromised hosts, are most at risk for invasive disease. Predisposing factors include diabetes mellitus, cancer, advanced age, liver cirrhosis, corticosteroid therapy, HIV, and other immunocompromised states. Bacteremia, skin and soft tissue infections, respiratory infections, and genitourinary infections in descending order of frequency are the major clinical manifestations.

GROUPS C AND G

- These streptococci occur sometimes in the nasopharynx and may cause pharyngitis, sinusitis, bacteremia, or endocarditis.
- They often look like group A *S pyogenes* on blood agar medium and are β-hemolytic. They are identified by reactions with specific antisera for groups C or G.
- Groups C and G streptococci have hemolysins and may have M proteins analogous to those of group A *S pyogenes*. Poststreptococcal sequelae of acute glomerulonephritis (AGN) and RF have been rarely reported.

GROUP D STREPTOCOCCI

- The group D streptococci have eight species, many of which do not cause infections in humans. The *Streptococcus bovis* group is of most importance to human disease.
- The species of S. *bovis* causes human endocarditis and is frequently epidemiologically associated with colon carcinoma.
- All group D streptococci are nonhemolytic and PYR negative. They grow in the

presence of bile and hydrolyze esculin (bile esculin positive) but do not grow in 6.5% NaCl. They are part of the normal enteric microorganism of humans and animals.

STREPTOCOCCUS ANGINOSUS GROUP

- Other species names in the *S anginosus* group are *Streptococcus constellatus* and *Streptococcus intermedius*. These streptococci are part of the normal microorganisms of the throat, colon, and urogenital tract.
- They may be β-, α-, or nonhemolytic.
- *S anginosus* group includes β-hemolytic streptococci that form minute colonies (<0.5 mm in diameter) and react with groups A, C, or G antisera and all β-hemolytic group F streptococci.
- Those that are group A are PYR negative. *S anginosus* are Voges-Proskauer test positive. They may be classified as viridans streptococci. These organisms are frequently associated with serious infections such as brain, lung, and liver abscesses. They can be easily detected in the laboratory by their characteristic butterscotch or caramel odor.

VIRIDANS STREPTOCOCCI

- The many species of the viridans streptococci are classified into groups and include the Streptococcus *mitis* group, S *anginosus* group, Streptococcus *mutans* group, Streptococcus *salivarius* group, and S *bovis* group.
- Typically they are α-hemolytic, but they may also be non-hemolytic. Members of the S *anginosus* group can be β-hemolytic.
- Their growth is not inhibited by optochin, and colonies are not soluble in bile (deoxycholate).
- The viridans streptococci are the most prevalent members of the normal microorganisms of the upper respiratory tract and are important for the healthy state of the mucous membranes there. They may reach the bloodstream as a result of trauma and are a principal cause of endocarditis on abnormal heart valves.
- Some viridans streptococci (eg, S mutans) synthesize large polysaccharides such as dextrans or levans from sucrose and contribute importantly to the genesis of dental caries.

STREPTOCOCCUS PNEUMONIAE

- *S pneumoniae* (pneumococci) is a member of the *S mitis* group and are indistinguishable from them on the basis of 16SrRNA.

- Pneumococci are gram-positive diplococci, often lancet shaped or arranged in chains, possessing a capsule of polysaccharide that permits typing with specific antisera.
- Pneumococci are readily lysed by surface-active agents, which probably remove or inactivate the inhibitors of cell wall autolysins.
- Pneumococci are normal inhabitants of the upper respiratory tract of 5–40% of humans and can cause pneumonia, sinusitis, otitis, bronchitis, bacteremia, meningitis, peritonitis, and other infectious processes.

Morphology and Identification

A. Typical Organisms

- The typical gram-positive, lancet-shaped diplococci are often seen in specimens of young cultures. In sputum orpus, single cocci or chains are also seen. With age, the organisms rapidly become gram negative and tend to lyse spontaneously.
- Autolysis of pneumococci is greatly enhanced by surface-active agents.
- On solid media, the growth of pneumococci is inhibited around a disk of optochin.
- Other identifying points include almost uniform virulence for mice when injected intraperitoneally and the “capsule swelling test,” or quellung reaction.

B. Culture

Pneumococci form small round colonies, at first dome-shaped and later developing a central depression with an elevated rim.

. Pneumococci are α-hemolytic on blood agar. Growth is enhanced by 5–10% CO_2.

C. Growth Characteristics

- Most energy is obtained from fermentation of glucose; this is accompanied by the rapid production of lactic acid, which limits growth. Neutralization of broth cultures with alkali at intervals results in massive growth.

D. Variation

- Pneumococcal isolates that produce large amounts of capsules appear as large mucoid colonies. Capsule production is not essential for growth on agar medium, and capsular production is therefore lost after a small number of subcultures.
- The pneumococci will, however, again produce capsules and have enhanced virulence if injected into mice.

Antigenic Structure

A. Component Structures

- The pneumococcal cell wall has peptidoglycan and teichoic acid, similar to other streptococci. The capsular polysaccharide that is found in the cell wall of all *S pneumoniae* can be detected in the urine and cerebrospinal fluid (CSF) as useful diagnostic tests for pneumococcal infections.

B. Quellung Reaction

- When pneumococci of a certain type are mixed with specific antipolysaccharide serum of the same type—or with polyvalent antiserum—on a microscope slide, the capsule swells markedly, and the organisms agglutinate by cross-linking of the antibodies. This reaction is useful for rapid identification and for typing of the organisms, either in sputum or in cultures.

Pathogenesis

A. Types of Pneumococci

In adults, types 1–8 are responsible for about 75% of cases of pneumococcal pneumonia and for more than half of all fatalities in pneumococcal bacteremia; in children, types 6,

14, 19, and 23 are frequent causes.

B. Production of Disease

Pneumococci produce disease through their ability to multiply in the tissues. The virulence of the organism is a function of its capsule, which prevents or delays ingestion by phagocytes.

A serum that contains antibodies against the type specific polysaccharide protects against infection. If such a serum is absorbed with the type-specific polysaccharide, it loses its protective power.

C. Loss of Natural Resistance

Because 40–70% of humans are at some time carriers of virulent pneumococci, the normal respiratory mucosa must possess great natural resistance to the pneumococcus. Among the factors that probably lower this resistance and thus predispose to pneumococcal infection are the following:

1. Viral and other respiratory tract infections that damage surface cells; abnormal accumulations of mucus (eg, allergy), which protect pneumococci from phagocytosis; bronchial obstruction (eg, atelectasis); and respiratory tract injury caused by irritants disturbing its mucociliary function.

2. Alcohol or drug intoxication, which depresses phagocytic activity, depresses the cough reflex, and facilitate aspiration of foreign material.
3. Abnormal circulatory dynamics (eg, pulmonary congestion, heart failure)
4. Other mechanisms, such as malnutrition, general debility, sickle cell anemia, hyposplenism, nephrosis, or complement deficiency

Pathology

- Pneumococcal infection causes an outpouring of fibrinous edema fluid into the alveoli followed by red blood cells and leukocytes, which results in consolidation of portions of the lung. Many pneumococci are found throughout this exudate, and they may reach the bloodstream via the lymphatic drainage of the lungs. The alveolar walls remain normally intact during the infection. Later, mononuclear cells actively phagocytose the debris, and this liquid phase is gradually reabsorbed. The pneumococci are taken up by phagocytes and digested intracellularly.

Clinical Findings

- The onset of pneumococcal pneumonia is usually sudden, with fever, chills, and sharp pleural pain. The sputum is similar to the alveolar exudate, being characteristically bloody or rusty colored. Early in the disease, when the fever is high, bacteremia is present in 10–20% of cases. With antimicrobial therapy, the illness is usually terminated promptly; if drugs are given early, the development of consolidation is interrupted.
- From the respiratory tract, pneumococci may reach other sites. The sinuses and middle ear are most frequently involved. Infection sometimes extends from the mastoid to the meninges. Bacteremia from pneumonia has a triad of severe complications: meningitis, endocarditis, and septic arthritis.

Diagnostic Laboratory Tests

- Blood is drawn for culture; CSF and sputum are collected for demonstration of pneumococci by smear and culture. CSF and urine can be used to detect pneumococcal C-polysaccharide by rapid immunochromatographic membrane assays.
- All specimens should be sent to the microbiology laboratory as soon as possible after collection because pneumococci tend to autolyse and delay will significantly impact recovery by culture.

A. Stained Smears

- A Gram-stained film of rusty-red sputum shows typical organisms, many polymorphonuclear neutrophils, and many red blood cells.

B. Capsule Swelling Tests

- Fresh emulsified sputum mixed with antiserum causes capsule swelling (the quellung reaction) for identification of pneumococci.

C. Culture

- The culture is created by inoculating sputum to blood agar and incubating the plate in CO2 at 37°C. A blood culture is also usually obtained.

D. Nucleic Acid Amplification Tests

- Several manufacturers have included *S pneumoniae* on panels for identification of positive blood culture bottles. Also, in development are panel tests for meningitis and separate molecular panels for direct detection of *S pneumoniae* in respiratory samples obtained from specimens in patients suspected of having community acquired or health care–associated pneumonia.

E. Immunity

- Immunity to infection with pneumococci is type specific and depends both on antibodies to capsular polysaccharide and on intact phagocytic function. Vaccines can induce production of antibodies to capsular polysaccharides.

Treatment

- Around 15% of pneumococci from non-meningeal sources are penicillin resistant (minimum inhibitory concentration [MIC] ≥8 µg/mL).
- High-dose penicillin G appears to be effective in treating pneumonia caused by pneumococci with MICs to penicillin below 8 µg/mL (resistant breakpoint) but would not be effective in treatment of meningitis caused by the same strains. Some penicillin-resistant strains are resistant to cefotaxime.
- Resistance to tetracycline, erythromycin, and fluoroquinolones also occurs.
- Pneumococci is susceptible to vancomycin.

Epidemiology, Prevention, and Control

- Pneumococcal pneumonia accounts for about 60% of all bacterial pneumonias. In the development of illness, predisposing factors are more important than exposure to the infectious agent, and a healthy carrier is more important in disseminating pneumococci than a sick patient.
- It is possible to immunize individuals with type-specific polysaccharides. Such vaccines can probably provide 90% protection against bacteremic pneumonia.

ENTEROCOCCI

- The enterococci have the group D group-specific substance and were previously classified as group D streptococci.
- Because the group D cell wall–specific antigen is a teichoic acid, it is not an antigenically good marker; enterococci are usually identified by characteristics other than immunologic reactions with group-specific antisera. They are part of the normal enteric microorganisms.
- They are usually non-hemolytic but are occasionally α-hemolytic or rarely β-hemolytic.
- Enterococci are PYR positive. They grow in the presence of bile, hydrolyze esculin (bile esculin positive) and in contrast to non-enterococcal group D streptococci, they grow well in
- 6.5% NaCl. Whereas enterococci grow well at between 10 and 45°C, streptococci generally grow at a much narrower temperature range.
- They are more resistant to penicillin G than the streptococci. Many isolates are vancomycin resistant.
- There are at least 47 species of enterococci, but less than one-third of these are associated with disease in humans. *Enterococcus faecalis* is the most common and causes 85–90% of enterococcal infections; *Enterococcus faecium* causes 5–10%.
- The enterococci are among the most frequent causes of health care associated infections, particularly in intensive care units, and are selected by therapy with cephalosporins and other antibiotics to which they are resistant.
- Enterococci are transmitted from one patient to another primarily on the hands of hospital personnel, some of whom may carry the enterococci in their gastrointestinal tracts. Enterococci occasionally are transmitted on medical devices.
- In patients, the most common sites of infection are the urinary tract, wounds, biliary tract, and blood. Enterococci may cause meningitis and bacteremia in neonates.
- In adults, enterococci can cause endocarditis. However, in intra-abdominal, wound, urine, and other infections, enterococci usually are cultured along with other species of bacteria, and it is difficult to define the pathogenic role of the enterococci in these clinical circumstances.

Antibiotic Resistance

A major problem with the enterococci is that they can be very resistant to antibiotics. *E faecium* is usually much more antibiotic-resistant than *E faecalis.*

Enterococci are intrinsically resistant to cephalosporins, penicillinase-resistant penicillins, and monobactams. They have intrinsic low-level resistance to many aminoglycosides, are of intermediate susceptibility or resistant to fluoroquinolones.

Therapy with combinations of a cell wall–active antibiotic (a penicillin or vancomycin) plus an aminoglycoside (streptomycin or gentamicin) is essential for severe enterococcal infections, such as endocarditis.

The glycopeptide vancomycin is the primary alternative drug to a penicillin (plus an aminoglycoside) for treating enterococcal infections.

Enterococci often show susceptibility to trimethoprim-sulfamethoxazole by in vitro testing, but the drugs are not effective in treating infections. This discrepancy is because enterococci are able to utilize exogenous folates available invivo and thus escape inhibition by the drugs.

GRAM-NEGATIVE COCCI

Neisseria is the medically important genus of gram-negative cocci. The genus *Neisseria* contains two important human pathogens: *Neisseria meningitidis* (meningococcus) and *Neisseria gonorrhoeae* (gonococcu).

N. meningitidis mainly causes meningitis and meningococcemia whereas *N. gonorrhoeae* causes gonorrhea, neonatal conjunctivitis (ophthalmia neonatorum) and pelvic inflammatory disease (PID).

Important properties

- Neisseriae are gram-negative cocci that resemble paired kidney beans and contain endotoxin (consist of lipo**oligo**saccharide) in their outer membrane.
- *N. meningitidis* (meningococcus) has a prominent **polysaccharide capsule** that enhances virulence by its antiphagocytic action and induces protective antibodies. They are divided into at least 13 serologic groups on the basis of the antigenicity of their capsular polysaccharides.
- N. *gonorrhoeae* (gonococcus) has no polysaccharide capsule but has multiple serotypes based on the antigenicity of its pilus protein (proteins I, II, and III). Protein II plays a role in attachment of the organism to cells and varies antigenically as well.
- They are cultured on “chocolate” agar containing blood heated to 80°C, which inactivates the inhibitors.
- Neisseriae are **oxidase-positive** (i.e., they possess the enzyme cytochrome *c*). This is an important laboratory diagnostic test in which colonies exposed to phenylenediamine turn purple or black as a result of oxidation of the reagent by the enzyme.

1. Neisseria meningitidis

Pathogenesis & Epidemiology

Humans are the only natural hosts for meningococci. The organisms are transmitted by airborne droplets; they colonize the membranes of the nasopharynx and become part of the normal flora of the upper respiratory tract. Carriers are usually asymptomatic. From the nasopharynx, the organism can enter the bloodstream and spread to specific sites, such as the meninges or joints, or be disseminated throughout the body (meningococcemia). About 5% of people become chronic carriers and serve as a source of infection for others.

Meningococci have three important virulence factors:

1. A **polysaccharide capsule** that enables the organism to resist phagocytosis by

polymorphonuclear leukocytes (PMNs).

2. **Endotoxin,** which causes fever, shock, and other pathophysiologic changes (in purified form, endotoxin can reproduce many of the clinical manifestations of meningococcemia).
3. An **immunoglobulin A (IgA) protease** that helps the bacteria attach to the membranes of the upper respiratory tract by cleaving secretory IgA.

Clinical Findings

The two most important manifestations of disease are meningococcemia and meningitis. The most severe form of meningococcemia is the life-threatening Waterhouse–Friderichsen syndrome, which is characterized by high fever, shock, widespread purpura, disseminated intravascular coagulation, thrombocytopenia, and adrenal insufficiency.

Bacteremia can result in the seeding of many organs, especially the meninges.

The symptoms of meningococcal meningitis are those of a typical bacterial meningitis, namely, fever, headache, stiff neck, and an increased level of polymorphonuclear leukocytes (PMNs) in spinal fluid.

Laboratory Diagnosis

- The principal laboratory procedures are smear and culture of blood and spinal fluid samples. A presumptive diagnosis of meningococcal meningitis can be made if gram-negative cocci are seen in a smear of spinal fluid.
- The organism grows best on chocolate agar incubated at 37°C in a 5% CO2 atmosphere. A presumptive diagnosis of Neisseria can be made if oxidase-positive colonies of gram-negative diplococci are found.
- The differentiation between N. *meningitidis* and N. *gonorrhoeae* is made on the basis of sugar fermentation: meningococci ferment maltose, whereas gonococci do not (both organisms ferment glucose).
- Immunofluorescence can also be used to identify these species.
- Tests for serum antibodies are not used for clinical diagnosis. However the rapid diagnosis of meningococcal meningitis by latex agglutination test can be used to detect capsular polysaccharide in the spinal fluid.

Treatment

Penicillin G is the treatment of choice for meningococcal infections. A third generation cephalosporin such as ceftriaxone can also be used. Resistance to sulfonamide is common.

Prevention

- Chemoprophylaxis and immunization are both used to prevent meningococcal disease.
- Either rifampin or ciprofloxacin can be used for prophylaxis in people who have had close contact with the index case. These drugs are preferred because they are efficiently secreted into the saliva, in contrast to penicillin G.
- The meningococcal vaccine which contain the capsular polysaccharide are used for immunization.

2. **Neisseria *gonorrhoeae***

Pathogenesis & Epidemiology

Gonococci, like meningococci, cause disease only in humans. The organism is usually transmitted **sexually;** newborns can be infected during birth. Because gonococcus is quite sensitive to dehydration and cool conditions, sexual transmission favors its survival.

Gonorrhea is usually symptomatic in men but often asymptomatic in women. Genital tract infections are the most common source of the organism, but anorectal and pharyngeal infections are important sources as well.

The most important virulence factors include;

1. **Pili** that mediate attachment to mucosal cell surfaces and are antiphagocytic.
2. **Endotoxin (lipooligosaccharide, LOS)** which causes fever, shock, and other pathophysiologic changes and the
3. **Outer membrane proteins** (The organism's **IgA protease)** hydrolyzes secretory IgA, which could otherwise block attachment to the mucosa.

   Note: Gonococci have no capsules.

The main host defenses against gonococci are antibodies (IgA and IgG), complement, and neutrophils. Antibody-mediated opsonization and killing within phagocytes occur, but repeated gonococcal infections are common, primarily as a result of antigenic changes of pili and the outer membrane proteins.

Gonococci infect primarily the mucosal surfaces (e.g., the urethra and vagina), but dissemination occurs.

Clinical Findings

Gonococci cause both localized infections, usually in the genital tract, and disseminated infections with seeding of various organs. Gonococci reach these organs via the bloodstream (gonococcal bacteremia).

Gonorrhea in men is characterized primarily by urethritis accompanied by dysuria and a purulent discharge. Epididymitis can occur.

In women, infection is located primarily in the endocervix, causing a purulent vaginal discharge and intermenstrual bleeding (cervicitis). The most frequent complication in women is an ascending infection of the uterine tubes (**salpingitis, PID**), which can result in **sterility** or ectopic pregnancy as a result of scarring of the tubes.

Disseminated gonococcal infections (DGI) commonly manifest as arthritis, tenosynovitis, or pustules in the skin. Disseminated infection is the most common cause of septic arthritis in sexually active adults.

Other infected sites include the anorectal area, throat, and eyes. Anorectal infections occur mostly in women and homosexual men. They are frequently asymptomatic, but a bloody or purulent discharge (proctitis) can occur.

In the throat, pharyngitis occurs, but many patients are asymptomatic. In newborn infants, purulent conjunctivitis (ophthalmia neonatorum) is the result of gonococcal infection acquired from the mother during passage through the birth canal. Gonococcal conjunctivitis also occurs in adults as a result of the transfer of organisms from the genitals to the eye.

Laboratory Diagnosis

- The diagnosis of urogenital infections depends on Gram staining and culture of the discharge However, nucleic acid amplification tests are widely used as screening tests.
- In **men,** the finding of gram-negative diplococci **within PMNs** in a urethral discharge specimen is sufficient for diagnosis.
- In **women,** the use of the Gram stain alone can be difficult to interpret; therefore, cultures should be done.
- Gram stains on cervical specimens can be falsely positive because of the presence of gram-negative diplococci in the normal flora and can be falsely negative.
- Specimens from mucosal sites, such as the urethra and cervix, are cultured on Thayer-Martin medium, which is a chocolate agar containing antibiotics (vancomycin, colistin, trimethoprim, and nystatin) to suppress the normal flora.
- The finding of an oxidase-positive colony composed of gram-negative diplococci is sufficient to identify the isolate as a member of the genus *Neisseria*.
- Specific identification of the gonococcus can be made either by its fermentation of glucose (but not maltose) or by fluorescent-antibody staining. Note that specimens from sterile sites, such as blood or joint fluid, can be cultured on

chocolate agar without antibiotics because there is no competing normal flora.

- Rapid tests are also used to detect the presence of gonococcal nucleic acids in patient specimens as a screening test.

Treatment

Ceftriaxone is the treatment of choice in uncomplicated gonococcal infections.

Azithromycin or ciprofloxacin should be used if the patient is allergic to penicillins or cephalosporins.

Prevention

- The prevention of gonorrhea involves the use of condoms and the prompt treatment of symptomatic patients and their contacts.
- Cases of gonorrhea must be reported to the public health department to ensure proper follow-up. A major problem is the detection of asymptomatic carriers.
- Gonococcal conjunctivitis in newborns is prevented most often by the use of erythromycin ointment. Silver nitrate drops are used less frequently.
- No vaccine is available.

GRAM POSITIVE RODS

There are four medically important genera of gram-positive rods: *Bacillus, Clostridium, Corynebacterium*, and *Listeria. Bacillus* and *Clostridium* form spores, whereas *Corynebacterium* and *Listeria* do not. Members of the genus *Bacillus* are aerobic, whereas those of the genus *Clostridium* are anaerobic.

These gram-positive rods can also be distinguished based on their appearance on Gram stain. *Bacillus* and *Clostridium* species are longer and more deeply staining than *Corynebacterium* and *Listeria* species. *Corynebacterium* species are club shaped (i.e., they are thinner on one end than the other). *Corynebacterium* and *Listeria* species characteristically appear as V- or L-shaped rods.

SPORE-FORMING GRAM-POSITIVE RODS

BACILLUS

There are two medically important *Bacillus* species: *Bacillus anthracis* and

Bacillus cereus.

1. ***Bacillus anthracis***

Disease

B. anthracis causes anthrax, which is common in animals but rare in humans. Human disease occurs in three main forms: cutaneous, pulmonary (inhalation), and gastrointestinal.

Important Properties

B. anthracis is a large gram-positive rod with square ends, frequently found in chains. Its antiphagocytic capsule is composed of D-glutamate.

It is non motile, whereas other members of the genus are motile. Anthrax toxin is encoded on one plasmid, and the polyglutamate capsule is encoded on a different plasmid.

Transmission

- Spores of the organism persist in soil for years. Humans are most often infected cutaneous at the time of trauma to the skin, which allows the **spores on animal products,** such as hides, bristles, and wool, to enter.
- Spores can also be inhaled into the respiratory tract. Pulmonary (inhalation) anthrax occurs when spores are inhaled into the lungs. Inhalation anthrax is not communicable from person to person, despite the severity of the infection. After being inhaled into the lung, the organism moves rapidly to the mediastinal lymph nodes, where it causes hemorrhagic mediastinitis. Because it leaves the lung so rapidly, it is not transmitted by the respiratory route to others
- Gastrointestinal anthrax occurs when contaminated meat is ingested.

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Pathogenesis

Pathogenesis is based primarily on the production of two exotoxins, collectively known as anthrax toxin. The two exotoxins, **edema factor** (causes an outpouring of fluid from the cell into the extracellular space, which manifests as edema) **and lethal factor** (inhibits cell growth), each consist of two proteins in an A–B subunit configuration. The B, or binding, subunit in each of the two exotoxins is **protective antigen**. The A, or active, subunit has enzymatic activity.

Clinical Findings

The typical lesion of cutaneous anthrax is a painless ulcer with a black eschar (crust, scab) Local edema is striking. The lesion is called a **malignant pustule**. Untreated cases progress to bacteremia and death.

Pulmonary (inhalation) anthrax, also known as “wool-sorter’s disease,” begins with nonspecific respiratory tract symptoms resembling influenza, especially a dry cough

and substernal pressure. This rapidly progresses to hemorrhagic mediastinitis, bloody pleural effusions, septic shock, and death. Although the lungs are infected, the classic features and X-ray picture of pneumonia are not present. Mediastinal widening seen on chest X-ray is an important diagnostic criterion.

The symptoms of gastrointestinal anthrax include vomiting, abdominal pain, and bloody diarrhea.

Laboratory Diagnosis

- Smears show large, gram-positive rods in chains. Spores are usually not seen in smears of exudate because spores form when nutrients are insufficient, and nutrients are plentiful in infected tissue. Non-hemolytic colonies form on blood agar aerobically.
- In case of a bioterror attack, rapid diagnosis can be performed in special laboratories using polymerase chain reaction (PCR)–based assays.
- Another rapid diagnostic procedure is the direct fluorescent antibody test that detects antigens of the organism in the lesion.
- Serologic tests, such as an enzyme-linked immunosorbent assay (ELISA) test for antibodies, require acute and convalescent serum samples and can only be used to make a diagnosis retrospectively.

Treatment

Ciprofloxacin is the drug of choice. Doxycycline is an alternative drug. No resistant strains have been isolated clinically.

Prevention

- Ciprofloxacin or doxycycline is used as prophylaxis in those exposed during the outbreak.
- People at high risk can be immunized with cell-free vaccine containing purified protective antigen as immunogen.
- Incinerating animals that die of anthrax, rather than burying them, will prevent the soil from becoming contaminated with spores.

2. ***Bacillus cereus***

Disease

B. cereus causes food poisoning.

Transmission

Spores on grains such as rice survive steaming and rapid frying. The spores germinate

when rice is kept warm for many hours (e.g., **reheated fried rice**). The portal of entry is the gastrointestinal tract.

Pathogenesis

B. cereus produces two enterotoxins. The mode of action of one of the enterotoxins leads to an increased concentration of cyclic AMP within the enterocyte. The mode of action of the other enterotoxin resembles that of staphylococcal enterotoxin (i.e., it is a superantigen).

Clinical Findings

There are two syndromes. One syndrome has a short incubation period (4 hours) and consists primarily of nausea and vomiting, similar to staphylococcal food poisoning. The other has a long incubation period (18 hours) and features watery, nonbloody diarrhea,

Laboratory Diagnosis

This is not usually done.

Treatment

Only symptomatic treatment is given.

Prevention

There is no specific means of prevention. Rice should not be kept warm for long periods.

CLOSTRIDIUM

There are four medically important species: *Clostridium tetani, Clostridium botulinum, Clostridium perfringens* (which causes either gas gangrene or food poisoning), and *Clostridium difficile* (causes antibiotic-associated pseudomembranous colitis). All clostridia are anaerobic, spore-forming,gram-positive rods

NON–SPORE-FORMING GRAM-POSITIVE RODS

There are two important pathogens in this group: *Corynebacterium diphtheriae* and

Listeria monocytogenes.

CORYNEBACTERIUM DIPHTHERIAE

Disease

C. diphtheriae causes diphtheria. Other *Corynebacterium* species (diphtheroids) are implicated in opportunistic infections.

Important Properties

Corynebacteria are gram-positive rods that appear **club-shaped** (wider at one end) and are arranged in palisades or in V- or L-shaped formations. The rods have a beaded appearance.

Transmission

Humans are the only natural host of *C. diphtheriae*. Both toxigenic and nontoxigenic organisms reside in the upper respiratory tract and are transmitted by **airborne droplets**. The organism can also infect the skin at the site of a preexisting skin lesion. This occurs primarily in the tropics but can occur worldwide in indigent persons with poor skin hygiene.

Pathogenesis

Although exotoxin production is essential for pathogenesis, invasiveness is also necessary because the organism must first establish and maintain itself in the throat.

Diphtheria toxin affects all eukaryotic cells regardless of tissue type but has no effect on the analogous factor in prokaryotic cells.

The host response to *C. diphtheriae* consists of the following:

- A local inflammation in the throat, with a fibrinous exudate that forms the tough, adherent, gray **pseudomembrane** characteristic of the disease.
- Antibody that can neutralize exotoxin activity by blocking the interaction of the binding domain with the receptors, thereby preventing entry into the cell.

Clinical Findings

Its most prominent sign is the thick, gray, adherent **pseudomembrane** over the tonsils and throat. The other aspects are nonspecific: fever, sore throat, and cervical adenopathy.

There are three prominent complications:

- Extension of the membrane into the larynx and trachea, causing airway obstruction.
- Myocarditis accompanied by arrhythmias and circulatory collapse.
- Nerve weakness or paralysis, especially of the cranial nerves. Paralysis of the muscles of the soft palate and pharynx can lead to regurgitation of fluids through the nose. Peripheral neuritis affecting the muscles of the extremities also occurs.

Cutaneous diphtheria causes ulcerating skin lesions covered by a gray membrane. These lesions are often indolent and often do not invade surrounding tissue. Systemic symptoms rarely occur.

Laboratory Diagnosis

Laboratory diagnosis involves both isolating the organism and demonstrating toxin production. It should be emphasized that the decision to treat with antitoxin is a clinical one and cannot wait for the laboratory results.

A throat swab should be cultured on Loeffler's medium, a **tellurite plate,** and a blood agar plate. If *C. diphtheriae* is recovered from the cultures, either animal inoculation or an antibody-based gel diffusion precipitin test is performed to document toxin production.

A PCR assay for the presence of the toxin gene in the organism isolated from the patient can also be used.

Smears of the throat swab should be stained with both Gram stain and methylene blue.

Treatment

The treatment of choice is **antitoxin,** which should be given immediately on the basis of clinical impression because there is a delay in laboratory diagnostic procedures. The toxin binds rapidly and irreversibly to cells and, once bound, cannot be neutralized by antitoxin. The function of antitoxin is therefore to neutralize unbound toxin in the blood.

Because the antiserum is made in horses, the patient must be tested for hypersensitivity, and medications for the treatment of anaphylaxis must be available. Serum sickness may occur after administration of antiserum made in horses.

Treatment with penicillin G or erythromycin is also recommended, but neither is a substitute for antitoxin. Antibiotics inhibit growth of the organism, reduce toxin production, and decrease the incidence of chronic carriers.

Prevention

Immunization of the children with diphtheria toxoid however this does not prevent nasopharyngeal carriage of the organism.

LISTERIA MONOCYTOGENES

Diseases

L. monocytogenes causes meningitis and sepsis in newborns, pregnant women, and immunosuppressed adults. It also causes outbreaks of febrile gastroenteritis. It is a major cause of concern for the food industry.

Important Properties

L. monocytogenes is a small gram-positive rod arranged in V- or L-shaped formations similar to corynebacteria. The organism exhibits an unusual **tumbling** movement that distinguishes it from the corynebacteria, which are nonmotile.

Colonies on a blood agar plate produce a narrow zone of β-hemolysis that resembles the hemolysis of some streptococci.

Listeria grows well at cold temperatures, so storage of contaminated food in the refrigerator can increase the risk of gastroenteritis. This paradoxical growth in the cold is called “cold enhancement.”

Pathogenesis

Listeria infections occur primarily in two clinical settings:

- In the fetus or in a newborn as a result of transmission **across the placenta** or **during delivery**
- In pregnant women and immunosuppressed adults, especially renal transplant patients. (Note that pregnant women have reduced cell-mediated immunity during the third trimester.)

The organism is distributed worldwide in animals, plants, and soil. From these reservoirs, it is transmitted to humans primarily by ingestion of unpasteurized milk products, undercooked meat, and raw vegetables. Contact with domestic farm animals and their feces is also an important source.

Following ingestion, the bacteria appear in the colon and then can colonize the female genital tract. From this location, they can infect the fetus if membranes rupture or infect the neonate during passage through the birth canal.

The pathogenesis of *Listeria* depends on the organism’s ability to invade and survive

within cells which is mediated by internalin made by *Listeria* and E-cadherin on the surface of human cells.

Upon entering the cell, the organism produces **listeriolysin,** which allows it to escape from the phagosome into the cytoplasm, thereby escaping destruction in the phagosome.

Because *Listeria* preferentially grows intracellularly, cell-mediated immunity is a more important host defense than humoral immunity. Suppression of **cell-mediated immunity** predisposes to *Listeria* infections.

L. monocytogenes can move from cell to cell by means of **actin rockets** —filaments of actin polymerize and propel the bacteria through the membrane of one human cell and into another.

Clinical Findings

Infection during pregnancy can cause abortion, premature delivery, or sepsis during the peripartum period.

Newborns infected at the time of delivery can have acute meningitis 1 to 4 weeks later. The bacteria reach the meninges via the bloodstream (bacteremia).

The infected mother either is asymptomatic or has an influenza-like illness.

L. monocytogenes infections in immunocompromised adults can be either sepsis or meningitis.

Gastroenteritis caused by *L. monocytogenes* is characterized by watery diarrhea, fever, headache, myalgias, and abdominal cramps but little vomiting.

Outbreaks are usually caused by contaminated dairy products, but undercooked meats such as chicken and hot dogs and some ready-to-eat foods.

Laboratory Diagnosis

Laboratory diagnosis is made primarily by Gram stain and culture. The appearance of gram-positive rods resembling **diphtheroids** and the formation of small, gray colonies with a narrow zone of β-hemolysis on a blood agar plate suggest the presence of *Listeria*.

The isolation of *Listeria* is confirmed by the presence of motile organisms, which differentiate them from the nonmotile corynebacteria.

Identification of the organism as *L. monocytogenes* is made by sugar fermentation tests.

Treatment

Treatment of invasive disease, such as meningitis and sepsis, consists of trimethoprim-

sulfamethoxazole.

Combinations, such as ampicillin and gentamicin or ampicillin and trimethoprim-sulfamethoxazole, can also be used.

Resistant strains are rare. *Listeria* gastroenteritis typically does not require treatment.

Prevention

Prevention is difficult because there is no immunization.

Limiting the exposure of pregnant women and immunosuppressed patients to potential sources such as farm animals, unpasteurized milk products, and raw vegetables is recommended.

Trimethoprim-sulfamethoxazole given to immunocompromised patients to prevent *Pneumocystis* pneumonia can also prevent listeriosis.

GRAM NEGATIVE RODS

They are a large group of diverse organisms that can be subdivided into three clinically relevant categories according to whether the organism is related primarily to the enteric or the respiratory tract or to animal sources.

Gram-negative rods related to the enteric tract include a large number of genera and have therefore been divided into three groups depending on the major anatomic location of disease, namely;

1. Pathogens both within and outside the enteric tract. (Escherichia and Salmonella)
2. Pathogens primarily within the enteric tract. (Shigella, Vibrio, Campylobacter and Helicobacter)
3. Pathogens outside the enteric tract. (Klebsiella - Enterobacter - Serratia group, Proteus – Providencia – Morganella group, Pseudomonas and Bacteroides)

ENTEROBACTERIACEAE & RELATED ORGANISMS

Enterobacteriaceae is a large family of gram-negative rods found primarily in the colon of humans and other animals, many as part of the normal flora.

They are the major facultative anaerobes in the large intestine.

They all ferment glucose (fermentation of other sugars varies).

None have cytochrome oxidase (i.e., they are oxidase-negative).

They reduce nitrates to nitrites as part of their energy-generating processes.

Pathogenesis

All members of the Enterobacteriaceae, being gram-negative, contain endotoxin in their cell walls and several exotoxins are produced (e.g., *E. coli* and *Vibrio cholerae* secrete exotoxins, called *enterotoxins*, that activate adenylate cyclase within the cells of the small intestine, causing diarrhea).

Antigens

The antigens especially *Salmonella* and *Shigella*, are important; they are used for identification purposes both in the clinical laboratory and in epidemiologic investigations. The three surface antigens are as follows:

1. The cell wall antigen (also known as the somatic, or O, antigen) is the outer polysaccharide portion of the lipopolysaccharide.
2. The H antigen is on the flagella protein. Only flagellated organisms, such as *Escherichia* and *Salmonella*, have H antigens, whereas the nonmotile ones, such as *Klebsiella* and *Shigella*, do not.
3. The capsular or K polysaccharide antigen is particularly prominent in heavily encapsulated organisms such as *Klebsiella*. The K antigen is identified by the quellung (capsular swelling) reaction in the presence of specific antisera.

Laboratory Diagnosis

Specimens are inoculated onto two media, a blood agar plate and a selective differential medium such as MacConkey's agar or eosin–methylene blue (EMB) agar. The *differential* ability of these latter media is based on **lactose fermentation.**

On these media, the non–lactose fermenters (e.g., *Salmonella* and *Shigella*) form colorless colonies, whereas the lactose fermenters (e.g., *E. coli*) form colored colonies.

Antibiotic Therapy

The appropriate treatment for infections caused by members of the Enterobacteriaceae and related organisms must be individually tailored to the antibiotic sensitivity of the organism.

A wide range of antimicrobial agents are potentially effective (e.g., some penicillins and cephalosporins, aminoglycosides, chloramphenicol, tetracyclines, quinolones, and sulfonamides). The specific choice usually depends on the results of antibiotic sensitivity tests.

ESCHERICHIA

Diseases

E. coli is the most common cause of urinary tract infection and gram-negative rod sepsis. It is one of the two important causes of neonatal meningitis and the agent most frequently associated with "traveler's diarrhea," a watery diarrhea. Some strains of *E. coli* are enterohemorrhagic and cause bloody diarrhea.

Important Properties

E. coli is a straight gram-negative rod.

It is the most abundant facultative anaerobe in the colon and feces.

It **ferments lactose,** a property that distinguishes it from the two major intestinal pathogens, *Shigella* and *Salmonella*.

It has three antigens that are used to identify the organism in clinical or epidemiologic investigations: the O, or cell wall, antigen; the H, or flagella, antigen; and the K, or capsular, antigen.

Pathogenesis

The reservoir of *E. coli* includes both humans and animals. The source of the *E. coli* that causes urinary tract infections is the patient's own colonic flora that colonizes the urogenital area. The source of the *E. coli* that causes neonatal meningitis is the mother's birth canal; the infection is acquired during birth. In contrast, the *E. coli* that causes traveler's diarrhea is acquired by ingestion of food or water contaminated with human feces. Note that the main reservoir of enterohemorrhagic *E. coli* O157 is cattle and the organism is acquired in undercooked meat.

E. coli has the ability to cause disease through: pili, a capsule, endotoxin, and three exotoxins (enterotoxins), two that cause watery diarrhea and one that causes bloody diarrhea and hemolytic–uremic syndrome.

Clinical Findings

E. coli causes a variety of diseases both within and outside the intestinal tract.

The clinical findings within the intestinal tract includes watery, non-bloody diarrhea (traveler's diarrhea) with no RBC or WBC in stool and bloody diarrhea caused by *E. coli* O157: hemolytic –uremic syndrome (HUS) with RBC in stool and schistocytes in blood smear.

Laboratory Diagnosis

E. coli, are grown initially on a blood agar plate and on a differential medium, such as EMB agar or MacConkey's agar.

E. coli, which ferments lactose, forms pink colonies, whereas lactose-negative organisms are colorless. On EMB agar, *E. coli* colonies have a characteristic **green sheen**.

Some of the important features that help distinguish *E. coli* from other lactose-fermenting gram-negative rods are as follows:

1. It produces indole from tryptophan.
2. It decarboxylates lysine.
3. It uses acetate as its only source of carbon.
4. It is motile. *E. coli* O157:H7 does not ferment sorbitol, which serves as an

important criterion that distinguishes it from other strains of *E. coli*.

The isolation of enterotoxigenic or enteropathogenic *E. coli* from patients with diarrhea is not a routine diagnostic procedure.

Treatment

Treatment of *E. coli* infections depends on the site of disease and the resistance pattern of the specific isolate. For example, an uncomplicated lower urinary tract infection (cystitis) can be treated using oral trimethoprim-sulfamethoxazole or nitrofurantoin. Pyelonephritis can be treated with ciprofloxacin or ceftriaxone.

E.coli sepsis requires treatment with parenteral antibiotics (e.g., a third generation cephalosporin, such as cefotaxime, with or without an aminoglycoside, such as gentamicin).

For the treatment of neonatal meningitis, a combination of ampicillin and cefotaxime is usually given. Antibiotic therapy is usually *not* indicated in *E. coli* diarrheal diseases. However, administration of trimethoprim-sulfamethoxazole or loperamide (Imodium) may shorten the duration of symptoms.

Rehydration is typically all that is necessary in this self-limited disease.

Prevention

There is no specific prevention for *E. coli* infections, however the incidence of urinary tract infections can be lowered by the judicious use and prompt withdrawal of catheters and, in recurrent infections, by prolonged prophylaxis with urinary antiseptic drugs (e.g., nitrofurantoin or trimethoprim-sulfamethoxazole).

Some cases of sepsis can be prevented by prompt removal of or switching the site of intravenous lines.

Traveler's diarrhea can sometimes be prevented by the prophylactic use of doxycycline, ciprofloxacin, trimethoprim-sulfamethoxazole, or Pepto-Bismol. Ingestion of uncooked foods and unpurified water should be avoided while traveling in certain countries.

SALMONELLA

Diseases

Salmonella species cause enterocolitis, enteric fevers such as typhoid fever, and septicemia with metastatic infections such as osteomyelitis.

Important Properties

Salmonellae are gram-negative rods that **do not ferment lactose** but do produce H_2S—features that are used in their laboratory identification.

Their antigens—cell wall O, flagella H, and capsular Vi (virulence)—are important for

taxonomic and epidemiologic purposes. The O antigens, which are the outer polysaccharides of the cell wall, are used to subdivide the salmonellae into groups A–I. There are two forms of the H antigens, phases 1 and 2.

The Vi antigens (capsular polysaccharides) are antiphagocytic and are an important virulence factor for *S. typhi*, the agent of typhoid fever. The Vi antigens are also used for the serotyping of *S. typhi* in the clinical laboratory.

There are three methods for naming the salmonellae. Ewing divides the genus into three species: *S. typhi, Salmonella choleraesuis*, and *Salmonella enteritidis*.

Pathogenesis & Epidemiology

The three types of *Salmonella* infections (enterocolitis, enteric fevers, and septicemia) have different pathogenic features.

Enterocolitis is characterized by an invasion of the epithelial and sub epithelial tissue of the small and large intestines resulting to inflammation and diarrhea. Gastric acid is an important host defense; gastrectomy or use of antacids lowers the infectious dose significantly.

In **typhoid** and other enteric fevers, infection begins in the small intestine, but few gastrointestinal symptoms occur. The organisms enter, multiply in the mononuclear phagocytes, and then spread to the phagocytes of the liver, gallbladder, and spleen. This leads to bacteremia, which is associated with the onset of fever and other symptoms, probably caused by endotoxin.

Septicemia accounts for only about 5% to 10% of *Salmonella* infections and occurs in one of two settings: a patient with an underlying chronic disease, such as **sickle cell anemia** or cancer, or a child with enterocolitis.

The epidemiology of *Salmonella* infections is related to the ingestion of food and water contaminated by human and animal wastes. *S. typhi*, the cause of typhoid fever, is **transmitted only by humans,** but all other species have a significant animal as well as human reservoir. The most frequent **animal source is poultry and eggs,** but meat products that are inadequately cooked have been implicated as well.

Clinical Findings

After an incubation period of 12 to 48 hours, enterocolitis begins with nausea and vomiting and then progresses to abdominal pain and diarrhea, which can vary from mild to severe, with or without blood. Usually the disease lasts a few days, is self-limited, causes non bloody diarrhea, and does not require medical care except in the very young and very old.

In typhoid fever, caused by *S. typhi*, and in enteric fever, caused by *S. paratyphi* the onset of illness is slow, with fever and constipation. Diarrhea may occur early but usually disappears by the time the fever and bacteremia occur. About 3% of typhoid

fever patients become chronic carriers.

Septicemia is most often caused by *S. choleraesuis*. The symptoms begin with fever but little or no enterocolitis and then proceed to focal symptoms associated with the affected organ, frequently bone, lung, or meninges.

Laboratory Diagnosis

In enterocolitis, the organism is most easily isolated from a stool sample. However, in the enteric fevers, a blood culture is the procedure most likely to reveal the organism during the first 2 weeks of illness. Stool cultures may also be positive, especially in chronic carriers in whom the organism is secreted in the bile into the intestinal tract.

Salmonellae form non–lactose-fermenting (colorless) colonies on MacConkey's or EMB agar. On TSI agar, an alkaline slant and an acid butt, frequently with both gas and H_2S (black color in the butt), are produced. *S. typhi* is the major exception;it does not form gas and produces only small amount of H2S.

Definitive serotyping of the O, H, and Vi antigens is performed for epidemiologic purposes.

In certain cases of enteric fever and sepsis the diagnosis can be made serologically by detecting a rise in antibody titer in the patient's serum (Widal test).

Treatment

Enterocolitis caused by *Salmonella* is usually a self-limited disease that resolves without treatment. Fluid and electrolyte replacement may be required. Antibiotic treatment does not shorten the illness or reduce the symptoms; in fact, it may prolong excretion of the organisms, increase the frequency of the carrier state, and select mutants resistant to the antibiotic. Antimicrobial agents are indicated only for neonates or persons with chronic diseases who are at risk for septicemia and disseminated abscesses.

The treatment of choice for enteric fevers such as typhoid fever and septicemia with metastatic infection is either ceftriaxone or ciprofloxacin. Ampicillin or ciprofloxacin should be used in patients who are chronic carriers of *S. typhi*.

Prevention

Salmonella infections are prevented mainly by public health and personal hygiene measures. Proper sewage treatment, a chlorinated water supply that is monitored for contamination by coliform bacteria, cultures of stool samples from food handlers to detect carriers, hand washing prior to food handling, pasteurization of milk, and proper cooking of poultry, eggs, and meat are all important.

Two vaccines are available, but they confer limited (50%–80%) protection against *S. typhi*. One contains the Vi capsular polysaccharide of *S. typhi* (given intramuscularly),

and the other contains a live, attenuated strain (Ty21a) of *S. typhi* (given orally). The two vaccines are equally effective.

SHIGELLA

Disease

Shigella species cause enterocolitis. Enterocolitis caused by *Shigella* is often called bacillary dysentery. The term *dysentery* refers to bloody diarrhea.

Important Properties

Shigellae are **non–lactose-fermenting,** gram-negative rods that can be distinguished from salmonellae by three criteria: they produce no gas from the fermentation of glucose, they **do not produce H_2S,** and they are **non-motile**. All shigellae have O antigens (polysaccharide) in their cell walls, and these antigens are used to divide the genus into four groups: A, B, C, and D.

Pathogenesis & Epidemiology

Shigellae are the most effective pathogens among the enteric bacteria.

Shigellosis is only a **human disease** (i.e., there is no animal reservoir). The organism is transmitted by the fecal–oral route. The four Fs—fingers, flies, food, and feces—are the principal factors in transmission. Foodborne outbreaks outnumber waterborne outbreaks by 2 to 1.

Shigellae, which cause disease almost exclusively in the gastrointestinal tract, produce bloody diarrhea (dysentery) by invading the cells of the mucosa of the distal ileum and colon. Local inflammation accompanied by ulceration occurs, but the organisms rarely penetrate through the wall or enter the bloodstream, unlike salmonellae. Although some strains produce an enterotoxin (called *Shiga toxin*), invasion is the critical factor in pathogenesis.

Shiga toxins are encoded by lysogenic bacteriophages. Shiga toxins very similar to those produced by enterohemorrhagic *E. coli* O157:H7 strains that cause enterocolitis and hemolytic –uremic syndrome (HUS).

Clinical Findings

After an incubation period of 1 to 4 days, symptoms begin with fever and abdominal cramps, followed by diarrhea, which may be watery at first but later contains blood and mucus. The disease varies from mild to severe depending on two major factors: the species of *Shigella* and the age of the patient, with young children and elderly people being the most severely affected.

The diarrhea frequently resolves in 2 or 3 days; in severe cases, antibiotics can shorten the course. Serum agglutinins appear after recovery but are not protective because the

organism does not enter the blood. The role of intestinal IgA in protection is uncertain.

Laboratory Diagnosis

Shigellae form non–lactose-fermenting (colorless) colonies on MacConkey's or

EMB agar. On TSI agar, they cause an alkaline slant and an acid butt, with no gas and no H2S. Confirmation of the organism as *Shigella* and determination of its group are done by slide agglutination.

One important adjunct to laboratory diagnosis is a methylene blue stain of a fecal sample to determine whether neutrophils are present. If they are found, an invasive organism such as *Shigella, Salmonella*, or *Campylobacter* is involved rather than a toxin-producing organism such as *V. cholerae, E. coli*, or *Clostridium perfringens*.

Treatment

The main treatment for shigellosis is fluid and electrolyte replacement. In mild cases, no antibiotics are indicated.

In severe cases, a fluoroquinolone (e.g., ciprofloxacin) is the drug of choice, but the incidence of plasmids conveying multiple drug resistance is high enough that antibiotic sensitivity tests must be performed. Trimethoprim-sulfamethoxazole is an alternative choice. Antiperistaltic drugs are contraindicated in shigellosis, because they prolong the fever, diarrhea, and excretion of the organism.

Prevention

Prevention of shigellosis is dependent on interruption of fecal–oral transmission by proper sewage disposal, chlorination of water, and personal hygiene (hand washing by food handlers). There is no vaccine, and prophylactic antibiotics are not recommended.

VIBRIO

Diseases

V. cholerae, the major pathogen in this genus, is the cause of cholera. *Vibrio parahaemolyticus* causes diarrhea associated with eating raw or improperly cooked seafood. *Vibrio vulnificus* causes cellulitis and sepsis.

Important Properties

Vibrios are curved, **comma-shaped,** gram-negative rods. *V. cholera* is divided into two groups according to the nature of its O cell wall antigen.

Members of the O1 group cause epidemic disease, whereas non-O1 organisms either cause sporadic disease or are non-pathogens. The O1 organisms have two biotypes, called classic and El Tor, and three serotypes, called Ogawa, Inaba, and Hikojima. (Biotypes are based on differences in biochemical reactions, whereas serotypes are

based on antigenic differences.) These features are used to characterize isolates in epidemiologic investigations.

V. parahaemolyticus and *V. vulnificus* are **marine organisms;** they live primarily in the ocean, especially in warm salt water. They are **halophilic** (i.e., they require a high NaCl concentration to grow).

1. Vibrio cholerae

Pathogenesis & Epidemiology

V. cholerae is transmitted by **fecal contamination** of water and food, primarily from human sources. Human carriers are frequently asymptomatic and include individuals who are either in the incubation period or convalescing. The main animal reservoirs are marine shellfish, such as shrimp and oysters. Ingestion of these without adequate cooking can transmit the disease.

The pathogenesis of cholera is dependent on colonization of the small intestine by the organism and secretion of enterotoxin. For colonization to occur, large numbers of bacteria must be ingested because the organism is particularly sensitive to stomach acid. Persons with little or no stomach acid, such as those taking antacids or those who have had gastrectomy, are much more susceptible.

After adhering, the organism multiplies and secretes an **enterotoxin** called choleragen (cholera toxin). This exotoxin can reproduce the symptoms of cholera even in the absence of the *Vibrio* organisms.

Clinical Findings

Watery diarrhea in large volumes is the hallmark of cholera. There are no red blood cells or white blood cells in the stool. **Rice-water stool** is the term often applied to the non-bloody effluent. There is no abdominal pain, and subsequent symptoms are referenced to the marked dehydration. The loss of fluid and electrolytes leads to cardiac and renal failure. Acidosis and hypokalemia also occur as a result of loss of bicarbonate and potassium in the stool. The mortality rate without treatment is 40%.

Laboratory Diagnosis

The approach to laboratory diagnosis depends on the situation. During an epidemic, a clinical judgment is made and there is little need for the laboratory.

For diagnosis of sporadic cases in this country, a culture of the diarrhea stool containing *V. cholerae* will show colorless colonies on MacConkey's agar because lactose is fermented slowly. The organism is oxidase-positive, which distinguishes it from members of the Enterobacteriaceae.

On TSI agar, an acid slant and an acid butt without gas or H2S are seen because the organism ferments sucrose. A presumptive diagnosis of *V. cholerae* can be confirmed

by agglutination of the organism by polyvalent O1 or non-O1 antiserum. A retrospective diagnosis can be made serologically by detecting a rise in antibody titer in acute- and convalescent phase sera.

Treatment

Treatment consists of prompt, adequate replacement of water and electrolytes, either orally or intravenously. Glucose is added to the solution to enhance the uptake of water and electrolytes. Antibiotics such as tetracycline are not necessary, but they do shorten the duration of symptoms and reduce the time of excretion of the organisms.

Prevention

Prevention is achieved mainly by public health measures that ensure a clean water and food supply. The vaccine, composed of killed organisms, has limited usefulness; it is only 50% effective in preventing disease for 3 to 6 months and does not interrupt transmission.

The use of tetracycline for prevention is effective in close contacts but cannot prevent the spread of a major epidemic.

Prompt detection of carriers is important in limiting outbreaks.

2. *Vibrio parahaemolyticus*

V. parahaemolyticus is a marine organism transmitted by **ingestion of raw or undercooked seafood,** especially shellfish such as oysters. It is a major cause of diarrhea in Japan, where raw fish is eaten in large quantities.

Little is known about its pathogenesis, except that an enterotoxin similar to choleragen is secreted and limited invasion sometimes occurs.

The clinical picture caused by *V. parahaemolyticus* varies from mild to quite severe watery diarrhea, nausea and vomiting, abdominal cramps, and fever. The illness is self-limited, lasting about 3 days. *V. parahaemolyticus* is distinguished from *V. cholerae* mainly on the basis of growth in NaCl: *V. parahaemolyticus* grows in 8% NaCl solution (as befits a marine organism), whereas *V. cholerae* does not. No specific treatment is indicated, because the disease is relatively mild and self-limited. Disease can be prevented by proper refrigeration and cooking of seafood.

3. *Vibrio vulnificus*

V. vulnificus is also a marine organism (i.e., it is found in warm salt waters such as the Caribbean Sea). It causes severe skin and soft tissue infections (**cellulitis**), **especially in shellfish handlers**, who often sustain skin wounds. It can also cause a rapidly fatal **septicemia in immunocompromised people who have eaten raw shellfish** containing the organism.

The recommended treatment is doxycycline.

CAMPYLOBACTER

Diseases

C. jejuni is a frequent cause of enterocolitis, especially in children. Other *Campylobacter* species are rare causes of systemic infection, particularly bacteremia.

Important Properties

Campylobacters are curved, gram-negative rods that appear either **comma**- or **S-shaped**.

They are **microaerophilic,** growing best in 5% oxygen rather than in the 20% present in the atmosphere. *C. jejuni* grows well at 42°C, whereas

Pathogenesis & Epidemiology

Domestic animals such as cattle, chickens, and dogs serve as a source of the organisms for humans. Transmission is usually **fecal–oral**. Food and water contaminated with animal feces are the major sources of human infection. Foods, such as poultry, meat, and unpasteurized milk, are commonly involved. Puppies with diarrhea are a common source for children. Human-to-human transmission occurs but is less frequent than animal-to-human transmission.

Inflammation of the intestinal mucosa often occurs, accompanied by blood in stools. Systemic infections (e.g., bacteremia) occur most often in neonates or debilitated adults.

Clinical Findings

Enterocolitis, caused primarily by *C. jejuni*, begins as watery, foul-smelling diarrhea followed by bloody stools accompanied by fever and severe abdominal pain. Systemic infections, most commonly bacteremia, are caused more often by *C. intestinalis*. The symptoms of bacteremia (e.g., fever and malaise) are associated with no specific physical findings.

Gastrointestinal infection with *C. jejuni* is associated with Guillain-Barré syndrome, the most common cause of acute neuromuscular paralysis. Guillain-Barré syndrome is an autoimmune disease attributed to the formation of antibodies against *C. jejuni* that cross-react with antigens on neurons.

Infection with *Campylobacter* is also associated with two other autoimmune diseases: reactive arthritis and Reiter's syndrome.

Laboratory Diagnosis

If the patient has diarrhea, a stool specimen is cultured on a blood agar plate containing antibiotics that inhibit most other fecal flora.

The plate is incubated at 42°C in a microaerophilic atmosphere containing 5% oxygen and 10% carbon dioxide, which favors the growth of *C. jejuni*. It is identified by failure to grow at 25°C, oxidase positivity, and sensitivity to nalidixic acid. Unlike *Shigella* and *Salmonella*, lactose fermentation is not used as a distinguishing feature. If bacteremia is suspected, a blood culture incubated under standard temperature and atmospheric conditions will reveal the growth of the characteristically comma- or S-shaped, motile, gram-negative rods.

Identification of the organism as *C. intestinalis* is confirmed by its failure to grow at 42° C, its ability to grow at 25°C, and its resistance to nalidixic acid.

Treatment

Erythromycin or ciprofloxacin is used successfully in *C. jejuni* enterocolitis. The treatment of choice for *C. intestinalis* bacteremia is an aminoglycoside.

Prevention

There is no vaccine or other specific preventive measure. Proper sewage disposal and personal hygiene (hand washing) are important.

HELICOBACTER

Diseases

Helicobacter pylori causes gastritis and peptic ulcers. Infection with *H. pylori* is a risk factor for gastric carcinoma and is linked to mucosal-associated lymphoid tissue (MALT) lymphomas.

Important Properties

Helicobacters are curved gram-negative rods similar in appearance to campylobacters, but because they differ sufficiently in certain biochemical and flagellar characteristics, they are classified as a separate genus. In particular, helicobacters are strongly urease-positive, whereas campylobacters are urease-negative.

Pathogenesis & Epidemiology

H. pylori attaches to the mucus-secreting cells of the gastric mucosa. The production of large amounts of ammonia from urea by the organism's urease, coupled with an inflammatory response, leads to damage to the mucosa. Loss of the protective mucus coating predisposes to gastritis and peptic ulcer. The ammonia also neutralizes stomach acid, allowing the organism to survive.

The natural habitat of *H. pylori* is the human stomach, and it is probably acquired by ingestion. However, it has not been isolated from stool, food, water, or animals.

Person-to-person transmission probably occurs because there is clustering of infection within families. The rate of infection with *H. pylori* in developing countries is very high—a

finding that is in accord with the high rate of gastric carcinoma in those countries.

MALT lymphomas are B-cell tumors located typically in the stomach, but they occur elsewhere in the gastrointestinal tract as well. *H. pylori* is often found in them MALT lesion, and the chronic inflammation induced by the organism is thought to stimulate B-cell proliferation and eventually a B-cell lymphoma.

Antibiotic treatment directed against the organism often causes the tumor to regress.

Clinical Findings

Gastritis and peptic ulcer are characterized by recurrent pain in the upper abdomen, frequently accompanied by bleeding into the gastrointestinal tract. No bacteremia or disseminated disease occurs.

Laboratory Diagnosis

The organism can be seen on Gram-stained smears of biopsy specimens of the gastric mucosa. It can be cultured on the same media as campylobacters. In contrast to *C. jejuni, H. pylori* is urease-positive. Urease production is the basis for a noninvasive diagnostic test called the “urea breath” test. In this test, radiolabeled urea is ingested. If the organism is present, urease will cleave the ingested urea, radiolabeled CO_2 is evolved, and the radioactivity is detected in the breath.

A test for *Helicobacter* antigen in the stool can be used for diagnosis and for confirmation that treatment has eliminated the organism. The presence of IgG antibodies in the patient’s serum can also be used as evidence of infection.

Treatment & Prevention

The concept that underlies the choice of drugs is to use antibiotics to eliminate *Helicobacter* plus a drug to reduce gastric acidity. A combination of two antibiotics is used because resistance, especially to metronidazole, has emerged. Treatment of duodenal ulcers with antibiotics (e.g., amoxicillin and metronidazole) and bismuth salts (Pepto-Bismol) results in a greatly decreased recurrence rate. Tetracycline can be used instead of amoxicillin. There is no vaccine or other specific preventive measure

KLEBSIELLA–ENTEROBACTER–SERRATIA GROUP

Diseases

These organisms are usually opportunistic pathogens that cause nosocomial infections, especially pneumonia and urinary tract infections. *K. pneumoniae* is an important respiratory tract pathogen outside hospitals as well.

Important Properties

K. pneumoniae, Enterobacter cloacae, and *Serratia marcescens* are the species most often involved in human infections. They are frequently found in the **large intestine** but are also present in soil and water. These organisms have very similar properties and are usually distinguished on the basis of biochemical reactions and motility. *K. pneumoniae* has a **very large polysaccharide capsule,** which gives its colonies a striking mucoid appearance. *S. marcescens* produces **red pigmented colonies.**

Pathogenesis & Epidemiology

Of the three organisms, *K. pneumoniae* is most likely to be a primary, non-opportunistic pathogen; this property is related to its anti-phagocytic capsule.

Although this organism is a primary pathogen, patients with *K. pneumoniae* infections frequently have predisposing conditions such as advanced age, chronic respiratory disease, diabetes, or alcoholism. The organism is carried in the respiratory tract of about 10% of healthy people, who are prone to pneumonia if host defenses are lowered.

Enterobacter and *Serratia* infections are clearly related to hospitalization, especially to invasive procedures such as intravenous catheterization, respiratory intubation, and urinary tract manipulations. In addition, outbreaks of *Serratia* pneumonia have been associated with contamination of the water in respiratory therapy devices.

Serratia also causes endocarditis in users of injection drugs. As with many other gram-negative rods, the pathogenesis of septic shock caused by these organisms is related to the endotoxins in their cell walls.

Clinical Findings

Urinary tract infections and pneumonia are the usual clinical entities associated with these three bacteria, but bacteremia and secondary spread to other areas such as the meninges and liver occur. It is difficult to distinguish infections caused by these organisms on clinical grounds, with the exception of pneumonia caused by *Klebsiella*, which produces a thick, bloody sputum (“currant-jelly” sputum) and can progress to necrosis and abscess formation.

Laboratory Diagnosis

Organisms of this group produce lactose-fermenting (colored) colonies on differential

agar such as MacConkey's or EMB, although *Serratia*, which is a late lactose fermenter, can produce a negative reaction. These organisms are differentiated by the use of biochemical tests.

Treatment

Because the antibiotic resistance of these organisms can vary greatly, the choice of drug depends on the results of sensitivity testing. Isolates from hospital-acquired infections are frequently resistant to multiple antibiotics.

An aminoglycoside (e.g., gentamicin) and cephalosporin (e.g., cefotaxime) are used empirically until the results of testing are known.

Prevention

Some hospital-acquired infections caused by gram-negative rods can be prevented by such general measures as changing the site of intravenous catheters, removing urinary catheters when they are no longer needed, and taking proper care of respiratory therapy devices. There is no vaccine.

PROTEUS–PROVIDENCIA–MORGANELLA GROUP

Diseases

These organisms primarily cause urinary tract infections, both community- and hospital-acquired.

In the past, there were four medically important species of *Proteus*. However, molecular studies of DNA relatedness showed that two of the four were significantly different. These species have been renamed: *Proteus morganii* is now *Morganella morganii*, and *Proteus rettgeri* is now *Providencia rettgeri*. In the clinical laboratory, these organisms are distinguished from *Proteus vulgaris* and *Proteus mirabilis* on the basis of several biochemical tests.

Important Properties

These gram-negative rods are distinguished from other members of the Enterobacteriaceae by their ability to produce the enzyme phenylalanine deaminase.

In addition, they produce the enzyme **urease,** which cleaves urea to form NH3 and

CO2. Certain species are very motile and produce a striking **swarming** effect on blood agar, characterized by expanding rings (waves) of organisms over the surface of the agar.

The cell wall O antigens of certain strains of *Proteus* cross-react with antigens of several species of rickettsiae. These *Proteus* antigens can be used in laboratory tests to detect the presence of antibodies against certain rickettsiae in patients' serum. This test, called the Weil-Felix reaction.

Pathogenesis & Epidemiology

The organisms are present in the human colon as well as in soil and water. Their tendency to cause urinary tract infections is probably due to their presence in the colon and to colonization of the urethra, especially in women.

The vigorous motility of *Proteus* organisms may contribute to their ability to invade the urinary tract.

Production of the enzyme urease is an important feature of the pathogenesis of urinary tract infections by this group. Urease hydrolyzes the urea in urine to form ammonia, which raises the pH, producing an alkaline urine. This encourages the formation of stones (calculi) called "**struvite**" composed of magnesium ammonium phosphate. Stones in the urinary tract obstruct urine flow, damage urinary epithelium, and serve as a nidus for recurrent infection by trapping bacteria within the stone.

Because alkaline urine also favors growth of the organisms and more extensive renal damage, treatment involves keeping the urine at a low pH.

Clinical Findings

The signs and symptoms of urinary tract infections caused by these organisms cannot be distinguished from those caused by *E. coli* or other members of the Enterobacteriaceae. *Proteus* species can also cause pneumonia, wound infections, and septicemia.

Laboratory Diagnosis

These organisms usually are highly motile and produce a "swarming" overgrowth on blood agar, which can frustrate efforts to recover pure cultures of other organisms.

Growth on blood agar containing phenylethyl alcohol inhibits swarming, thus allowing isolated colonies of *Proteus* and other organisms to be obtained.

They produce non–lactose-fermenting (colorless) colonies on MacConkey's or EMB agar.

P. vulgaris and *P. mirabilis* produce H_2S, which blackens the butt of TSI agar, whereas neither *M. morganii* nor *P. rettgeri* does. *P. mirabilis* is indole-negative, whereas the other three species are indole-positive—a distinction that can be used clinically to guide the choice of antibiotics. These four medically important species are urease-positive. Identification of these organisms in the clinical laboratory is based on a variety of biochemical reactions.

Treatment

Most strains are sensitive to aminoglycosides and trimethoprim-sulfamethoxazole, but

because individual isolates can vary, antibiotic sensitivity tests should be performed. *P. mirabilis* is the species most frequently sensitive to ampicillin. The indole-positive species (*P. vulgaris, M. morganii*, and *P. rettgeri*) are more resistant to antibiotics than is *P. mirabilis*, which is indole-negative. The treatment of choice for the indole-positive species is a cephalosporin (e.g., cefotaxime). *P. rettgeri* is frequently resistant to multiple antibiotics.

Prevention

There are no specific preventive measures, but many hospital-acquired urinary tract infections can be prevented by prompt removal of urinary catheters.

PSEUDOMONAS

Diseases

Pseudomonas aeruginosa causes infections (e.g., sepsis, pneumonia, and urinary tract infections) primarily in patients with lowered host defenses. It also causes chronic lower respiratory tract infections in patients with cystic fibrosis, wound infections (cellulitis) in burn patients and malignant otitis externa in diabetic patients. It is the most common cause of ventilator-associated pneumonia.

(*P. aeruginosa* is also known as *Burkholderia aeruginosa*.) *Pseudomonas cepacia*(renamed *Burkholderia cepacia*) and *Pseudomonas maltophilia* (renamed *Xanthomonas maltophilia* and now called *Stenotrophomonas maltophilia*) also cause these infections, but much less frequently. *Pseudomonas pseudomallei* (also known as *Burkholderia pseudomallei*), the cause of melioidosis.

Important Properties

Pseudomonads are gram-negative rods that resemble the members of the Enterobacteriaceae but differ in that they are strict aerobes (i.e., they derive their energy only by oxidation of sugars rather than by fermentation). Because they do not ferment glucose, they are called **non fermenters,** in contrast to the members of the Enterobacteriaceae, which do ferment glucose. Oxidation involves electron transport by cytochrome c (i.e., they are **oxidase-positive**).

Pseudomonads are able to grow in **water** containing only traces of nutrients (e.g., tap water), and this favors their persistence in the hospital environment. *P. aeruginosa* and *B. cepacia* have a remarkable ability to withstand disinfectants.

P. aeruginosa produces two pigments useful in clinical and laboratory diagnosis:

1. **Pyocyanin,** which can **color the pus in a wound blue.**
2. Pyoverdin (fluorescein), a yellow-green pigment that fluoresces under ultraviolet light, a property that can be used in the early detection of skin infection in burn patients.

In the laboratory, these **pigments diffuse into the agar, imparting a blue-green color** that is useful in identification. *P. aeruginosa* is the only species of *Pseudomonas* that synthesizes pyocyanin.

Strains of *P. aeruginosa* isolated from cystic fibrosis patients have a prominent slime layer (glycocalyx), which gives their colonies a very mucoid appearance. The slime layer mediates adherence of the organism to mucous membranes of the respiratory tract and prevents antibody from binding to the organism.

Pathogenesis & Epidemiology

P. aeruginosa is found chiefly in soil and water, although approximately 10% of people

carry it in the normal flora of the colon. It is found on the skin in moist areas and can colonize the upper respiratory tract of hospitalized patients. It contaminates the respiratory therapy and anesthesia equipment, intravenous fluids, and even distilled water.

P. aeruginosa is primarily an opportunistic pathogen that causes infections in hospitalized patients (e.g., those with extensive burns), in whom the skin host defenses are destroyed; in those with chronic respiratory disease (e.g., cystic fibrosis), in whom the normal clearance mechanisms are impaired; in those who are immunosuppressed; in those with neutrophil counts of less than 500/mL; and in those with indwelling catheters. It causes 10% to 20% of hospital-acquired infections and in many hospitals, is the most common cause of gram-negative nosocomial pneumonia, especially ventilator -associated pneumonia.

Pathogenesis is based on multiple virulence factors: endotoxin, exotoxins, and enzymes. Its endotoxin, like that of other gram-negative bacteria, causes the symptoms of sepsis and septic shock. The best known of the exotoxins is exotoxin A, which causes tissue necrosis.

It also produces enzymes, such as elastase and proteases that are histotoxic and facilitate invasion of the organism into the bloodstream. Pyocyanin damages the cilia and mucosal cells of the respiratory tract.

Clinical Findings

P. aeruginosa can cause infections virtually anywhere in the body, but urinary tract infections, pneumonia (especially in **cystic fibrosis** patients), and wound infections (especially burns) predominate. It is an important cause of hospital acquired pneumonia, especially in those undergoing mechanical ventilation (ventilator-associated pneumonia). From these sites, the organism can enter the blood, causing sepsis. The bacteria can spread to the skin, where they cause black, necrotic lesions called **ecthyma gangrenosum.**

Severe external otitis (malignant otitis externa) and other skin lesions (e.g., folliculitis) occur in users of swimming pools and hot tubs (hot tub folliculitis) in which the chlorination is inadequate. *P. aeruginosa* is the most common cause of osteochondritis of the foot in those who sustain puncture wounds through the soles of gym shoes. Corneal infections caused by *P. aeruginosa* are seen in contact lens users.

Laboratory Diagnosis

P. aeruginosa grows as non–lactose-fermenting (colorless) colonies on MacConkey's or EMB agar. It is **oxidase-positive**. A typical metallic sheen of the growth on TSI agar, coupled with the blue-green pigment on ordinary nutrient agar and a fruity aroma are sufficient to make a presumptive diagnosis.

The diagnosis is confirmed by biochemical reactions. Identification for epidemiologic

purposes is done by bacteriophage or pyocin6 typing.

Treatment

Treatment must be tailored to the sensitivity of each isolate and monitored frequently; resistant strains can emerge during therapy. The treatment of choice is an antipseudomonal penicillin (e.g., piperacillin/tazobactam or ticarcillin/clavulanate) plus an aminoglycoside (e.g., gentamicin or amikacin). For infections caused by highly resistant strains, colistin (polymyxin E) is useful. The drug of choice for urinary tract infections is ciprofloxacin.

Prevention

Prevention of *P. aeruginosa* infections involves keeping neutrophil counts above 500/mL, removing indwelling catheters promptly, taking special care of burned skin, and taking other similar measures to limit infection in patients with reduced host defenses.

BACTEROIDES & PREVOTELLA assignment

GRAM NEGATIVE RODS OF THE RESPIRATORY TRACT

There are three medically important gram-negative rods typically associated with the respiratory tract, namely, *Haemophilus influenzae, Bordetella pertussis*, and *Legionella pneumophila*. *H. influenzae* and *B. pertussis* are found only in humans, whereas *L. pneumophila* is found primarily in environmental water sources.

HAEMOPHILUS

Diseases

H. influenzae used to be the leading cause of meningitis in young children, but the use of the highly effective "conjugate" vaccine has greatly reduced the incidence of meningitis caused by this organism. It is still an important cause of upper respiratory tract infections (otitis media, sinusitis, conjunctivitis, and epiglottitis) and sepsis in children. It also causes pneumonia in adults, particularly in those with chronic obstructive lung disease.

Important Properties

H. influenzae is a small gram-negative rod (coccobacillus) with a polysaccharide capsule. It is one of the three important **encapsulated pyogens,** along with the pneumococcus and the meningococcus. Serologic typing is based on the antigenicity of the capsular polysaccharide.

Growth of the organism on laboratory media requires the addition of two components, **heme (factor X)** and **NAD (factor V),** for adequate energy production.

Pathogenesis & Epidemiology

H. influenzae infects only humans; there is no animal reservoir. It enters the body by the inhalation of airborne droplets into the **respiratory tract,** resulting in either asymptomatic colonization or infections such as otitis media, sinusitis, or pneumonia.

The organism produces an IgA protease that degrades secretory IgA, thus facilitating attachment to the respiratory mucosa. After becoming established in the upper respiratory tract, the organism can enter the bloodstream (bacteremia) and spread to the meninges.

Pathogenesis of *H. influenzae* involves its antiphagocytic capsule and endotoxin; no exotoxin is produced.

Most infections occur in children between the ages of 6 months and 6 years, with a peak in the age group from 6 months to 1 year. This is due to a decline in maternal IgG in the child coupled with the inability of the child to generate sufficient antibody against the polysaccharide capsular antigen until the age of approximately 2 years.

Clinical Findings

Meningitis caused by *H. influenzae* is typical with rapid onset of fever, headache, and stiff neck, along with drowsiness.

Sinusitis and otitis media cause pain in the affected area.

Other serious infections caused by this organism include septic arthritis, cellulitis, and sepsis, the latter occurring especially in splenectomized patients.

Rarely, **epiglottitis,** which can obstruct the airway, occurs with a swollen “cherry-red” epiglottis seen.

Pneumonia in elderly adults, especially those with chronic respiratory disease,

Laboratory Diagnosis

Laboratory diagnosis depends on isolation of the organism on heated-blood

(“chocolate”) agar enriched with two growth factors namely, factor X (a heme compound) and factor V (NAD).

An organism that grows only in the presence of both growth factors is presumptively identified as *H. influenza.*

Definitive identification can be made with either biochemical tests or the capsular swelling (quellung) reaction.

Other tests include fluorescent antibody staining of the organism and counter immune-electrophoresis or latex agglutination tests to detect the capsular polysaccharide.

Treatment

The treatment of choice for meningitis or other serious systemic infections caused by

H. influenzae is ceftriaxone.

H. influenzae upper respiratory tract infections, such as otitis media and sinusitis, are treated with either amoxicillin-clavulanate or trimethoprim-sulfamethoxazole.

Prevention

Vaccination of children between the age of 2 and 15 moths. The vaccine contains the capsular polysaccharide of *H. influenzae* type b **conjugated to diphtheria toxoid** or other carrier protein.

Meningitis in close contacts of the patient can be prevented by rifampin as it decreases respiratory carriage of the organism, thereby reducing transmission.

BORDETELLA

Disease

B. pertussis causes whooping cough (pertussis).

Important Properties

B. pertussis is a small, coccobacillary, encapsulated gram-negative rod.

Pathogenesis & Epidemiology

B. pertussis, a pathogen **only for humans,** is transmitted by **airborne droplets** produced during the severe coughing episodes. The organisms attach to the ciliated epithelium of the upper respiratory tract but do not invade the underlying tissue.

Decreased cilia activity and subsequent death of the ciliated epithelial cells are important aspects of pathogenesis.

Pertussis is a highly contagious disease that occurs primarily in infants and young children and has a worldwide distribution.

Several factors play a role in the pathogenesis:

1. Attachment of the organism to the cilia of the epithelial cells is mediated by a protein on the pili called filamentous hemagglutinin.
2. **Pertussis toxin** stimulates adenylate cyclase resulting to edema of the respiratory mucosa that contributes to the severe cough of pertussis.
3. The organisms also synthesize and export adenylate cyclase which when taken up by phagocytic cells (e.g., neutrophils), inhibits their bactericidal activity.
4. Tracheal cytotoxin damages ciliated cells of the respiratory tract.

Clinical Findings

Whooping cough is an acute tracheobronchitis that begins with mild upper respiratory tract symptoms followed by a severe paroxysmal cough, which lasts from 1 to 4 weeks. The paroxysmal pattern is characterized by a series of hacking coughs, accompanied by production of copious amounts of mucus, that end with an inspiratory “whoop” as air rushes past the narrowed glottis. Despite the severity of

Leukocytosis with up to 70% lymphocytes is seen and death occurs mainly due to pneumonia.

In adults, *B. pertussis* infection often manifests as a paroxysmal cough of varying severity lasting weeks. The characteristic whoop is often absent, leading to difficulty in recognizing the cough as caused by this organism and therefore adults with a cough lasting several weeks (often called the 100-day cough) should be evaluated for infection with *B. pertussis*.

Laboratory Diagnosis

The organism can be isolated from nasopharyngeal swabs taken during the paroxysmal stage.

Identification of the isolated organism can be made by agglutination with specific antiserum or by fluorescent-antibody staining.

Polymerase chain reaction-based tests are highly specific and sensitive and should be used if available.

Serologic tests that detect antibody in the patient's serum can be used for diagnosis in patients with prolonged cough.

Treatment

Azithromycin is the drug of choice as it reduces the number of organisms in the throat and decreases the risk of secondary complications.

Supportive care (e.g., oxygen therapy and suction of mucus) during the paroxysmal stage is important, especially in infants.

Prevention

Vaccination with an acellular vaccine containing purified proteins from the organism and a killed vaccine containing inactivated *B. pertussis* organisms.

Azithromycin is used in prevention of disease in exposed, unimmunized individuals and should also be given to immunized children younger than 4 years who have been exposed because vaccine-induced immunity is not completely protective.

LEGIONELLA

Disease

L. pneumophila (and other legionellae) causes pneumonia, both in the community and in hospitalized immunocompromised patients. The genus is named after the famous outbreak of pneumonia among people attending the American Legion convention in Philadelphia in 1976 (Legionnaires' disease).

Important Properties

Legionellae are gram-negative rods that **stain faintly with the standard Gram stain**. They do, however, have a gram-negative type of cell wall, and increasing the time of the safranin counterstain enhances visibility.

Legionellae grow in culture media supplemented with high concentration of iron and cysteine.

L. pneumophila causes approximately 90% of pneumonia attributed to legionellae and the remaining 10% of cases are caused by two species, *Legionella micdadei* and *Legionella bozemanii*.

Pathogenesis & Epidemiology

Legionellae are associated chiefly with **environmental water sources** such as air conditioners and water-cooling towers. Outbreaks of pneumonia in hospitals have been attributed to the presence of the organism in water taps, sinks, and showers.

The portal of entry is the respiratory tract, and pathologic changes occur primarily in the lung. However, in severe cases, bacteremia occurs, accompanied by damage to the vascular endothelium in multiple organs, especially the brain and kidneys.

The major virulence factor of the organism is lipopolysaccharide (endotoxin). No exotoxins are produced.

The typical candidate for Legionnaires' disease is an older man who smokes and consumes substantial amounts of alcohol. Patients with acquired immunodeficiency syndrome (AIDS), cancer, or transplants (especially renal transplants) or patients being treated with corticosteroids are predisposed to *Legionella* pneumonia, which indicates that **cell-mediated immunity** is the most important defense mechanism.

Despite airborne transmission of the organism, person-to-person spread does *not* occur.

Clinical Findings

The clinical picture varies from a mild influenza-like illness to a severe pneumonia accompanied by mental confusion, non-bloody diarrhea, proteinuria, and microscopic hematuria. Cough is a prominent symptom with sputum that is scanty and non-purulent.

Hyponatremia is an important laboratory finding that occurs more often in *Legionella* pneumonia than in pneumonia caused by other bacteria.

Legionellosis is an **atypical pneumonia** and must be distinguished from other similar pneumonias

Laboratory Diagnosis

Sputum Gram stains reveals many neutrophils but no bacteria. The organism grows only on charcoal-yeast agar, a special medium supplemented with iron and cysteine.

Diagnosis usually depends on a significant increase in antibody titer in convalescent-phase serum by the indirect immunofluorescence assay.

Detection of *L. pneumophila* antigens in the urine is a rapid means of making a diagnosis.

Treatment

Azithromycin or erythromycin (with or without rifampin) is the treatment of choice.

Certain fluoroquinolones, such as levofloxacin and trovafloxacin, are also drugs of choice.

The organism frequently produces β-lactamase, and so penicillins and cephalosporins

are less effective.

Prevention

Prevention involves reducing cigarette and alcohol consumption, eliminating aerosols from water sources, and reducing the incidence of *Legionella* in hospital water supplies by using high temperatures and hyperchlorination.

There is no vaccine.

GRAM-NEGATIVE RODS RELATED TO ANIMAL SOURCES (ZOONOTIC ORGANISMS)

Zoonoses are human diseases caused by organisms that are acquired from animals.

There are bacterial, viral, fungal, and parasitic zoonoses. Some zoonotic organisms are acquired directly from the animal reservoir, whereas others are transmitted by vectors, such as mosquitoes, fleas, or ticks.

There are four medically important gram-negative rods that have significant animal reservoirs: *Brucella* species, *Francisella tularensis, Yersinia pestis*, and *Pasteurella multocida.*

BRUCELLA

Disease

Brucella species cause brucellosis (undulant fever).

Important Properties

Brucellae are small gram-negative rods without a capsule. The three major human pathogens and their animal reservoirs are *Brucella melitensis* (goats and sheep), *Brucella abortus* (cattle), and *Brucella suis* (pigs).

Pathogenesis & Epidemiology

The organisms enter the body either by ingestion of **contaminated milk products** or **through the skin** by direct contact in an occupational setting such as an abattoir.

They localize in the **reticuloendothelial system,** namely, the lymph nodes, liver, spleen, and bone marrow. Many organisms are killed by macrophages, but some survive within these cells, where they are protected from antibody. The host response is granulomatous, with lymphocytes and epithelioid giant cells, which can progress to form focal abscesses. The mechanism of pathogenesis of these organisms is not well defined, except that endotoxin is involved. No exotoxins are produced.

Clinical Findings

After an incubation period of 1 to 3 weeks, non specific symptoms such as fever, chills, fatigue, malaise, anorexia, and weight loss occur. The onset can be acute or gradual. The undulating (rising-and-falling) fever pattern that gives the disease its name occurs in a minority of patients. Enlarged lymph nodes, liver, and spleen are frequently found. Pancytopenia occurs.

B. melitensis infections tend to be more severe and prolonged, whereas those caused by *B. abortus* are more self-limited.

Osteomyelitis is the most frequent complication.

Secondary spread from person to person is rare.

Laboratory Diagnosis

Isolation of the organism requires the use of enriched culture media and incubation in 10% CO2.

The organisms can be presumptively identified by using a slide agglutination test with *Brucella* antiserum, and the species can be identified by biochemical tests.

Treatment

The treatment of choice is tetracycline plus rifampin. There is no significant resistance to these drugs.

Prevention

Prevention of brucellosis involves pasteurization of milk, immunization of animals, and slaughtering of infected animals. There is no human vaccine.

FRANCISELLA

Disease

Francisella tularensis causes tularemia.

Important Properties

F. tularensis is a small, pleomorphic gram-negative rod. It has a single serologic type. There are two biotypes, A and B, which are distinguished primarily on their virulence. Type A is more virulent, whereas type B is less virulent.

Pathogenesis & Epidemiology

F. tularensis is remarkable in rabbits, deer, and a variety of rodents. The bacteria are transmitted among these animals by vectors such as **ticks,** mites, and lice, especially the *Dermacentor* ticks that feed on the blood of wild rabbits. The tick maintains the chain of transmission by passing the bacteria to its offspring by the transovarian route. In this process, the bacteria are passed through ovum, larva, and nymph stages to adult ticks capable of transmitting the infection.

Humans are accidental “dead-end” hosts who acquire the infection most often by being bitten by the vector or by having skin contact with the animal during removal of the hide. Rarely, the organism is ingested in infected meat, causing gastrointestinal tularemia, or is inhaled, causing pneumonia. There is no person-to-person spread.

It then localizes to the cells of the reticuloendothelial system, and granulomas are formed. Necrosis and abscesses can also occur. Symptoms are caused primarily by endotoxin. No exotoxins have been identified.

Clinical Findings

Presentation varies from sudden onset of influenza like syndrome to prolonged onset of a low-grade fever and adenopathy. The site of entry ulcerates and the regional lymph nodes are swollen and painful.

Disease usually confers lifelong immunity.

Laboratory Diagnosis

The most frequently used diagnostic method is the agglutination test with acute- and convalescent-phase serum samples.

Fluorescent-antibody staining of infected tissue can be used if available.

Treatment

Streptomycin is the drug of choice. There is no significant antibiotic resistance.

Prevention

Prevention involves avoiding both being bitten by ticks and handling wild animals.

There is a live, attenuated bacterial vaccine that is given only to persons whose occupation brings them into close contact with wild animals.

YERSINIA

Disease

Yersinia pestis is the cause of plague, also known as the Black Death.

Two less important species, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*,

Important Properties

Y. pestis is a small gram-negative rod that exhibits bipolar staining (i.e., it **resembles a safety pin**, with a central clear area). Freshly isolated organisms possess a capsule composed of a polysaccharide–protein complex. The capsule can be lost with passage in the laboratory; loss of the capsule is accompanied by a loss of virulence.

It is one of the **most virulent** bacteria.

Pathogenesis & Epidemiology

The enzootic (sylvatic) cycle consists of transmission among **wild rodents by fleas**.

Humans are accidental hosts, and cases of plague occur as a result of being bitten by a flea that is part of the sylvatic cycle.

The cycle consists of transmission of the bacteria among rats (the reservoir), with the **rat flea** as vector. This cycle predominates in poor sanitation, when rats proliferate and come in contact with the fleas in the sylvatic cycle.

The flea ingests the bacteria while taking a blood meal from a bacteremic rodent. A thick biofilm containing many organisms forms in the upper gastrointestinal tract that prevents any food from proceeding down the gastrointestinal tract of the flea. This “blocked flea” then regurgitates the organisms into the bloodstream of the next animal or human it bites.

The organisms inoculated at the time of the bite spread to the regional lymph nodes, which become swollen and tender. These swollen lymph nodes are the **buboes** that have led to the name **bubonic plague**.

The **endotoxin-related symptoms,** including disseminated intravascular coagulation and cutaneous hemorrhages, probably were the genesis of the term **black death**.

The organism has several factors that contribute to its virulence:

1. The envelope capsular antigen,
2. Endotoxin
3. Exotoxin
4. Two proteins known as V antigen and W antigen

Clinical Findings

Bubonic plague, which is the most frequent form, begins with pain and swelling of the lymph nodes draining the site of the flea bite and systemic symptoms such as high fever, myalgias, and prostration. The affected nodes enlarge and become exquisitely tender. These buboes are an early characteristic finding.

Septic shock and pneumonia are the main life-threatening subsequent events. Pneumonic plague can arise either from inhalation of an aerosol or from septic emboli that reach the lungs.

Laboratory Diagnosis

Smear and culture of blood or pus from the bubo is the best diagnostic procedure.

Giemsa or Wayson stain reveals the typical safety-pin appearance of the organism better than does Gram stain.

Fluorescent-antibody staining can be used to identify the organism in tissues.

Treatment

The treatment of choice is a combination of streptomycin and a tetracycline such as

doxycycline, although streptomycin alone can be used. Levofloxacin can also be used. There is no significant antibiotic resistance.

Prevention

Prevention of plague involves controlling the spread of rats in urban areas, preventing rats from entering the country by ship or airplane, and avoiding both flea bites and contact with dead wild rodents.

A patient with plague must be placed in strict isolation (quarantine) for 72 hours after antibiotic therapy is started. Only close contacts need to receive prophylactic tetracycline, but all contacts should be observed for fever. Reporting a case of plague to the public health authorities is mandatory.

A vaccine consisting of formalin-killed organisms provides partial protection gainst bubonic but not pneumonic plague.

PASTEURELLA and BARTONELLA assignment

MYCOBACTERIA

Mycobacteria are aerobic, **acid-fast** bacilli (rods). They are neither gram-positive nor gram-negative (i.e., they are stained poorly by the dyes used in Gram stain). They are the only bacteria that are acid-fast with an exception of *Nocardia asteroides*, the major cause of nocardiosis.

The term *acid-fast* refers to an organism's ability to retain the carbolfuchsin stain despite subsequent treatment with an ethanol–hydrochloric acid mixture. The high lipid (mycolic acid) content (approximately 60%) of their cell wall makes mycobacteria acid-fast.

The major pathogens are *Mycobacterium tuberculosis*, the cause of tuberculosis, and *Mycobacterium leprae*, the cause of leprosy. Atypical mycobacteria, such as *Mycobacterium avium-intracellulare* complex and *Mycobacterium kansasii*, can cause tuberculosis-like disease but are less frequent pathogens.

MYCOBACTERIUM TUBERCULOSIS

Disease

It causes tuberculosis.

Important Properties

M. tuberculosis **grows slowly.** Because growth is so slow, cultures of clinical specimens must be held for 6 to 8 weeks before being recorded as negative.

Media used for its growth (e.g., Löwenstein-Jensen medium) contain complex nutrients (e.g., egg yolk) and dyes (e.g., malachite green) which inhibit the unwanted normal flora present in sputum samples.

M. tuberculosis is an **obligate aerobe**; this explains its predilection for causing disease in highly oxygenated tissues such as the upper lobe of the lung and the kidney.

Cord factor (trehalose dimycolate) is correlated with virulence of the organism.

The organism also contains proteins, which, when combined with waxes, elicit delayed hypersensitivity. These proteins are the antigens in the **purified protein derivative (PPD)** skin test (also known as the tuberculin skin test).

A lipid located in the bacterial cell wall called phthiocerol dimycocerosate is required for pathogenesis in the lung.

M. tuberculosis is relatively resistant to acids and alkalis. NaOH is used to concentrate clinical specimens; it destroys unwanted bacteria

M. tuberculosis is resistant to dehydration and survives in dried expectorated sputum; this property may be important in its transmission by aerosol.

Some strains of *M. tuberculosis* are resistant to the main antimycobacterial drug, isoniazid as well as to multiple antibiotics (called **multidrug-resistant** or **MDR** strains).

Transmission & Epidemiology

M. tuberculosis is transmitted from person to person by respiratory aerosol, and its initial site of infection is the lung. In the body, it resides chiefly within reticuloendothelial cells (e.g., **macrophages**). **Humans are the natural reservoir** of *M. tuberculosis* though some animals can be infected, and are not a reservoir for human infection.

Most transmission occurs by aerosols generated by the coughing of "smear-positive" people. However, about 20% of people are infected by aerosols produced by the coughing of "smear-negative" people.

The risk of infection and disease is highest among socioeconomically disadvantaged people, who have poor housing and poor nutrition.

Pathogenesis

M. tuberculosis produces no exotoxins and does not contain endotoxin in its cell wall. The organism infects macrophages and other reticuloendothelial cells. *M. tuberculosis* survives and multiplies within a cellular vacuole called a phagosome.

It produces a protein called "exported repetitive protein" that prevents the phagosome from fusing with the lysosome, thereby allowing the organism to escape the degradative enzymes in the lysosome.

Lesions are dependent on the presence of the organism and the host response.

There are two types of lesions:

1. **Exudative lesions,** which consist of an acute inflammatory response and occur chiefly in the lungs at the initial site of infection.
2. **Granulomatous lesions,** which consist of a central area of giant cells containing tubercle bacilli surrounded by a zone of epithelioid cells. A **tubercle** is a granuloma surrounded by fibrous tissue.

Spread of the organism within the body occurs by two mechanisms:

1. A tubercle can erode into a bronchus and spread the organism to other parts of the lungs, to the gastrointestinal tract if swallowed, and to other persons if expectorated.
2. It can disseminate via the bloodstream to many internal organs. Dissemination can occur at an early stage if cell-mediated immunity fails to contain the initial infection or at a late stage if a person becomes immunocompromised.

Clinical Findings

Many organs can be involved with fever, fatigue, night sweats, and weight loss being common. Pulmonary tuberculosis causes cough and hemoptysis. **Scrofula** is mycobacterial cervical lymphadenitis that presents as swollen, non-tender lymph nodes.

Lymphadenitis is the most common extrapulmonary manifestation of tuberculosis, affecting mostly the patients infected with human immunodeficiency virus (HIV).

Erythema nodosum, characterized by tender nodules along the extensor surfaces of the tibia and ulna, is a manifestation of primary infection seen in patients who are controlling the infection with a potent cell-mediated response.

Miliary tuberculosis is characterized by multiple disseminated lesions that resemble millet seeds. **Tuberculous meningitis** and **tuberculous osteomyelitis**, especially vertebral osteomyelitis (Pott's disease), are important disseminated forms.

Gastrointestinal tuberculosis is characterized by abdominal pain and diarrhea accompanied by more generalized symptoms of fever and weight loss. Intestinal obstruction or hemorrhage may occur.

Oropharyngeal tuberculosis typically presents as a painless ulcer accompanied by local adenopathy.

Renal tuberculosis presents with dysuria, hematuria, and flank pain occur. "Sterile pyuria"is a characteristic finding. The urine contains white blood cells, and mycobacterial cultures are often positive.

Laboratory Diagnosis

Acid-fast staining of sputum or other specimens is the usual initial test.

After digestion of the specimen by treatment with NaOH and concentration by centrifugation, the material is cultured on special media, such as Löwenstein-Jensen agar, for up to 8 weeks. It will *not* grow on a blood agar plate.

In liquid BACTEC medium, radioactive metabolites are present, and growth can be detected by the production of radioactive carbon dioxide in about 2 weeks.

The organism can be identified by biochemical tests,as *M. tuberculosis* produces **niacin,** whereas almost no other mycobacteria do. It also produces catalase.

Nucleic acid amplification tests can be used to detect the presence of *M. tuberculosis* directly in clinical specimens such as sputum.

Treatment & Resistance

Multidrug therapy is used to prevent the emergence of drug-resistant mutants during the long (6- to 9-month) duration of treatment.

Isoniazid (INH), a bactericidal drug, is the mainstay of treatment. Treatment for most

patients with pulmonary tuberculosis is with three drugs: INH, rifampin, and pyrazinamide. INH and rifampin are given for 6 months, but pyrazinamide treatment is stopped after 2 months.

In patients who are immunocompromised, who have disseminated disease, or who are likely to have INH-resistant organisms, a fourth drug, ethambutol, is added, and all four drugs are given for 9 to 12 months.

Although therapy is usually given for months, the patient's sputum becomes **noninfectious within 2 to 3 weeks**.

Prevention

Prevention of the spread of the organism depends largely on the prompt identification and adequate treatment of patients who are coughing up the organism.

The use of masks and other respiratory isolation procedures to prevent spread to medical personnel is also important.

Contact tracing of individuals exposed to patients with active pulmonary disease who are coughing should be done.

BCG vaccine can be used to induce partial resistance to tuberculosis.

MYCOBACTERIUM LEPRAE

Disease

This organism causes leprosy (Hansen's disease).

Important Properties

M. leprae can only be grown in experimental animals, such as mice and armadillos.

Humans are the natural hosts, although the armadillo appears to be a reservoir for human infection.

The optimal temperature for growth (30°C) is lower than body temperature; therefore, *M. leprae* grows preferentially in the skin and superficial nerves.

It grows very slowly, with a doubling time of 14 days.

Transmission

Infection is acquired by **prolonged contact with patients** with lepromatous leprosy, who discharge *M. leprae* in large numbers in nasal secretions and from skin lesions.

Pathogenesis

The organism replicates intracellularly, typically within skin histiocytes, endothelial cells, and the Schwann cells of nerves. The nerve damage in leprosy is the result of two

processes: damage caused by direct contact with the bacterium and damage caused by CMI attack on the nerves.

There are two distinct forms of leprosy—**tuberculoid** and **lepromatous**—with several intermediate forms between the two extremes.

1. In tuberculoid (also known as paucibacillary) leprosy, the CMI response to the organism limits its growth, very few acid-fast bacilli are seen, and granulomas containing giant cells form. The nerve damage seems likely to be caused by cell-mediated immunity as there are few organisms and the CMI response is strong.
2. In lepromatous (also known as multibacillary) leprosy, the cell-mediated response to the organism is poor, the skin and mucous membrane lesions contain large numbers of organisms, foamy histiocytes rather than granulomas are found.

Clinical Findings

The incubation period averages several years, and the onset of the disease is gradual.

In tuberculoid leprosy, hypopigmented macular or plaque-like skin lesions, thickened superficial nerves, and significant anesthesia of the skin lesions occur

In lepromatous leprosy, multiple nodular skin lesions occur, resulting in the typical **leonine** (lion-like) **facies**. After the onset of therapy, patients with lepromatous leprosy often develop **erythema nodosum leprosum** (ENL), which is interpreted as a sign that cell-mediated immunity is being restored.

The disfiguring appearance of the disease results from several factors:

1. The skin anesthesia results in burns and other traumas, which often become infected.
2. Resorption of bone leads to loss of features such as the nose and fingertips.
3. Infiltration of the skin and nerves leads to thickening and folding of the skin.

Laboratory Diagnosis

In lepromatous leprosy, the bacilli are easily demonstrated by performing an acid fast stain of skin lesions or nasal scrapings. Lipid-laden macrophages called “foam cells” containing many acid-fast bacilli are seen in the skin.

In the tuberculoid form, very few organisms are seen, and the appearance of typical granulomas is sufficient for diagnosis.

Cultures are negative because the organism does not grow on artificial media.

A serologic test for IgM against phenolic glycolipid-1 is useful in the diagnosis of lepromatous leprosy but is not useful in the diagnosis of tuberculoid leprosy.

The diagnosis of lepromatous leprosy can be confirmed by using the polymerase chain reaction (PCR) test on a skin sample.

Treatment

The mainstay of therapy is **dapsone** (diaminodiphenylsulfone), but because sufficient resistance to the drug has emerged, combination therapy is now recommended (e.g., dapsone, rifampin, and clofazimine for lepromatous leprosy and dapsone and rifampin for the tuberculoid form). Treatment is given for at least 2 years or until the lesions are free of organisms.

A combination of ofloxacin plus clarithromycin is an alternative regimen. Thalidomide is the treatment of choice for severe ENL reactions.

Prevention

Isolation of all lepromatous patients, coupled with chemoprophylaxis with dapsone for exposed children, is required.

There is no vaccine.

ATYPICAL MYCOBACTERIA assignment