

INTRODUCTION TO BACTERIOLOGY AND BACTERIAL STRUCTURE/FUNCTION

LEARNING OBJECTIVES

- To describe historical landmarks of medical microbiology
- To describe Koch's Postulates
- To describe the characteristic structures and chemical nature of cellular constituents that distinguish eukaryotic and prokaryotic cells
- To describe chemical, structural, and functional components of the bacterial cytoplasmic and outer membranes, cell wall and surface appendages
- To name the general structures, and polymers that make up bacterial cell walls
- To explain the differences between gram negative and gram positive cells
- To describe the chemical composition, function and serological classification as H antigen of bacterial flagella and how they differ from flagella of eucaryotic cells
- To describe the chemical composition and function of pili
- To explain the unique chemical composition of bacterial spores
- To list medically relevant bacteria that form spores
- To explain the function of spores in terms of chemical and heat resistance
- To describe characteristics of different types of membrane transport
- To describe the exact cellular location and serological classification as O antigen of Lipopolysaccharide (LPS)
- To explain how the structure of LPS confers antigenic specificity and toxicity
- To describe the exact cellular location of Lipid A
- To explain the term endotoxin in terms of its chemical composition and location in bacterial cells

INTRODUCTION TO BACTERIOLOGY

1. Two main threads in the history of bacteriology: 1) the natural history of bacteria and 2) the contagious nature of infectious diseases, were united in the latter half of the 19th century. During that period many of the bacteria that cause human disease were identified and characterized.
2. Individual bacteria were first observed microscopically by Antony van Leeuwenhoek at the end of the 17th century.
3. Bacteria are readily visible when present in large numbers because they make a turbid suspension. The controversy over spontaneous generation of bacterial life in liquid cultures led to the development of two important bacteriological procedures.
 - a. Sterilization: the preparation of medium or instruments such that no living bacteria are present.
 - b. Aseptic technique: laboratory technique that allows the manipulation of sterilized material without bacteriological contamination.
4. Bacteria are most easily studied in pure cultures in which only a single species is present. Pure cultures were originally produced by limiting dilution in liquid medium. Today pure cultures are usually prepared on medium solidified with agar, a gelling agent derived from seaweed. A mixed bacterial suspension is mechanically spread on the agar surface to yield isolated individual bacterial cells. These grow to yield macroscopic colonies (clones) that can be used to prepare pure cultures.
5. The ability to prepare pure cultures led to the study of bacterial classification and taxonomy.

- a. The first basis for classification was shape. Round bacteria are called cocci (singular coccus). Rod shaped bacteria are called bacilli (singular bacillus). Other shapes will be considered later in the course.
 - b. Bacteria are very difficult to study microscopically unless stained. The staining characteristics of bacteria in the Gram stain are very useful in classification. Gram positives are violet, while gram negatives are red.
 - c. Bacterial taxonomy today depends upon the extent of DNA sequence homology. An important laboratory technique for the amplification and detection of specific DNA sequences (as, for example, in a bacterium or a virus) is the polymerase chain reaction (PCR). Examples of when PCR is used for clinical diagnostics will be considered later in this course. However, for routine laboratory diagnosis the most important bacterial characteristics are:
 1. The morphology of colonies on appropriate agar medium.
 2. Microscopic morphology and staining of individual bacteria.
 3. Simple biochemical characteristics such as the ability to ferment a given carbohydrate.
 4. Specific antigens detected by known antisera.
6. **Koch's Postulates.** The use of pure cultures has made possible the identification of the bacterial etiology of many infectious diseases. The original rules for the proof of microbial etiology (**Koch's Postulates**):
- a. Find the bacteria in all cases of the disease.
 - b. Grow the bacteria in pure cultures.
 - c. Reproduce the disease (in animals) using the pure culture.
 - d. Reisolate the bacteria in pure culture from the experimental infection.
- These rules cannot be applied to all infectious diseases. Some infectious diseases, such as obligate intracellular pathogens (i.e., those organisms that cannot grow on laboratory medium but require a host cell to grow) will not answer all of Koch's postulates.
7. **Koch's Molecular Postulates.** Koch's Molecular Postulates were put forth by Stanley Falkow in 1988 to deal with defining the molecular basis by which a specific infectious disease is caused.
- a. The phenotype under investigation should be associated significantly more often with a pathogenic organism than with a nonpathogenic member or strain.
 - b. Specific inactivation of a gene (or genes) associated with the suspected virulence trait should lead to a measurable decrease in virulence.
 - c. Restoration of full pathogenicity should accompany replacement of the mutated gene with the wild type original.

BACTERIAL STRUCTURE AND FUNCTION: THE MICROBIAL WORLD
(Introduction to the Prokaryotic Cell)

Reading assignment: Levinson, Chapter 1, 2 (omit plasmids and transposons until genetics lectures), and 5

Classes of Microorganisms (which classes contain human pathogens?)

Distinguishing Characteristics

Algae	:	no pathogens, all photosynthetic
Fungi	:	some pathogens, nonphotosynthetic; rigid cell wall
Protozoa	:	some pathogens, no rigid cell wall; unicellular, nonphotosynthetic (cysts have rigid walls)
Bacteria	:	many pathogens; mostly require organic compounds as energy source but some of the non-pathogens are photosynthetic; all (but one) have a rigid cell wall

Microorganisms have been traditionally differentiated from animals and true plants on the basis of their *relatively simple biological-organization*. The higher plants and animals are multicellular and develop *distinct tissue regions* that differ from one another with respect to the *kinds of cells* of which they are composed. A further level of internal complexity may be achieved by the combination of different tissues into a specialized local structure known as an organ (e.g. liver or leaf). Microorganisms are unicellular, but there is an increasing realization that they can act as multicellular groups and show differentiation into functionally distinct regions. Examples include stalk and spore formation in the soil microbe *Myxococcus xanthus*, and the formation of surface microbial communities on implants by pathogenic microbes.

Microorganisms are divided into two subgroups on basis of *structure of the individual cell*. (This has clinical importance, since different classes of antibiotics are used to treat pathogens in each group.)

Higher Microorganisms: (Eukarya domain)	Fungi, Protozoa, Algae (Eucaryotic cells) (Eucaryotic = "true" nucleus)
Lower Microorganisms: (Bacterial domain)	Bacteria (Prokaryotic cells)

Characteristics of structure and function exhibited by Eucaryotic as compared to Procaryotic cells. (These differences are often important for understanding the mechanism of action of chemotherapeutic agents. Antibiotics useful for combating bacterial infections are often useless against fungal infections.)

1. Chromosome(s)

Eucaryotic: Each cell contains a number of different linear chromosomes contained *Within the Nuclear Membrane*. Mitosis occurs.


Procaryotic: Each cell generally contains one circular chromosome. *Not bound by a nuclear membrane*. The mechanism of chromosome segregation during division does not involve mitosis.

2. Mitochondria and other *membrane bound structures within* the cytoplasm housing specific parts of the functional machinery of the cell.

Eucaryotic: Eucaryotic mitochondrion contains the oxidative enzymes and carries out oxidative phosphorylation. Eucaryotic cells also contain other membrane-bound structures, such as vacuoles, peroxisomes, etc.

Procaryotic: Procaryotic cells contain no mitochondria, although mitochondria likely evolved from prokaryotes (“endosymbiotic hypothesis”). Oxidative enzymes are associated with cytoplasmic membrane of cell. Oxidative phosphorylation is associated with the cytoplasmic membrane.

A general characteristic of procaryotic cell: No membrane bound structures smaller than the cell itself. There are a few exceptions to this generalization, but there are no elaborate membrane bound structures inside of the cytoplasmic membrane in the procaryotic cell. Procaryotic cells do demonstrate organization at the level of protein localization (e.g., some proteins are localized to the pole of the cell and some to the center of the cell)

3. Mechanism of  Cellular movement—a third way to distinguish eucaryotic from procaryotic cells.

Eucaryotic: Movement may be accomplished by cytoplasmic streaming (amoeboid movement) or by contraction of flagella or cilia.

(The *flagellum* or cilium of eucaryotic cells, when present, is comprised of microtubules in a specific 9 doublet:2 singlet arrangement that is surrounded by a membrane continuous with the cell membrane.)

Procaryotic: There is no cytoplasmic streaming or amoeboid movement. (In fact, the cytoplasm of the bacterial cell is very dense, due to a high content of ribosomes necessary for the rapid protein synthesis required for rapid growth.) Some bacterial cells also have flagella. However, the structure of bacterial flagella is very different (a long, helical filament composed of repeating protein (flagellin) subunits with a hollow tube down the middle). There are no microtubules in the procaryotic flagellum and it typically has no membrane coat.

4. Cell wall: A protective structure; strength due to macro-molecular mesh of polysaccharide. Animal cells do not have cell walls.
- Eucaryotic: In higher plants and green algae the cell wall is composed of the polysaccharide cellulose (polymer of glucose). In most fungi the cell wall is composed of chitin (polymer of acetyl glucosamine) and beta 1,3 glucan (a polymer of glucose).
- Procaryotic: In most procaryotes, the cell wall is composed of a peptidoglycan polymer, containing muramic acid (derivative of acetyl glucosamine), D-amino acids and other unusual amino acids as unique components, which are not found in eucaryotic cells. (The extraordinary ability of an antibiotic such as penicillin to kill without harming human cells was explained when it was discovered that penicillin interfered with the formation of this peptidoglycan.)
5. RNA:
- Eucaryotic: RNA is transcribed in the nucleus, spliced, and transported to the endoplasmic reticulum where it is translated into protein.
- Procaryotic: Since there is no nucleus, the nascent RNA is translated as it is transcribed. There is no RNA splicing.

NOTE: Procaryotic cells are divided into two major groups, the bacteria (true bacteria) and the archaea. All prokaryotes of medical importance are bacteria, while the archaea inhabit unusual environmental niches. Archaea exhibit considerable differences in cellular structures such as in their membranes and cell walls. We will only discuss bacteria in these notes. Therefore, when we use the term "bacteria", we are not including archaea. Sometimes bacteria are referred to as "eubacteria" for "true bacteria", but this term is rarely used within the context of infectious disease.

COMPARISON OF PROCARYOTIC AND EUCARYOTIC CELLS
(A Summary Chart)

CHARACTER	PROCARYOTIC	EUCARYOTIC
Nuclear division	Nonmitotic	Mitotic
Nuclear structure: Membrane Chromosome number	Not present Circular (usually 1-2) plus plasmids	Present More than one
Sexual reproduction	Partial, unidirectional transfer of DNA	Meiosis
Cytoplasmic structure: Mitochondria Endoplasmic reticulum Lysosomes and other organelles	Absent Absent Absent	Present Present Present
Protein expression: Ribosomes	70S	80S (except in mito- chondria - 70S)
mRNA splicing	Absent	Present
Chemical composition: Sterols Muramic acid	Absent* Present in most	Present Absent
Cell wall	Peptidoglycan with muramic acid	Not present or of another polymer
Contractile locomotor organelles: Bacterial flagella Multistranded flagella or cilia	Present in some Absent	Absent Present in some

* The one exception is in the Mycoplasma, which may contain sterols in their membrane. The presence of sterols in eucaryotic cells such as fungi is important in chemotherapy, since certain antifungal antibiotics react with sterols in membranes.

RELATIVE SIZE OF MICROORGANISMS



(Most *bacteria* of medical interest have dimensions of ~1 micrometer [μm].)

Some specific examples are given below to illustrate the size variation of microscopic forms. (This material is illustrative, and should not be memorized.)

(compare to) lymphocyte (one of the smallest mammalian cells)	10 mm (diameter)
<i>Bacillus anthracis</i> (one of the larger bacteria)	1 x 3 μm
<i>Francisella tularensis</i> (a small bacterium)	0.2 x 0.5 μm
Mycoplasma (smallest free-living organism)	0.175 μm (175nm)
Large viruses (pox) (note how these overlap the smallest bacteria in size)	200 x 300 nm
Polio virus (a small virus)	25 nm
(compare to) egg albumin molecule	2.5 x 10 nm

1 μm (micrometer, older term micron) = 0.001mm = 1000 nm (nanometer)

Since the resolving power of the light microscope is 0.2 μm , it is obvious that the highest possible magnification must be used to detect bacteria in clinical specimens. Therefore, stained clinical specimens are always examined with the use of the oil immersion lens. Lower magnifications are used to locate the specimen, but definitive determination of shape and color require the oil immersion objective (900x to 1,000x magnification).

Note that even the largest viruses and a few of the smallest bacteria are below the resolving power of the light microscope.

A theoretical comment, not for memorization for examination.

In principle, the smallest size for a procaryotic cell is set by molecular limitations. In order to reproduce itself, any cell requires a large number of different enzymes and other proteins. We do not know the precise minimum, but the number is probably on the order of several hundred. Furthermore, many molecules of most enzymes are needed. To these must be added the cellular nucleic acids and the various other organic constituents of the cell such as lipids and carbohydrates. All in all, it seems likely that the smallest free-living organisms, the mycoplasmas, have a size very close to this molecular limit for the maintenance of cellular function. Since viruses are not cellular organisms, they can obviously be much smaller.

The practical lesson is to always note the approximate size of a cell in the microscope. This will aid in determining whether bacteria are present in clinical specimens. In clinical specimens, cells larger than 10 μm in diameter are not bacteria. They may be cells from the host, or they may be eucaryotic microorganisms such as fungi or protozoa.

GENERAL MORPHOLOGICAL TYPES OF BACTERIA



(For the physician, the ability to recognize the important bacterial pathogens by microscopic examination of clinical specimens is essential for diagnosis and treatment of infections. Appropriate antibiotic choice can be guided by microscopic examination. However, this examination is typically done these days in the clinical lab.)

Because of the strength and rigidity of its wall, the bacterial cell has a characteristic size and shape. As long as the wall remains intact, large changes in osmotic pressure of the environment have little effect on cell shape. *

1. Spherical form or "coccus"

a) In general, a diameter of 0.8 to 1.0 μm

b) Some organisms exhibit characteristic small variations from the sphere: e.g., the Pneumococcus (causes pneumonia) and the Gonococcus (causes Gonorrhoea) are elongated cocci. In contrast, the Staphylococcus (causes abscesses) is a perfect sphere.

2. Rod shaped or "bacillus" (cylindrical shape of cell)

The shape of individual rods is different for different species and may be useful for identification. For instance, the rod may be long and slender or short and thick. The ends of the rod may appear square or rounded or tapered. Some rods, such as *Corynebacterium diphtheriae* are characteristically pleomorphic. (Note: With some organisms, the line of demarcation between a coccus and a rod is difficult to draw, with the result that we call some forms "cocci bacilli"). This is a problem for gram negative rods, as you will find in the laboratory.

3. Curved rod or spiral shape

The vibrios (e.g. the cholera vibrio) are curved rods.

The spirochetes are bacteria with a spiral shape. However, they do not have a rigid wall and are therefore classified differently. (*Treponema pallidum* the causative agent of syphilis, is a spirochete as is *Borrelia burgdorferi*, the cause of Lyme Disease.)

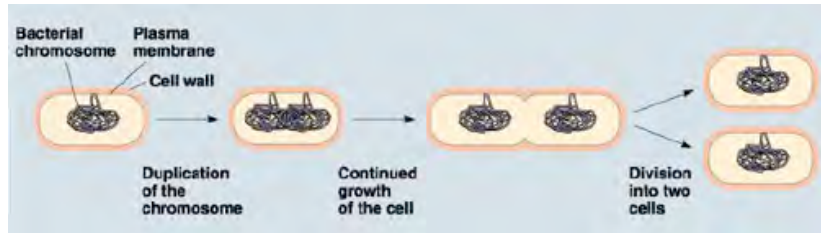
4. Other rare bacterial shapes will be considered later in the course.

* It is interesting to contrast bacterial cells to mammalian cells, which lack a cell wall. The shape of mammalian cells will change, depending upon the ionic strength of the surrounding medium. In distilled water, mammalian cells will swell and burst, but bacterial cells remain stable.

ARRANGEMENT OF CELLS

(This property is also a useful aid in identifying pathogens.)

Most bacteria multiply by binary fission. This binary fission occurs by the formation and subsequent joining of a central transverse wall. If the daughter cells do not separate after completion of the transverse wall, many-celled aggregates called "filaments" may result (these filaments should not be confused with filamentous structures formed by many fungi). This is common among the cocci, where the specific form of the aggregate is a stable characteristic of an organism, and is therefore useful for identification.



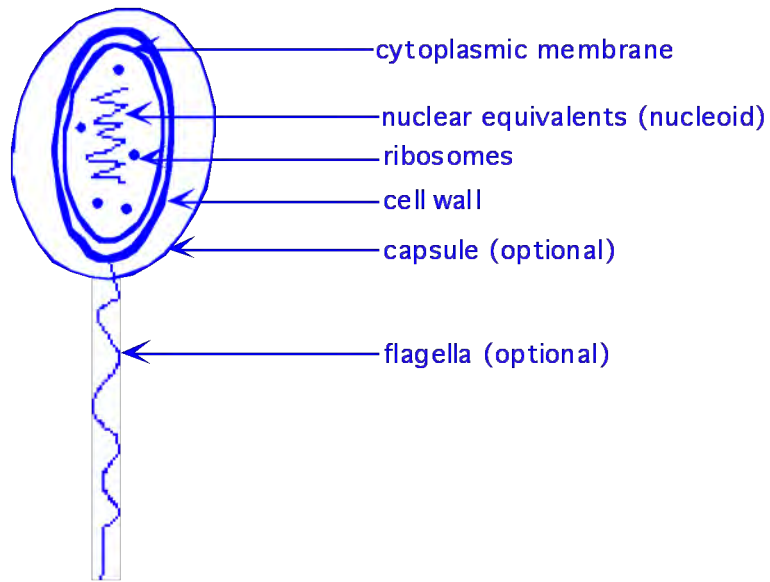
The form of the aggregate is determined by the pattern of successive division.

- Successive divisions of cocci along the same axis will result in a chain of cocci (Streptococci).
- Successive divisions that take place regularly at right angles to one another will result in tetrads or more extensive flat plates of cells. (No medically important bacteria have this arrangement.)
- Three successive divisions at right angles to one another will result in cuboidal arrangement. (Not very common.)
- If successive divisions of cocci can occur in any direction, irregular clusters will result, as found in the Staphylococci.

In rod shaped bacteria, cell division always takes place at right angles to the long axis, and only chains can result. This is common among some aerobic Bacilli.

In rod shaped bacteria of the genus *Corynebacterium* (one species causes diphtheria), the rods exhibit a unique characteristic of sticking together at the ends. However, the rods may slip under each other without separating. This leads to stacks of rods, or to a variety of groupings that resemble letters of the Chinese alphabet.

A TYPICAL BACTERIAL CELL



1. Cytoplasmic membrane:

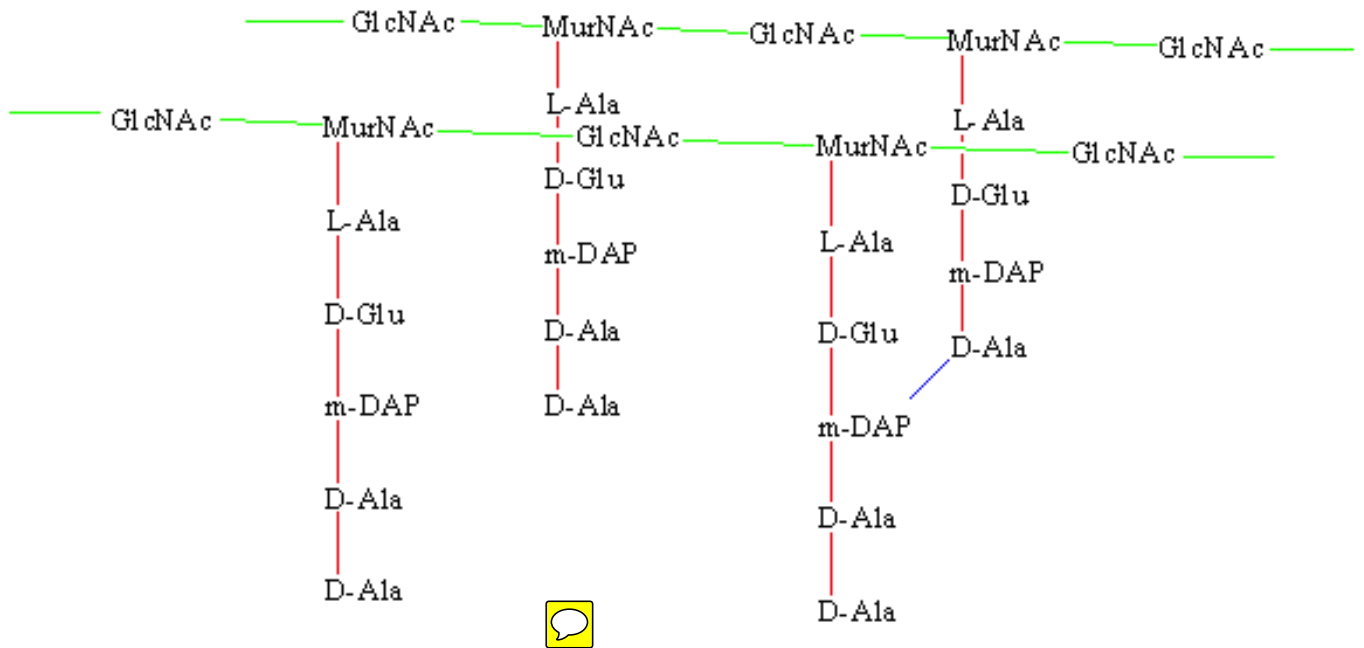
- a phospholipid bilayer such as found in the membrane of mammalian cells.
- overall chemical composition similar to that of membranes in mammalian cell: 20-30% lipid (mainly phospholipid), 60% protein. The bacterial membrane rarely contains sterols, in contrast to the membranes of mammalian cells and fungi.
- a semi-permeable membrane; if a bacterium is suspended in hypertonic media, the membrane contracts from its normal position (plastered against the rigid cell wall). Water is freely permeable, but all ions and non-ionized molecules larger than glycerol penetrate very slowly except by specific transport.
- stable *protoplasts* can be made by digesting all or most of the cell wall with specific enzymes. Under these conditions, a rod-shaped bacterium will assume a spherical shape because the cell wall (not the cytoplasmic membrane) gives shape to the cell. Without the wall, the cell will now burst if placed in media of low osmotic strength (hypotonic media).
- functions of this membrane:
 1. membrane contains oxidative enzymes (cytochromes, quinones, ATPase) and resembles inner membrane of mitochondria, both in structure and function.
 2. membrane also contains enzymes which function in external cell wall synthesis.

3. membrane must have ability to pump in nutrients from dilute external media and thus contains *selective* transport systems for specific sugars, amino acids, metals, etc. Simple passive diffusion is insufficient for the bacterial cell in its usual environment of dilute nutrients. There are three different mechanisms for transporting across the membrane. One of these is called *active transport*, which requires energy and transports nutrients without chemical modification. See the end of these notes for a further discussion of transport.
 4. the membrane contains mechanisms for secreting toxins and certain enzymes into the extracellular medium.
- between the cytoplasmic membrane and the outer membrane is the *periplasmic space* where certain enzymes are located, especially enzymes degrading extracellular substances of high molecular weight.
2. Nuclear equivalent in bacteria (also called nucleoid) (see lectures on Bacterial Genetics): no nuclear membrane; no organization of DNA into visible chromosomes.
 3. Components seen in cytoplasm in electron micrographs:
 - a) *Ribosomes*: Composed of a subunit structure similar to that found in mammalian cells. Function is same as mammalian ribosomes, to participate in protein synthesis. Differences in structure and function between mammalian and bacterial ribosomes explain the selective action of some antibiotics, which inhibit protein synthesis (see lectures on antibiotics). The bacterial ribosome is smaller (70S) than most mammalian ribosomes (80S).
 - b) Cytoplasmic granules
 - bodies found in the cytoplasm of some bacteria under certain conditions.
 - are storage bodies formed under growth conditions where excess food is available.
 - one type is a high molecular weight lipid. Glycogen, a polymer of sugars, accumulates in other bacteria.
 - another type is metachromatic granules. They consist mostly of polymerized phosphate, which stains deeply with basic dyes. The bacterium causing Diphtheria has these granules, which aids in identification since this is a distinguishing characteristic seen microscopically.
 - these high molecular weight polymers allow storage of large quantities of nutrients without increasing the osmolarity of the cytoplasm.

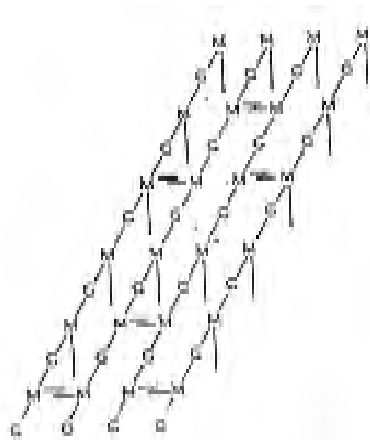
4. Cell wall: (properties help explain many aspects of disease production, including toxicity and mechanism of antibiotic action)

- A relatively large structure, making up 20-35% of the cell weight.
- All bacteria (except Mycoplasma) have a cell wall. All bacteria have walls that contain a unique polymer of N-acetyl glucosamine (NAG) linked to muramic acid by a glycosidic bond. (Muramic acid is N-acetyl glucosamine linked to lactic acid.) (See figure on next page.)
- A short peptide consisting of 4 amino acids (a tetra peptide) is linked to the lactic acid residue. Some of these amino acids are in the uncommon D-configuration. There is some variation in these amino acids in different species of bacteria. For instance, Staphylococci substitute lysine for diaminopimelic acid. (See figure on next page.)
- This polymer is usually called "peptidoglycan" because of its peptide and sugar components.
- "Cross-linking" between polymer chains occurs by bonding between amino acids side chains. This gives strength and rigidity to the wall. The bonding is through a peptide bridge from the terminal carboxyl of the tetrapeptide to a neighboring amino or carboxyl group on another tetrapeptide chain. There is variation in the composition of this peptide bridge among various species. For instance, *E. coli* has a single amide bond, while Staphylococci have an interposed peptide bridge (pentaglycine bridge). (See figure on next page.) *Note that in E. coli, some of these side chains are not cross-linked. In other bacteria, almost all of the side chains are cross-linked, resulting in a thicker, stronger peptidoglycan layer.*
- Penicillin blocks this cross-linking (see lectures on antibiotics).
- The enzyme, lysozyme, which is found in tears and other body fluids, splits the glycosidic bond between N-acetyl glucosamine and muramic acid. Lysozyme can digest the wall of some bacteria, causing lysis and thus acts as an antibacterial agent (ex. In tears).

Structure of Peptidoglycan Layer



General structure of a peptidoglycan. Glycosidic bonds form between the repeating glycoside subunits of GlcNAc and MurNAc. These chains are crosslinked by peptide chains. In this representation, the mode of peptide cross-linking occurs with a peptide bond between the terminal carboxyl group of D-alanine on one subunit and the free amino group of the diaminopimelic acid on an adjacent subunit.



Legend:

M = N-acetylmuramic acid
G = N-acetylglucosamine

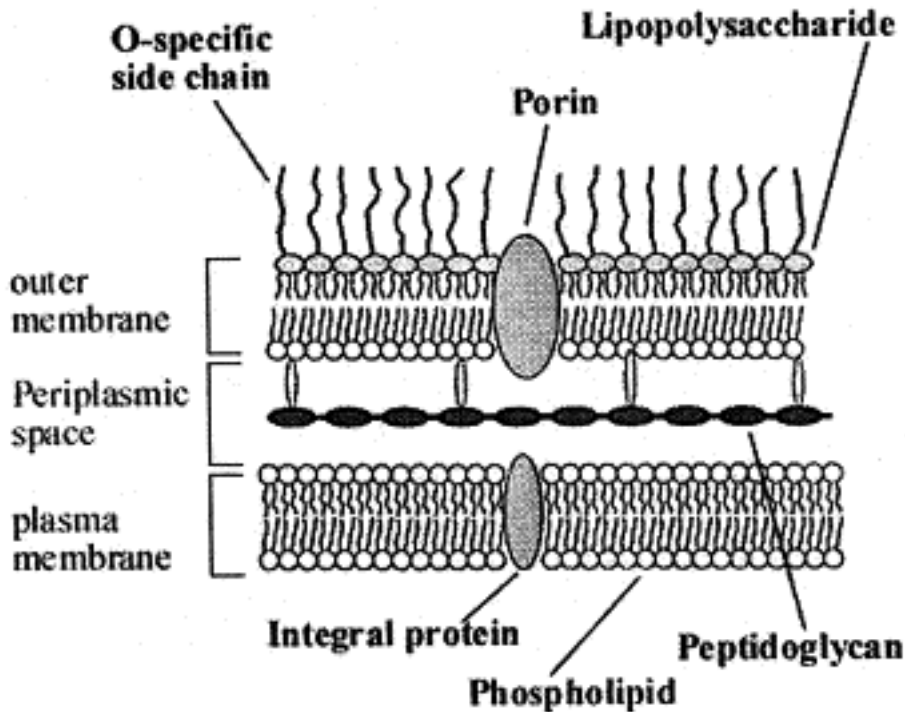
M
= Tetrapeptide side chain
(not cross linked)

M --- M = cross linked
tetrapeptide side chains

A schematic representation of the organization of the intact peptidoglycan sacculus of *E. coli*. G and M designate residues of N-acetylglucosamine and N-acetylmuramic acid, respectively, joined (diagonal lines) by β -1-4-glycosidic bonds. The vertical lines represent free tetrapeptide side chains, attached to muramic acid residues. The symbol (---) represents cross-linked tetrapeptide side chains.

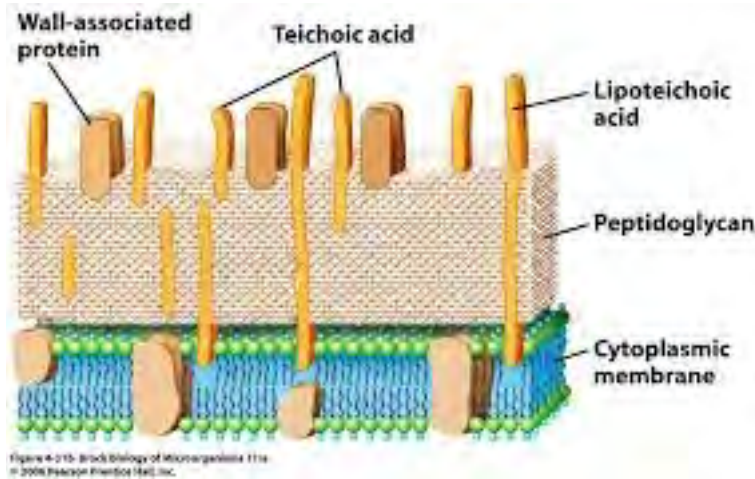
Differentiation of Gram Positive and Gram Negative Bacteria

- The cell wall and membrane structure of gram positive bacteria are much different in composition than those of gram negatives. (The diagnostic usefulness of this differential reaction in the gram stain was discovered long before its chemical basis was suspected. In addition, early observations showed that gram positive bacteria were more susceptible to disinfectants and antibiotics.)
- In addition to a thick layer of peptidoglycan, *Gram positive* (Staphylococcus, Streptococcus, etc.) walls are composed mostly of different carbohydrate polymers. Perhaps the key aspect is that there is no phospholipid outer membrane in the gram positive bacteria.
- For instance, a polymer of ribitol (you should remember this as a sugar alcohol) phosphate (a teichoic acid) in Staphylococcus or a polymer of other sugars in Streptococcus. The teichoic acids linked to the peptidoglycan are important antigens.
- The antibody response of the host seems mainly directed against these polymers, rather than against the peptidoglycan. Thus, these polymers probably are on the outermost surface of the cell, with the peptidoglycan underneath closest to the cytoplasmic membrane. Teichoic acids, for instance, may be slightly modified by the addition of an amino acid, and this will impart immunological specificity to the cell surface.
- *Gram negative*: the peptidoglycan (mucopeptide) layer is thinner.
- *Gram negative* organisms also have an outer membrane (OM). The outer surface of the OM bilayer contains lipopolysaccharide (LPS).
- As in gram positives, the peptidoglycan layer of the gram-negative cell wall is closest to the cytoplasmic membrane. Outside of the peptidoglycan, and attached to it by lipoprotein, is the important outer membrane, containing lipopolysaccharide. In summary, gram negatives have the inner cytoplasmic membrane surrounded by a thin layer of peptidoglycan, surrounded by the unique outer membrane.
- Thus, gram negative bacteria have a unique, 3 layered envelope (see figure on next page), with the outer membrane being unique to gram negative bacteria. A periplasmic space, containing some binding proteins and some digestive or hydrolytic enzymes occurs in gram negative, but not gram positive bacteria. This periplasmic space is located between the inner and the outer membrane.

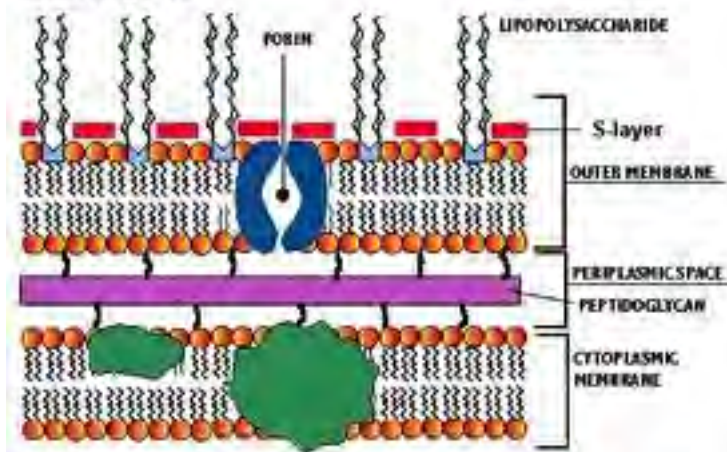


Schematic representation of the gram-negative cell envelope. The lower structure portrays the cytoplasmic membrane, a phospholipid bilayer in which various proteins, many of which span the membrane, are embedded. These membrane-spanning proteins may be involved in active transport mechanisms. Overlying this membrane is the peptidoglycan layer to which the outer membrane is bound through the helical murein lipoprotein. (Note the thin nature of this layer in gram-negative organisms.) Spanning the outer membrane are a number of proteins including the trimeric porins that allow entry of nutrients by passive diffusion (not by active transport). The outer membrane is shown with an inner leaflet of phospholipids and an outer leaflet of lipopolysaccharide (LPS) from which the polysaccharide chains extend in the medium. Note that the lipid A portion of LPS is imbedded at the base of this outer leaflet. The polysaccharide chains of LPS cover the entire surface.

Gram Positive



Gram Negative



Comparison of gram positive and gram negative surface layers.

Top. Diagrammatic representation of surface profile of a gram-positive organism shows thick peptidoglycan cell wall structure, with intertwined chains of teichoic acid emerging at the cell surface (only present in some species such as staphylococci). Organization of underlying cytoplasmic membrane shows bilayer phospholipids, transmembrane and inner and outer leaflet proteins, transmembrane proteins involved in active transport, and proton channel (the energy-transducing system). Bottom. Profile through the gram-negative envelope diagrammatically depicts lipopolysaccharides (LPS) of the outer leaflet of the outer membrane (OM) organized to form a bilayer with phospholipids and the porin proteins. (Note that lipid A of LPS is imbedded in the membrane, and the polysaccharides in LPS are outside of the membrane and cover the exterior of the cell.) These OM proteins form a pore allowing passage of small molecules (up to about 600-700 molecular weight). Other OM proteins are indicated; lipoprotein attached to the underlying peptidoglycan forms an anchor with OM lipids. Essential features of the cytoplasmic membrane are similar to those of gram-positive membrane structures.

- This outer membrane reacts with antibodies, and also blocks the entry of large molecules into the periplasmic space (although it doesn't contain the transport systems of the inner, cytoplasmic membrane). For instance, lysozyme cannot reach its site of action unless part of the lipopolysaccharide is removed from the outer membrane. The outer membrane serves as a barrier to antimicrobial agents and detergents. The outer membrane of gram negatives makes them generally more resistant to antibiotics, disinfectants and detergent-like molecules (i.e., bile salts in the intestine) than are gram positives. For example, while the antibiotic vancomycin, which targets the cell wall, works against Gram positive organisms this antibiotic is not effective versus Gram negative bacteria because it cannot cross the OM. Part of this barrier is due to matrix proteins known as "porins," which allow passage only of smaller molecules. Note that this is a non-specific entry of small molecules, in contrast to the active transport of the cytoplasmic membrane. **The assembled subunits of porin form a channel that limits passage of hydrophilic molecules across the outer membrane to molecules of less than M.W. 600-700. (See figure for a diagram of porins in the outer membrane.)** These porins are of medical importance since antibiotics may gain entrance to the periplasmic space through porins. Thus a change in porin structure (by mutation) could alter sensitivity of a gram-negative bacterium to antibiotics.
- The lipopolysaccharide located on the outer layer of the outer membrane is known as endotoxin since it is bound to the bacterial cell (hence "endo") and is toxic (causes shock and fever in gram negative sepsis). **It is composed of a polysaccharide (which is responsible for antigenic specificity) and lipid A (which confers the toxicity). Lipid A is composed of glucosamine linked to fatty acids and pyrophosphate (see figure). The lipopolysaccharide layer has many toxic biological effects, which will be discussed in a later lecture. (Although the chemical details in this figure are not to be learned, you should remember that the lipid A is imbedded within the lipid bilayer of the outer membrane.)**
- The polysaccharide is a polymer of many different monosaccharides. **The outer layer is composed of repeating oligosaccharide units, termed "O" specific region.** The sugars impart immunological specificity to the cell since they predominate at the very surface of the cell [remember this]. **For instance, the Salmonella can be divided into more than 500 different "serotypes."** A comparison of the lipopolysaccharides from different serotypes reveals minor differences in sugar composition, which is the basis of the immunological specificity. **These polysaccharides are called "O" antigens. This designation is from the German word "ohne" (without), since it was first recognized in strains *without* a characteristic colony morphology.**
- There is a "periplasmic space" in gram negative bacteria located between the cytoplasmic membrane and the outer membrane. Certain enzymes, especially degradative enzymes are located here (i.e., amylases which degrade starch). Such enzymes can digest large molecules in the external environment such as proteins or polysaccharides. This periplasmic space has added medical importance since enzymes that decompose antibiotics (for example, penicillinase) may be located in this space. In gram positive bacteria, there is no periplasmic space, and these bacteria excrete degradative enzymes into the extracellular environment or tether them to their cell wall.**



THE GRAM STAIN

Although the exact basis for the differential reactions of bacteria in the gram reaction is not known, there is a very good correlation between the gram reaction and many other important properties. For instance, gram negatives are characteristically more resistant to penicillin and detergents. Endotoxin, the substance responsible for shock and fever during blood infections, is only found in gram negative bacteria.

Procedure:

1. Stain cell with crystal violet, a basic dye.
2. Treat with iodine, which forms a water insoluble complex with crystal violet, inside of the bacterium.
3. Decolorize by a brief treatment with 70% ethanol. This is the differential step. Only gram negatives decolorize. Gram positives retain the dye-iodine complex and will appear blue after this step. (Note: Prolonged treatment with alcohol also decolorizes gram positives.)
4. Counterstain the decolorized (gram negative) cells with a red stain.

Some gram positive organisms lose their gram positive property with increasing age.

The term "gram-variable" is encountered for a few important species of bacteria.

The most likely mechanism of the gram stain is based on a permeability difference in the walls of the two types of bacteria. *Cell walls* of gram positives become largely *impermeable* to low molecular weight compounds *when the organisms are in 70% alcohol*. Perhaps it is the nature of the gram positive walls *after dehydration with alcohol* which *traps* the dye-iodine complex in the cytoplasm. (Alcohol may dehydrate the thick carbohydrate polymer (mucopeptide) and decrease pore size.) In gram negatives the dye-I₂ complex rapidly leaks out through the wall. This difference may be based on the fact that gram negative walls contain only a thin layer of mucopeptide polymer, combined with a large amount of the outer, lipid containing membrane, whereas gram positives contain mostly mucopeptides and other polysaccharides. It is probably the much higher mucopeptide (carbohydrate) content which retains the dye in the gram positive cell wall.

* If the cell wall is removed from a gram positive cell, it reacts as a gram negative in the staining procedure. NOTE: some gram positive bacteria tend to become gram negative when the culture is old. This loss in "gram positiveness" may be caused by loss of integrity of the cell wall through autolytic activity. This characteristic can lead to confusion in the clinical laboratory. For instance, a gram positive bacterium grown in culture for more than 30 hours may stain as gram negative. Take home message: ALWAYS USE FRESH CULTURES FOR GRAM STAINS.

5. *Capsule*: A non-essential secretion on cell surface. Usually polysaccharide in composition and thus different from cell wall. A capsule is hard to see since it doesn't stain in the gram stain. When present, it plays an important role in disease production.

- mutants of *Pneumococcus* which have lost ability to produce capsule are able to grow in culture media but have lost all pathogenicity. (Capsule plays a key role in resisting phagocytosis.)
- protective antibodies often directed mainly against capsular substance. (Some vaccines are capsule or contain capsule components.)
- many bacteria do not have capsules.
- the capsule layer may be a rather loose slime rather than a well-formed capsule. Capsular material can often be found in the cell-free filtrate of cultures or in infected body fluids, such as blood or cerebro-spinal fluid or urine. In fact, some capsular antigens are excreted from the blood into the urine. The presence of such capsular material in urine or spinal fluid is an important diagnostic test for infections caused by encapsulated bacteria (e.g., *Haemophilus influenzae*, *Streptococcus pneumoniae*)



6. *Flagella*: Organs of locomotion, 10-20 nm thick (much thinner than the cell itself): 8-12 μm long (much longer than the cell).

- chemical composition: composed of proteins (flagellins) - a long, helical filament composed of repeating protein (flagellin) subunits with a hollow tube down the middle
- since the diameter is below the resolving power of the light microscope, flagella can be seen only in the electron microscope or by special stains that deposit molecules to increase the diameter.
- the position of flagella (or singular flagellum) on the cell surface is a stable characteristic useful for identifying bacteria. For instance:
 - many flagella over whole surface of cell. These are called peritrichous flagella, although it is not necessary to remember this name.
 - originate from one end of cell only. These are called polar flagella.
- all motile bacteria contain flagella (with a few exceptions).
- not all bacteria are motile.
- motile bacteria may become non-motile in later stages of growth. (Motility ceases in energy-poor conditions, but cells will remain viable.)
- flagella propel the cell much like a propeller. The rigid flagella spin around their short axis.

7. *Pili (Fimbriae)*: filamentous, surface appendages, shorter than flagella. (Pili and fimbriae are very similar structures, but historically, pili were considered usually larger and in fewer numbers on the cell surface. However, the terms pili and fimbriae are now commonly used interchangeably), There are different types of pili, exhibiting slight differences in size. The terms pili and fimbriae are sometimes used interchangeably, except with respect to DNA transfer as described below. Most, but not all, bacteria of medical importance have pili.

- Function: not generally involved in motility (although there are some exceptions); function of pili is to provide a means for adherence to other cells, either bacterial or animal (see below). One type (F pili), is found only on "male" donor strains of bacteria and is necessary for bacterial conjugation which results in the transfer of DNA from one cell to another. The sex pili are responsible for bacterial attachment during conjugation. Some pili (known as type IV pili) can extend and retract and thereby push or pull bacteria across a surface (contrast this with the liquid "swimming" motility which requires flagella). This pili-mediate movement is known as "twitching motility" and may play a role during infection by allowing bacteria to form small clusters or "microcolonies" on host cells.
- molecular structure: hollow tubes (assembled by polymerization of presynthesized protein molecules).

Fimbriae (Pili) are an example of a class of surface structures that allow attachment of the bacterial cell to surfaces. They are important for bacterial survival within a host. For instance, urinary tract pathogens are richly piliated, and the virulence of *Gonococcus* seems correlated with formation of pili. Pili facilitate adherence to various epithelial cells. In fact, pili belong to a class of proteins that recognize and bind to specific sugar residues on polysaccharides found on the surface of mammalian cells.

The most virulent strains of *Gonococci* produce fimbriae (pili) that bind to specific receptors of cervical epithelial cells, and thereby prevent these bacteria (the cause of gonorrhoea) from being washed out by bulk flow. Thus the gonococcal pilus is an important virulence factor for gonorrhoea.

(Important Note: There are other surface components that permit bacteria to adhere to specific tissue cells. In gram positive bacteria, for example, these may be carbohydrate polymers located on the surface of the cell wall. These are called *glycocalyx* or slime layer. These allow gram positive bacteria to adhere firmly to structures such as heart valves and intravenous catheters, and also to the surface of teeth, forming dental plaque). A variety of proteins localized to the outer membrane of gram negative organisms or the cell wall of gram positive organisms can also serve as adhesins.

8. Spores: only found in a few species of bacteria (the large gram positive rods in the genera *Bacillus* and *Clostridia*). During the life cycle of these bacteria, nutrient deprivation (and other signals) trigger the formation of one spore from each bacterium. Sporulation occurs after the rapid (logarithmic) phase of growth is finished, and is a response to nutritional deprivation.
- the spore is a highly differentiated structure. The spore is produced within the bacterial cell (endospore). Certain chemicals are found only in the spore and are not made by actively growing bacteria. (Dipicolinic acid is an example). Many other spore components are also quite different from those found in the actively growing bacterial cell.
 - spores are dormant (resting state) and very resistant to drying, heat and chemicals. Unlike other cells, many bacterial spores can survive boiling and must be heated to at least 121°C (steam under pressure) to be killed (moist heat).
 - the chemical basis for this extreme resistance is not understood. The low water content within the spore may explain heat resistance. A keratin-like proteinaceous outer coat, which is not present in the actively growing bacterium may explain resistance of the spore to killing by disinfectants which readily kill vegetative cells.
 - spores are also resistant to staining. For instance, they will remain colorless in the gram staining procedure.
 - under appropriate conditions, a spore will "germinate," giving rise to one bacterial cell. The germination occurs in response to a specific trigger, such as the presence of a specific nutrient. Germination is accompanied by loss of heat resistance, a swelling, and an uptake of water. The spore coat then ruptures and a vegetative cell grows out. (The vegetative form is the term used to describe the reproductive, or non-spore form.)
 - it is important to remember that a cell which is capable of forming a spore will only do so under some conditions. Spores are rarely seen in very young cultures. Some *Clostridia*, such as the tetanus organism, readily form spores in older cultures. Other *Clostridia*, such as the gangrene organism, very rarely form spores.
 - spores can survive for a long period in soil. Tetanus spores survive and germinate when deposited in deep wounds causing tetanus.
 - *Bacillus anthracis*: spores are likely required for survival in the environment, but unfortunately, they also serve as a bioterror agent. The spores are stable for years, are powder-like in consistency and germinate in the warm, wet nutrient rich environment of the lung (causing inhalation anthrax).
 - the position and size of the round spore in the rod-shaped cell is characteristic for different species of bacteria. (The spore may be located centrally or at one end of the cell. The spore may have a larger diameter than that of rod that produced it, or the spore may fit within the vegetative cell. A spore may be round or oval in shape. These shapes are very useful in identifying different species.)

CHEMOTAXIS

- Motile bacteria may exhibit chemotaxis. Various sugars and amino acids present in the growth medium serve as "attractants" (positive chemotaxis) while other substances (phenols, acids) serve as repellants (negative chemotaxis).
- Bacteria possess specific sensory chemoreceptors ("tasters") in the membrane that control a phospho-cascade which in turn controls the direction of flagellar rotation.
- Net movement is controlled by the direction in which the flagellum is rotating. Thus, when rotation is counter-clockwise, the bacteria travel in a straight line, but if the direction of flagellar rotation is reversed, the organism will tumble in place. When moving up a gradient of "attractant," the straight line movements last longer than the tumbles. When moving away from an attractant or toward a repellent, the tumbling occurs frequently until the net movement is properly redirected.

BACTERIAL GROWTH

Learning objectives

- To describe the roles and sources of carbon, nitrogen and inorganic ions for bacterial growth, and distinguish their roles from those of growth factors.
- To describe the physical requirements for bacterial growth or inhibition of growth
- To describe how growth can be measured by colony formation (plate count) and by various physical or chemical measurements.
- To describe the phases of the bacterial growth curve and the relationship between growth rate and disease.

Reading assignment: Levinson, Chapter 3

Disease occurs when bacteria or fungi grow (and survive) where they should not be.

- Bacterial growth is essential for infections of different body sites because of disruption of physical barriers (such as surgical sites or burns), immune dysfunction, or other host perturbations (antibiotics).
- The control of bacterial growth is key in control of food, water, and drug supplies.
- Impacts almost every medical specialty.

1. Bacterial and fungi can grow very quickly.

The “**generation time**” is often used to describe the growth rate. The generation time is the time required for the population to double.

The maximal generation time varies for various organisms. It may be as short as 20 minutes for some rapidly growing pathogens, and as long as 20 hours for others, such as *Mycobacterium tuberculosis*.

Many pathogens have generation times of less than 30 minutes, thus their populations can quickly become very large. One bacterium with a 30 min generation time will grow overnight (12 hrs) into more than one billion cells. After less than three days of exponential growth (with an unlimited supply of nutrients), this culture would weigh as much as planet earth!

Luckily, growth is limited by:

- Exhausted nutrient supplies or key resources
- Accumulation of toxic metabolic products
- Antibiotics from neighboring microbes (or humans)
- Immune system
- Environmental conditions

What must happen for growth to occur? New synthesis of many cellular components.

2. Growth Requirements:

A. Major Nutrient Requirements for Bacterial Growth:

Carbon-to make all molecules

Nitrogen-proteins and DNA are nitrogen-rich compounds

Phosphorus is in membrane phospholipids and DNA

Sulfur-in proteins

Iron-in many enzymes, especially those involved in metabolism.

1. Major nutrient sources:

Glucose (C₆H₁₂O₆) (4-6 mM in serum). Almost all pathogenic bacteria can grow on glucose.

Other sugars

Proteins, peptides, and amino acids

Lipids

Organic acids and alcohols

Many nutrient sources serve as both an energy source and a source of carbon for the synthesis of all cellular components.

For example, in the presence of O₂ many bacteria can oxidize about 50% of the glucose to CO₂ and water, and this produces enough energy to convert the remaining 50% of the glucose to cell material.

Many bacteria can also use a wide variety of organic compounds (amino acids, DNA, lipids, other sugars), depending upon what degradative enzymes they have.

The carbon sources that a bacterium use can be diagnostic (see *Neisseria* as an example).

Amino acids peptides and proteins are excellent sources of C, N, and S.

Bacteria need large amounts of nitrogen to synthesize proteins and nucleic acids.

Many pathogenic bacteria require specific, pre-formed amino acids.

Amino acids and peptides can be taken up by bacteria, proteins cannot (too big). Thus, many pathogens secrete proteases.

Ammonia (NH₄⁺) and nitrate are also possible nitrogen sources.

Microbes secrete nucleases to break down available DNA and RNA.

Microbes can take up nucleotides that can be used as C, N, and P sources, or for their own nucleic acid synthesis.

Microbes may produce phospholipases that act on host cell membranes or lung surfactant.

Degradation of phospholipids from host cell membranes provides microbes with C, N, P


Low phosphorus or low iron levels can induce phospholipase production

Host cell lysis by phospholipase activity yields iron

Iron (Fe) plays an important role in host-pathogen relationships. The host makes iron-binding factors that make iron unavailable to microbial invaders. Bacteria and fungi make iron chelators (siderophores) that can extract iron from host reserves.

2. Pathogenesis often results from microbes accessing host nutrients. Microflora organisms are "provided for" by the host.

B. Growth factors:

Growth factors are organic (carbon) compounds that are not metabolized to supply energy but are used to make metabolites that the bacterium cannot synthesize themselves. 

While *E. coli* needs no growth factors and can grow on glucose, NH_4^+ , and inorganic ions, many host-associated microbes require one or more specific growth factors in addition to the carbon source.

Different organisms require different growth factors. Some are “fastidious”.

The requirement for growth factors can be diagnostic. (See *Haemophilus* as an example.)

C. Physical requirements for bacterial growth:

Growth rates (generation times) are affected by the environment.

Temperature: For each organism there is an optimal temperature for growth and a range of temperatures at which growth will occur.

Most pathogens usually grow best at 37°C , body temperature. Environmental bacteria often grow better at room temperature (22 to 30°C).

An interesting exception is *Mycobacterium leprae*: growth *in vivo* is poor at 37°C (core body temperature). This is reflected by the distribution of lesions in clinical cases of leprosy. Skin shows obvious lesions but internal organs are not usually involved. Lab animals are not susceptible to *M. leprae* unless inoculated in the footpad, a site of reduced body temperature.

Some bacteria (*Legionella*) are capable of growing at high temperatures (near 45°C).

A good refrigerator (below $40^\circ\text{F}/4^\circ\text{C}$) will prevent growth of most pathogens. However, *Listeria*, a causative agent of food poisoning, can grow at or below 4°C .

pH affects the growth rate of bacteria and (as for temperature) there exists a range and an optimum for each species. For pathogens, this is usually pH 6.0 to 8.0, with an optimum at 7.4.

Clostridium botulinum (which produces the botulinum toxin that causes botulism) is more of a problem in canned foods with less acidity.

Osmotic conditions:

Very high salt and sugar concentrations (sauerkraut, jams, and jellies) inhibit growth of many pathogens.

Most bacteria do not need to regulate their internal osmolarity with precision because they are enclosed by a cell wall capable of withstanding a considerable internal osmotic pressure. Contrast this to the red blood cell, which will burst if placed in distilled water, and must be maintained in physiological saline (0.85% NaCl). The osmotic strength of a medium can be adjusted by addition of ions such as NaCl or non-metabolized sugars such as sucrose.

3. Growth of bacteria in the lab:

A. Growth medium



Common medium: peptone broth with glucose added.

Peptone is a peptic digest of meat (digested with pepsin) containing peptides and amino acids. The addition of glucose serves as an additional source of energy.

Blood agar supports the growth of ~90% of pathogens.

To obtain pure cultures of bacteria, and to observe colonial morphology, bacteria must be grown in solid medium. This is accomplished by adding agar, an indigestible polymer, to liquid media. Agar melts at 100°C, and solidifies at 45°C. Molten and sterile media are poured into "Petri" plates above 45°C and allowed to solidify at room temperature before use. You will work with these plates in the laboratory.

Agar medium is most useful when you can get isolated colonies.

B. Measurement of bacteria:

- Knowing the size of a bacterial population can help you determine if there is an infection.
 - In some clinical samples, there should be no bacteria (CSF).
 - In others (urine), you always expect to find some organisms, so levels matter.
- Antibiotic susceptibility testing needs to be done with cultures at a known density to be accurate.
- Measurement of bacteria is important in research.

1. Optical measurements of cell mass

- Determined by the amount of light scatter by a suspension of cells; small particles scatter light in proportion to their concentration.
- The reduction in the amount of light transmitted as a result of light scattering is a measurement of cell density.
- Increased turbidity can be measured using a spectrophotometer.
- Low cell densities cannot be detected, the limit of detection is ~1 million bacteria per ml.
- Bacterial cultures become faintly turbid to the naked eye at about 1×10^6 bacteria per ml. and are very turbid at 1×10^8 bacteria per ml.
- For a given organism, a robust correlation between the amount of scattered light and cell number can be established.

2. Determination of metabolic activity is a sensitive method in current clinical use (production of metabolic products such as CO₂ or ATP). This is a sensitive method for detecting bacteremia.

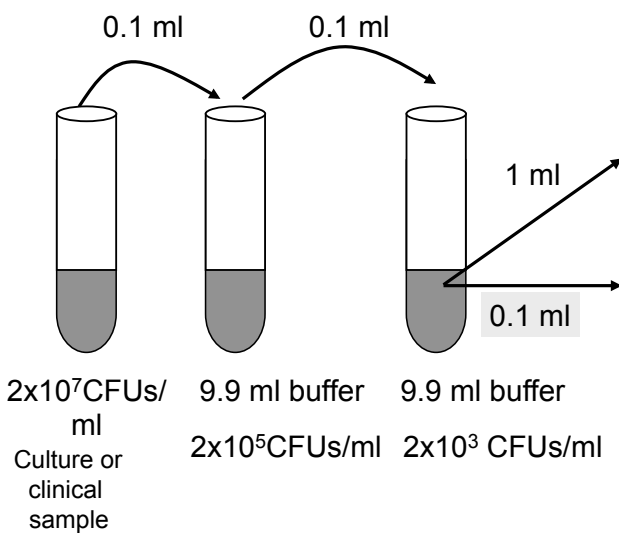
3. Direct measurement of cell number:

- Direct counting in a counting chamber using a microscope.
 - Includes both viable and nonviable cells.
 - Infrequently used since high concentrations of bacteria are required (10^5 /ml).
 - Note that if any bacteria are seen microscopically in a clinical sample, it indicates a high level of infection. Therefore, if even only a few bacteria are seen microscopically in an unconcentrated urine sample, this suggests more than 100,000 (10^5) bacteria per ml and indicates infection.

4. Viable or Plate counts:

- A single bacterium can give rise to a macroscopic colony containing millions of bacteria on a nutrient agar petri dish after 24 hours incubation.
- The number of viable cells in a sample is determined by plating the sample and counting the number of colonies on the plate the next day. Because every bacterium might not give rise to a colony, the number is expressed as colony forming units.
- In most cases, the sample is serially diluted and plated on a nutrient medium. After counting the number of colonies that arise from plating the diluted sample, one can calculate the number of viable cells that were in the undiluted sample.

5. Sample dilution scheme for viable counts and determination of CFUs/ml:



To calculate the number of bacteria in the starting sample,

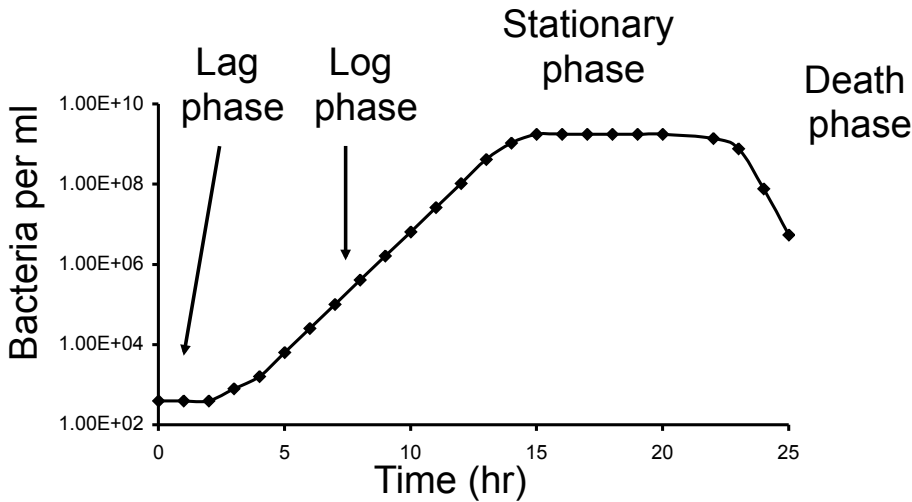
Divide the number colonies on the plate, by the volume plated. Then divide the result by the dilution factor.

$$200 \text{ colonies} \div 0.1 \text{ ml} = 2000 \text{ (} 2 \times 10^3 \text{) CFUs per ml}$$

$$2 \times 10^3 \div 10^{-4} = 2 \times 10^7 \text{ CFUs/ml}$$

C. Phases of growth in the bacterial growth curve:

The growth of a bacterial culture is usually portrayed by plotting the number (or mass) of cells on a logarithmic scale as a function of time (see figure). A growth curve contains four phases.



a. Lag Phase (Metabolic activity, but no increase in numbers)

- When bacteria are inoculated into fresh medium, reproduction does not usually begin immediately.
- During the lag phase, cell mass and size begin to increase before cell numbers increase and the synthesis of macromolecules (RNAs, proteins, etc.) needed for growth in the new medium occurs.
- The length of the lag phase depends on the kind of bacteria, the age and size of the inoculum, the nature of the medium from which they were taken, and the nutrients present in the new medium.

b. Log or Exponential Growth Phase

- During the exponential growth phase, cell numbers increase in a logarithmic manner with a constant generation time. Each cell generation results in a doubling of the population. Most bacteria reproduce by binary fission.
- Both cell number and mass increase in a co-ordinate manner during log phase, and measurements of either parameter can be used to determine the generation time.
- The rate of cell division is dependent on the type of organism, the nature of the medium, the temperature, and, for aerobic organisms, the rate of aeration.
- *Definition of balanced growth:* An orderly increase of all cellular components. In *balanced growth*, a doubling of biomass is accompanied by a doubling of all other components (i.e. protein, RNA and DNA). After doubling in size, each cell divides, yielding two identical cells.

Exponential phase growth rates of *Salmonella typhimurium* in different growth media at 37 C

Growth medium	Growth rate (Doubling time (min))
Brain-heart infusion (an extract of beef brain and heart)	15
Amino acid mix (no vitamins or growth factors)	23
Minimal medium	35

c. Stationary Phase

- Logarithmic growth eventually slows because of accumulation of waste products, exhaustion of nutrients, change in pH, or a decrease in oxygen tension. The population then enters the stationary phase in which the number of viable cells remains about constant.
- Usually, there is a steady state in which some cells die and others continue to divide. Entry into the stationary phase or even starvation for a required nutrient need not result in the killing of most bacteria. This phase may last for only a few hours, but in some cases can last for days, months or years depending on the species and conditions.
- *Almost all pathogenic bacteria will grow to a concentration of between 5×10^8 to 1×10^9 bacteria per ml at the stationary phase when grown in a test tube in a lab.*

d. Death Phase

- Eventually the rate of death exceeds the rate of reproduction, and the number of viable cells declines.
- The length of time before all cells have died differs markedly for various organisms. Some species have a very short death phase, whereas others may take weeks or even years before all the cells in the culture have died.
- During this phase, cells often assume unusual shapes, making it difficult to recognize bacteria in old cultures.

e. Growth phases and disease (Adapted from Trends in Microbiology 6:239-243 by H. Smith)

Lag phase: Adaptation to the host environment

Exponential phase:

Rapid growth of small numbers to replace the losses to host defenses

Slow growth in log phase may occur in chronic disease states

Rapid growth in tissues produces the effects of disease before host defenses mount

Little is known about growth rates during infection. Increases or decreases in bacterial populations can be measured, but these will represent the net results of multiplication and destruction by host defenses.

Stationary phase: Increased resistance to host defenses and antibiotics
Latent infections

Death phase: Growth rate is lower than the death or clearance rate.

4. Review-Why study growth characteristics of a microbe?

- Identify ways to kill or inhibit microbial growth
- To better understand disease
- To identify and quantify a microbe in the clinical laboratory and research lab

BACTERIAL METABOLISM


Learning objectives:

- To compare aerobic respiration, and fermentation
 - To understand the how different fermentation products impact host-microbe interactions
 - To explain the biochemical basis for obligate aerobes, facultative anaerobes, aerotolerant anaerobes, obligate anaerobes, and microaerophilic bacteria.
 - To explain where anaerobes reside in the host.
 - To understand the importance of bacterial metabolism in diagnostics.
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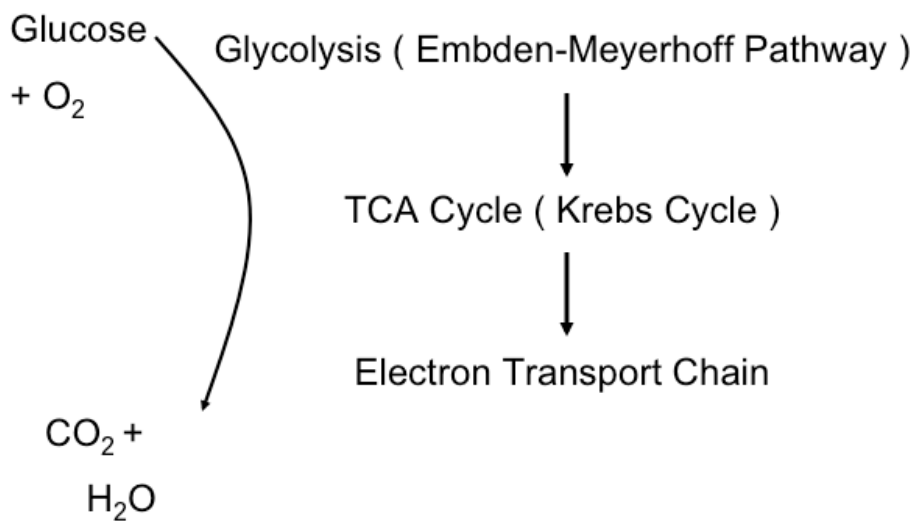
Reading assignment: Levinson, Chapter 3

1. BASIC PRINCIPLES OF BACTERIAL METABOLISM:

In general, bacterial cells have the same metabolic activities as eukaryotic cells.

Although bacteria can use a number of different organic carbon sources, we will use glucose as the growth substrate or carbon source in our discussion of energy generation via respiration and fermentation. 

Overview of respiration

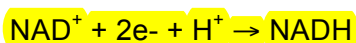


A. Glycolysis (Embden-Meyerhof Pathway)

In glycolysis, glucose is oxidized to pyruvate.

The transfer of electrons FROM the glucose allows the bacterium to produce ATP via direct **substrate level phosphorylation**.

Other electrons from the oxidized substrate are never free in solution, but rather are transferred to NAD⁺ (nicotinamide adenine dinucleotide) to generate the reduced form of the molecule called NADH.



During glycolysis, 2 moles of ATP are consumed and 4 moles of ATP are produced for every mole of glucose. One equivalent of NADH is formed from NAD^+ .

B. Tricarboxylic acid cycle

The **Tricarboxylic acid cycle** liberates reducing equivalents (H which equals a proton and an electron), that drive ATP formation via oxidative phosphorylation.

The **glyoxylate shunt** functions similarly to the Krebs cycle but lacks many of the Krebs cycle enzyme reactions. The glyoxylate cycle consumes acetylCoA which is generated from acetate. Acetate is a major product when microbes grow on fatty acids. (This pathway is generally absent in mammals.)

C. Electron transport chain

NADH donates electrons to a respiratory chain (a series of electron carriers such as cytochromes located in the inner membrane of the bacteria cell).

Transfer of electrons through the electron transport chain converts the energy of the electrons into a proton gradient. This proton gradient, in turn, is used to generate ATP via an ATPase in the inner membrane.

In aerobic respiration, the terminal electron acceptor is O_2 . $\text{O}_2 + 2\text{e}^-$ yields H_2O .

While mammalian cells can only respire with oxygen as the final (terminal) electron acceptor, some bacteria can respire **anaerobically** by using a terminal electron acceptor other than oxygen (for example, NO_3^- which can be reduced to N_2).

The production of ATP by this process is known as **oxidative phosphorylation**. In mammalian cells, this occurs in mitochondria, in bacteria this occurs across the cytoplasmic membrane. Mitochondria very likely evolved from an endosymbiotic bacterium.

D. Fermentation

If there is a lack of oxygen causing the ETC and TCA cycle to “back up,” or if an organism lacks TCA cycle enzymes, some of pyruvic acid formed from glucose during glycolysis is reduced using NADH. This regenerates NAD⁺.

The resulting fermentation product is excreted.

ATP is made directly via the transfer of a high-energy phosphate bond to ADP. No respiratory chain is involved.

The direct synthesis of ATP during fermentation via **substrate-level phosphorylation** only.

Aerobic respiration yields much more energy per mole of glucose than fermentation does.

Aerobic respiration: $C_6H_{12}O_6$ (glucose) + $6O_2 \rightarrow 6 CO_2 + 6 H_2O$ ($-\Delta F = 688,000$ cal).

Fermentation: $C_6H_{12}O_6 \rightarrow 2 C_3H_6O_3$ (lactic acid) ($-\Delta F = 58,000$ cal).

While most ATP in eukaryotic cells is made via respiration, fermentation is an important way for many bacterial pathogens to gain energy in the absence of oxygen.

In all cases, the fermentation product is reduced compared to pyruvate and the electrons for this reduction were those accumulated during the oxidation of the growth substrate (glucose).

Lactic acid fermentation is the most simple fermentation pattern, featuring the direct reduction of pyruvic acid by NADH and the enzyme lactic dehydrogenase. (Lactate dehydrogenase is also present in human muscle tissue.)

A comparison of respiration and fermentation

Respiration	Fermentation
During glycolysis, glucose is oxidized to form pyruvate, generating NADH and a small amount of ATP.	During glycolysis, glucose is oxidized to form pyruvate, generating NADH and a small amount of ATP.
NADH donates its electrons to the electron transport chain in the cytoplasmic membrane. This regenerates NAD ⁺ and the transfer of electrons allows for the generation of additional ATP.	NADH donates its electrons to a carbon-based substrate, often pyruvate, to generate a fermentation product that is excreted. While NAD ⁺ is once again regenerated, no additional ATP is made in this step.
A large amount of ATP is generated via oxidative phosphorylation.	ATP is made by substrate level phosphorylation.
A large portion of the carbon from the growth substrate appears as either CO ₂ or is assimilated into cellular material. The growth substrate is nearly completely oxidized.	Much of the carbon from the growth substrate appears as reduced fermentation products in order to regenerate NAD ⁺ .
O ₂ is reduced to water during aerobic respiration. Some bacteria have specialized electron transport chains that can use other electron acceptors (such as nitrate, which is reduced to N ₂).	The fermentation products that bacterial species accumulate are characteristic.

E. How does bacterial metabolism affect humans?

1. Lactic acid production.

The resulting low pH discourages growth of competing microbes.

Lactobacilli live in very high numbers in the vaginal tract and in the intestine. *Lactobacillus* spp. are commensal organisms. Their production of lactic acid creates a low pH environment that is very important for health in places including the vagina. Lactobacilli are thought to be particularly important for protecting against *Candida albicans* (yeast infections).

Pre-puberty and post-menopausal women don't have Lactobacilli in the vagina and the pH is neutral.

Some species of lactic acid-producing bacteria are important in fermenting foods, such as sauerkraut, yogurt, and certain cheeses.

The Streptococci such *Streptococcus pyogenes* as those that cause strep throat use this pathway.



2. Butyric acid fermentations, as well as the **butanol-acetone** fermentation, are run by the clostridia, the masters of fermentation. In addition to butyric acid, the clostridia form acetic acid, CO₂ and H₂ from the fermentation of sugars. Small amounts of ethanol and isopropanol may also be formed. If we detect hydrogen gas or butyric acid in tissue, we know that *Clostridium* is present.

Butyric acid has a very distinctive (and unpleasant) odor, but in animal models butyric acid produced by intestinal microbes such as *Clostridium butyricum* are protective against intestinal pathogens such as the enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 has been considered as an agent responsible for outbreak of hemorrhagic colitis and the hemolytic uremic syndrome.

3. Propionic acid fermentation. This is an unusual fermentation carried out by the propionic acid bacteria which include corynebacteria, *Propionibacterium* and *Bifidobacterium*. Propionic acid bacteria will ferment lactate (the end product of lactic acid fermentation) to acetic acid, CO₂ and propionic acid.

The propionic acid bacteria are used in the manufacture of Swiss cheese, which is distinguished by the distinct flavor of propionate and acetate, and holes caused by entrapment of CO₂.

Propionibacterium acnes are found in the deeper follicles in the dermis and is implicated in the pathogenesis of acne. Propionic and butyric acid are thought to keep other microbes from growing in these niches.

4. Mixed Acid Fermentations by enteric microbes. Mainly the pathway of the *Enterobacteriaceae*, a medically important group of Gram-negative rods including *E. coli* and *Salmonella*. In this fermentation, pyruvic acid is converted to a two carbon compound (acetyl-CoA) and to formic acid (HCOOH).

The formic acid can then be converted to carbon dioxide and hydrogen gas, if the appropriate enzyme is present. *Salmonella* spp. contain this enzyme and form hydrogen gas, but a related pathogen, *Shigella*, lacks this enzyme and accumulates formic acid instead of gas. This property is used to differentiate between these two intestinal pathogens.)

The hydrogen gas formed by this fermentation is a uniquely bacterial product never produced by mammalian cells. Since it is an insoluble gas, it is readily detected in tissues during infections by these bacteria. Gas gangrene results from bacterial production of gas.

The remainder of the products formed during the mixed acid fermentation is derived from acetyl CoA. Some of this is converted to ethanol, some to acetic acid, and some to succinic acid. Thus, a mixture of acid fermentation products accumulate.

5. Some bacteria form **2,3 butanediol** and its distinctive intermediate, **acetoin**, along with the mixed acids and gases described above. The use of the pathway decreases acid formation (butanediol is neutral).

Water microbiologists have specific tests to detect low acid and acetoin in order to distinguish non-fecal enteric bacteria (butanediol formers, such as *Klebsiella* and *Enterobacter*) from fecal enterics (mixed acid fermenters, such as *E. coli*, *Salmonella* and *Shigella*).

6. **Ethanol fermentation** is performed by yeasts including *Saccharomyces cerevisiae* (baker's and brewer's yeast) and the human pathogenic fungus *Candida albicans*. In this fermentation, pyruvic acid is converted to CO₂ and ethanol using NADH as reductant and the enzyme alcohol dehydrogenase. At slightly acidic pH, this carbon dioxide appears as the bubbles in fermented beverages. Note that the carbon dioxide produced in this fermentation appears as a gas at low pH, but would appear as non-gaseous carbonate if the pH were alkaline as in some tissue infections.

7. **Stickland Reaction.** Some *Clostridia* (associated with wound infection) carry out a specialized fermentation in which energy is generated by fermentation of pairs of amino acids. One amino acid serves as electron donor, the other as electron acceptor. This fermentation, which occurs in putrefactive wounds, produce decarboxylated derivatives of amino acids, which are often volatile and malodorous. These products are responsible for the extremely foul odor associated with some anaerobic infections.

Fermentation products also create an environment that is harmful to competing microbes due to the production of acids or alcohols. Bacteria are very diverse and have involved many clever ways to get rid of extra electrons.

2. RELATIONSHIPS WITH OXYGEN:

The relationship that microbes have with oxygen is linked to the organism's metabolism (respiratory or fermentative) and their ability to detoxify oxygen radicals.

Bacteria can be classified in five different categories based on their relationship to oxygen:

- A. **Obligate aerobes:** O₂ is absolutely required for growth of these organisms. They do not have the enzymes necessary for fermentation and thus gain all of their energy through respiration via the electron transport chain.

**Some bacteria, such as *Pseudomonas aeruginosa*, are referred to as obligate aerobes in clinical microbiology texts because they do not ferment sugars. They can, however, respire in the absence

of oxygen. For example, *P. aeruginosa* can grow via nitrate (NO_3^-) respiration even in the absence of oxygen. When a substance other than oxygen is used as an electron acceptor, this may be termed anaerobic respiration.

Mycobacterium tuberculosis, an obligate aerobe, resides in upper lobes of the lung which have the highest O_2 levels.

B. **Facultative anaerobes** exhibit growth under both aerobic and anaerobic conditions. These contain a functional respiratory system, and also have fermentative capacity. Because more ATP is generated during respiration, they exhibit better growth aerobically than anaerobically.

Many pathogens fall into this category. Key examples include *E. coli* and *Staphylococcus* spp. These organisms may exhibit both modes of growth in the same site.

C. **Obligate anaerobes.**

Many obligate anaerobes can gain energy only from fermentation mechanisms, and do not possess cytochromes or an electron transport chain.

Examples include *Bacteroides fragilis* (found in the largely anaerobic intestine and anoxic abscesses).

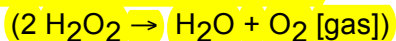
Many obligate anaerobes cannot grow in the presence of oxygen and require reducing conditions for growth.

-Production of toxic H_2O_2 (hydrogen peroxide) upon exposure to oxygen.

Many anaerobes contain respiratory-like enzymes containing flavins (flavins are electron carriers) that produce peroxide (H_2O_2) when O_2 is around. Reactive oxygen species (ROS) from H_2O_2 can damage DNA and proteins.

Many enzymes in anaerobes are inhibited or inactivated by oxygen and reactive oxygen species (superoxide, peroxide).

Obligate anaerobes lack **catalase** which is a heme-containing enzyme which decomposes H_2O_2 . Most aerobes contain catalase.



Anaerobes also lack the enzyme called "superoxide dismutase." Superoxide is a highly reactive free radical reactive form of oxygen (O_2^-) formed by flavoenzymes. Superoxide dismutase breaks down superoxide by the following reaction ($2 \text{O}_2^- + 2 \text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$).

D. **Aerotolerant anaerobes:** No functional respiratory system capable of oxidative phosphorylation, so they grow exclusively via fermentation, but these bacteria are indifferent to oxygen and thus can grow in air.

The lactic acid bacteria (*Lactobacillus* and *Streptococcus* species) are aerotolerant anaerobes.

Some *Clostridium* spp. are found in the anaerobic environment of the intestine while others such as *Clostridium tetani* (which grows in deep anoxic puncture wounds) and *Clostridium botulinum* (which

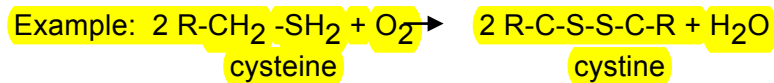
can grow in canned goods) are soil bugs. These organisms can survive oxygen exposure but only grow robustly in anaerobic conditions.

E. **Microaerophiles:** an organism that requires or will tolerate oxygen only if it is at a lower concentration than is found in air (20% oxygen). For instance a microaerophilic organism might grow in an atmosphere containing 5% oxygen. (A key example is *Campylobacter jejuni*. Special growth conditions are necessary to culture this organism.)

F. **Where do the anaerobes exist within the host?**

Anaerobic conditions exist in mouth and GI tract (at least in some regions or niches). Anaerobes can grow in surface tissue we usually think of as bathed in air. This occurs because the other bacteria (aerobic or facultative) present or the host tissues are using up the oxygen and allowing the anaerobes to grow.

Anaerobes can grow in the presence of some oxygen if reducing agents (cysteine, thioglycolic acid) are present in the growth medium. These reducing agents remove oxygen from the growth medium by chemical reaction.



3. MICROBIAL GROWTH SUBSTRATES OTHER THAN GLUCOSE:

Most pathogenic bacteria are more constrained in what they can use as energy sources; not surprisingly, bacterial pathogens use those nutrients that are readily available in the host such as carbohydrates, proteins and lipids.

A. Carbohydrates:

- Glucose is a growth substrate of most microbial pathogens.
- Some pathogens can use other carbohydrates and this substrate utilization pattern is characteristic. Enteric organisms that reside in the intestine are generally good at using carbohydrates.

	Glucose fermentation	Lactose fermentation
<i>Escherichia coli</i>	+	+ (fast)
<i>Klebsiella</i>	+	+ (fast)
<i>Salmonella</i>	+	-
<i>Shigella</i>	+	-
<i>Citrobacter</i>	+	(slow)
<i>Pseudomonas</i>	-	-

- Glucose is a monosaccharide while lactose is a disaccharide that requires cleavage by a galactosidase prior to catabolism. Organisms that cannot ferment lactose lack this enzyme.
- pH indicators and gas detection systems can allow one to easily determine if a strain can ferment a particular substrate.

Why know about bacterial metabolism?

- Properties of bacterial metabolism are exploited as the primary means of identifying microbes.
- Knowing how microbes grow provides insight into which microbe may be causing disease.
- Metabolism may affect antibiotic susceptibility and therapies may target metabolic processes.

Bacterial Genetics

Learning Objectives:

- 1) To explain the relationships between types of mutation, genotypes and phenotypes
- 2) To explain that mutations are spontaneous and not induced by selective pressure
- 3) To explain the two basic genetic mechanisms that generate antibiotic resistance (chromosomal mutation versus acquisition by transfer)
- 4) To explain the three types of genetic recombination
- 5) To explain the three types of bacterial genetic transfer; how they occur and how they can modulate antibiotic resistance profiles and evolution of pathogenic bacteria by acquisition of virulence traits
- 6) To explain the properties and lifestyles of different representative phage
- 7) To describe the general properties of transposons and how they spread antibiotic resistance
- 8) To explain the differences between generalized and specialized transduction
- 9) To explain the relationships between F, F', and Hfr strains
- 10) To describe the similarities and differences between an F' and a resistance transfer factor

1. Mutation (Brief review from Biochemistry Course)

A. Phenotype and Genotype

1. Mutation is any change in the base sequence of DNA. Genotype is the exact nucleotide sequence of the genome. Mutations are changes in genotype.
2. Wild type is the designation of the genotype of an organism found in the wild.
3. Phenotype refers to the observable characteristics of an organism.

B. Types of mutations

1. Point mutations are single base changes.
 - a. Transitions - a purine is replaced by a purine, or pyrimidine is replaced by pyrimidine (i.e. A to G; or C to T).
 - b. Transversions - a purine is replaced by a pyrimidine, or vice versa.
 - c. A point mutation in a codon of a gene may change it to another resulting in an amino acid change (missense mutation).
 - d. A point mutation in a codon may change it to a stop codon (nonsense mutation) resulting in a shortened protein.
2. Deletion - the removal of one or more nucleotides.

3. Insertion - the addition of one or more nucleotides.
4. Frameshift - a shift in reading frame caused by an insertion or deletion of nucleotides.

met phe ser cys thr pro val
 AUG UUU UCU UGU ACU CCA GUG... wild type

met phe ser cys asp ser ser
 AUG UUU UCU UGU (G AC)(U CC)(A GU)G... +1 frame shift

5. A mutation does not always give rise to a change in phenotype. Such mutations are referred to as silent mutations.

Why are some mutations silent?

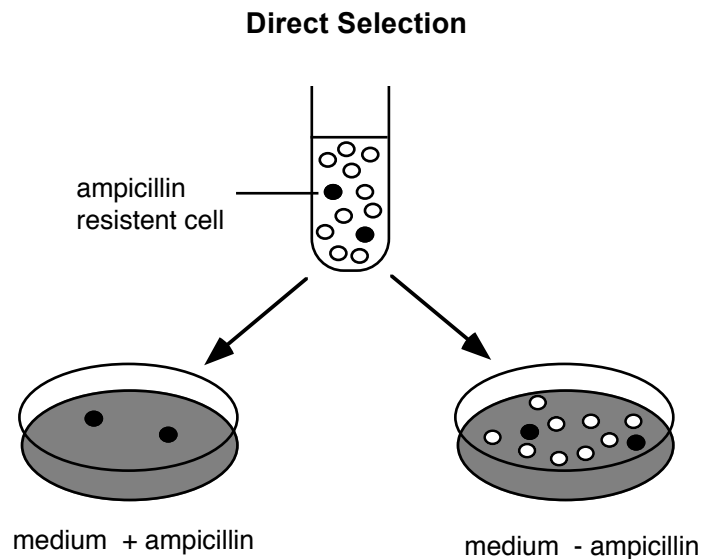
1. The genetic code is redundant.

C
 The triplet GCA codes for alanine.
 G
 U

2. Conservative changes in amino acids may not affect function.

[Valine to alanine - both are nonpolar.]
 Arginine to lysine - both are basic.]

- C. Direct selection of mutants.** An example of a direct selection - bacteria grown in liquid are plated on solid medium (agar plates) containing ampicillin. Only the ampicillin resistant organisms will grow and form colonies (on the left).



2. Are mutations spontaneous or induced?

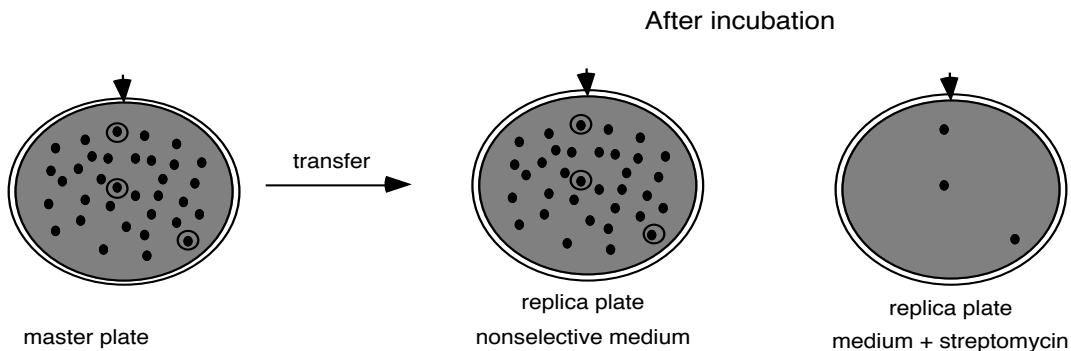
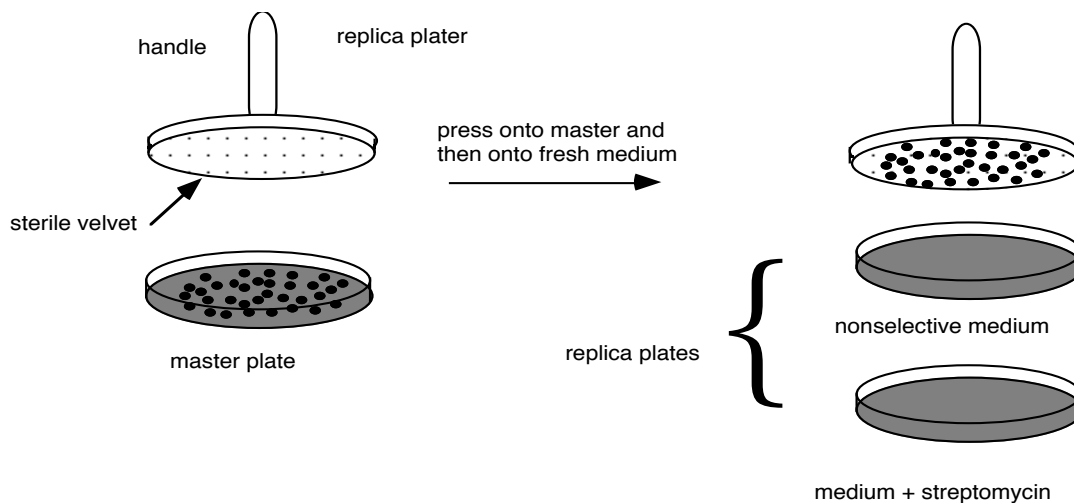
1. In most cases bacterial infections are treated with antibiotics. In addition, antibiotics are added to live stock feed to increase yields.
2. A long-term problem associated with increased antibiotic usage is a dramatic increase in the appearance of resistant organisms.
3. How do antibiotic resistant strains arise?

Two basic mechanisms exist:

- a. mutation to antibiotic resistance
- b. transfer of antibiotic resistance genes.

4. In patients treated with an antibiotic, the percentage of antibiotic resistant organisms is proportional to the length of treatment. At face value, this would suggest that the resistant organisms arise in response to the treatment (Adaptation theory, Lamarckism). But this is not so.
5. In fact, in a population of organisms rare mutations occur spontaneously and give rise to many small subpopulations.
6. Under the selective pressure imposed by antibiotic treatment only the antibiotic resistant organisms are able to survive (Darwinism; survival of the fittest) and over time become the principal component of the new population.

Lederberg Experiment



Conclusions:

1. The results showed that resistant mutants appeared spontaneously prior to antibiotic exposure.
2. Therefore, resistance following antibiotic exposure is due to the selection of rare individual cells with chromosomal mutations to antibiotic resistance that preexisted prior to the antibiotic treatment (natural selection).

3. Genetic Recombination

1. Genetic recombination is the process by which two genetic elements combine to form one.
2. Three types:
 - a. General (homologous) recombination - requires extensive DNA homology.
 - b. Site-specific - requires a small region of homology (e.g. lysogen formation, section 10).
 - c. Illegitimate recombination - requires no homology; occurs at a very low frequency (nonhomologous recombination, e.g transposition)

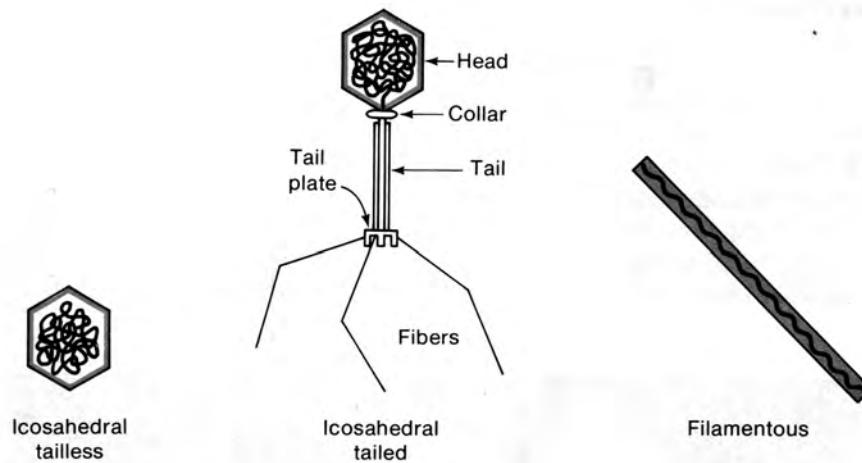
4. Bacteriophage

Bacterial viruses, called bacteriophage, have provided convenient model systems for research on the molecular biology and genetics of virus reproduction. As viruses, they are among the simplest life forms consisting of a nucleic acid genome surrounded by a protein coat.

A. Structure of bacteriophage

1. Bacteriophage consist of a DNA or RNA genome within a protein shell or capsid.
2. Some bacteriophage structures are simple consisting of a nucleocapsid with either icosahedral symmetry or helical symmetry.
3. Other bacteriophage structures are complex consisting of a head, tail, and tail fibers.
4. Bacteriophage may be small, having a genome with less than 10 genes, or large with more than 150 genes.
3. The viral coat proteins of the phage determine the species of bacteria it infects.

Types of Bacteriophage

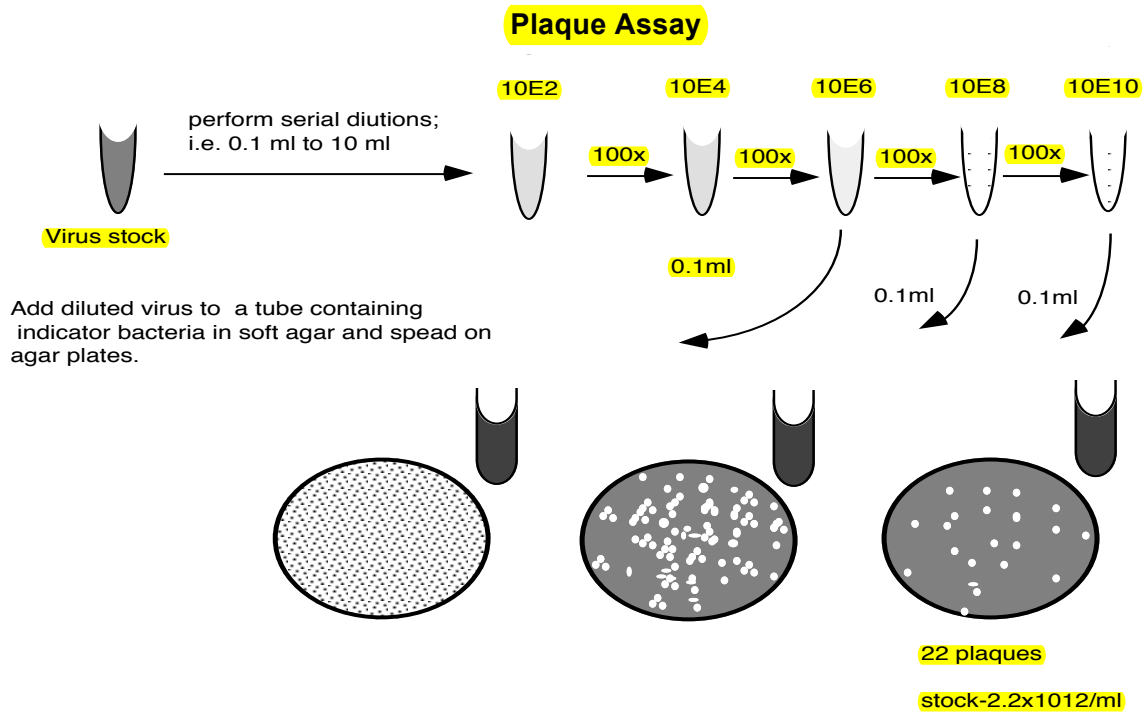


B. Phage growth and assay

1. Bacteriophage are grown by infecting an actively growing bacterial culture. The bacteriophage reproduce, and in most cases, cause host cell lysis with the release of progeny phage. The culture is centrifuged to remove bacterial debris from the supernatant containing the bacteriophage.

2. Bacteriophage are quantified by plaque assay. Phage are placed on a lawn of sensitive bacteria. Their growth (infection of a bacterium, multiplication, release of progeny phage by bacterial lysis, reinfection of adjacent bacteria, etc.) will produce a plaque, a circular zone of bacterial death.

Plaques produced by a particular phage species have a characteristic morphology (i.e. size, clear or turbid, etc.). A plaque contains several million phage.



C. Life cycle of virulent phage

1. The phage life cycle fits into one of two distinct categories - lytic and lysogenic.

1. The lytic pathway involves phage multiplication and the release of newly formed phage following host cell lysis. Virulent phage follow the lytic pathway only.

2. The lysogenic pathway does not result in the production of progeny phage or bacterial killing.

3. Temperate phage can follow either the lytic or lysogenic pathway.

2. Steps in the life cycle of a virulent DNA phage.

1. Adsorption - binding of phage coat protein to cell membrane receptors.

2. Introduction of the DNA - nucleic acid goes in; coat protein stays out.

3. Transcription of phage DNA and inhibition of host transcription.

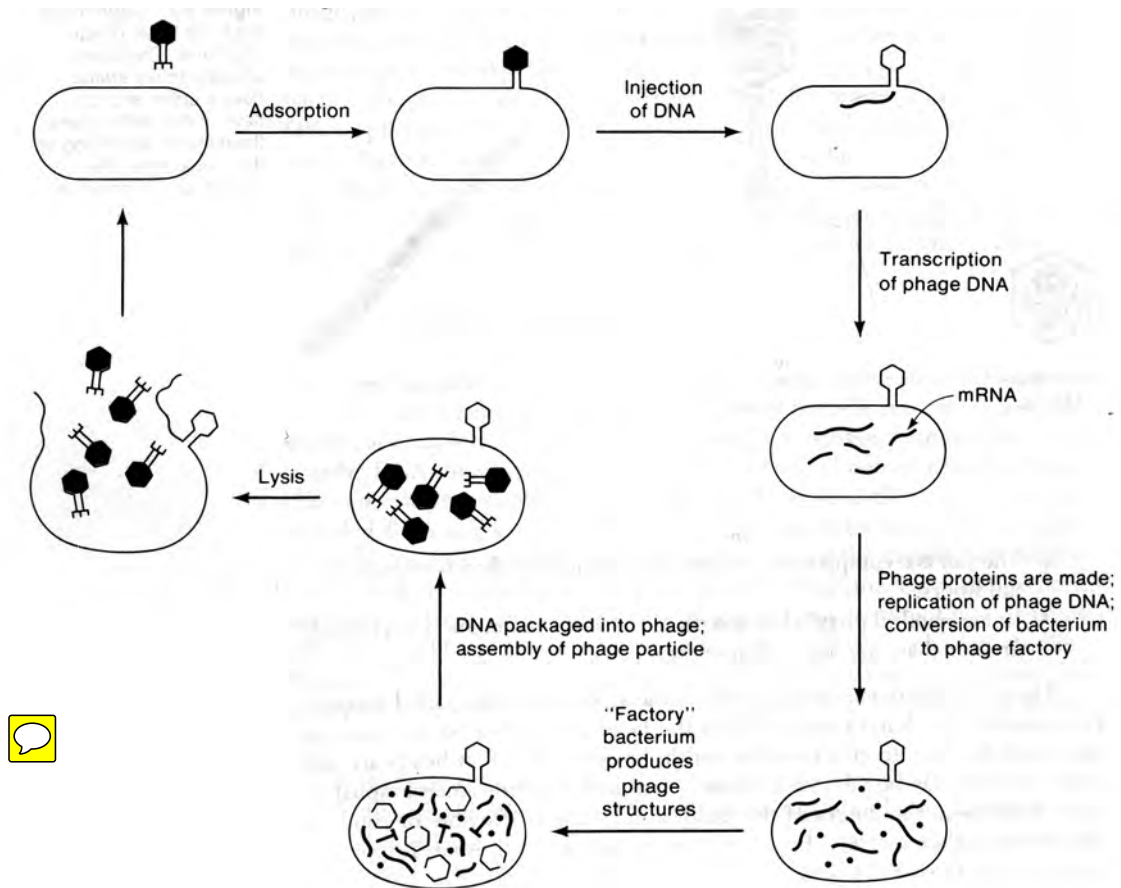
4. Replication of phage DNA.

5. Synthesis of phage capsid proteins.

6. Morphogenesis and packaging of the phage genomes.

7. Lysis and release.

Life cycle of virulent phage



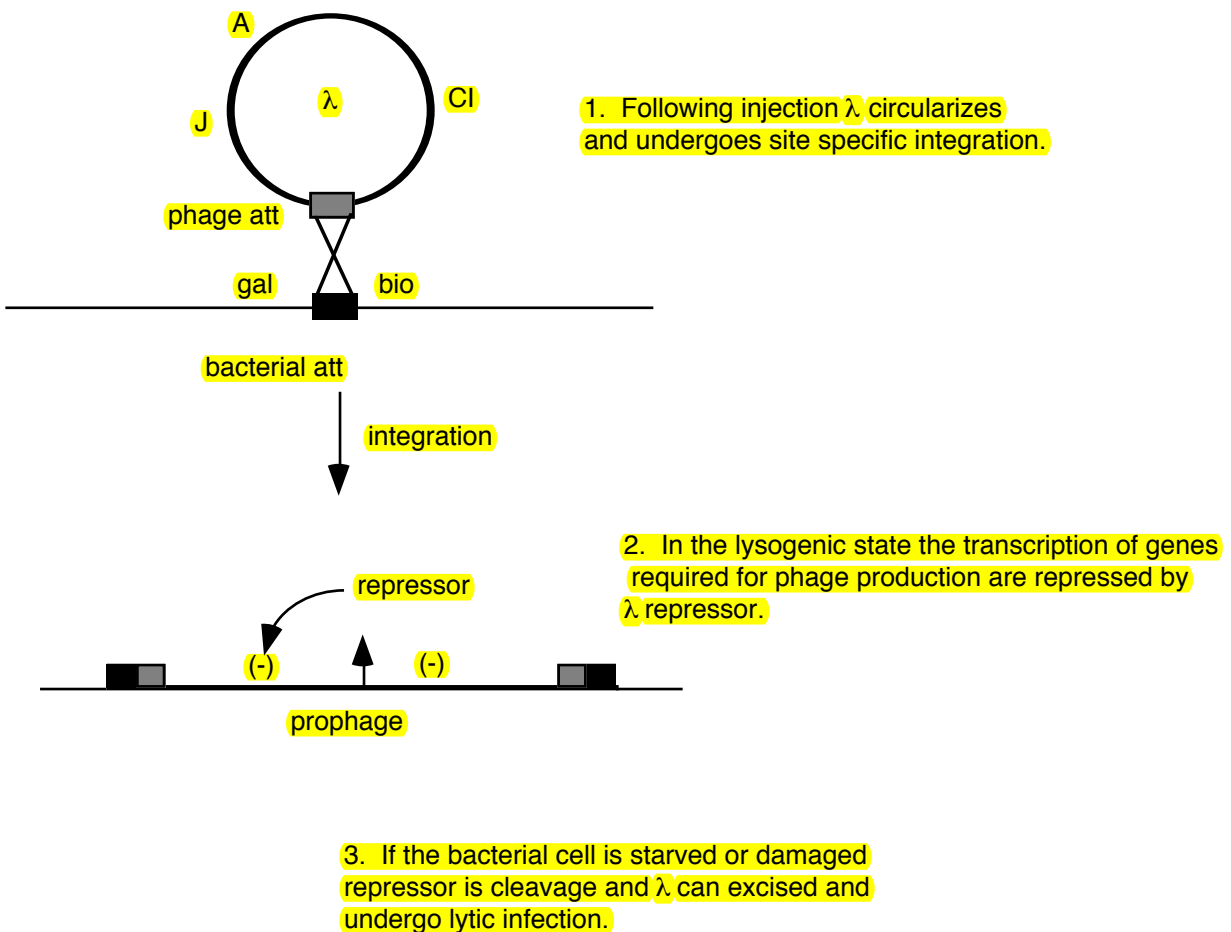
3. A kinetic analysis of phage reproduction, called the one step growth curve, indicates that phage do not reproduce by binary fission. Instead, only the phage nucleic acid enters the cell turning it into a "phage factory". Viral components (proteins and nucleic acid) are synthesized and then assembled into mature phage particles late in infection. Once assembled the phage all simultaneously burst out of the cell, lysing it in the process.

D. Temperate phage and lysogeny

1. Virulent phage produce clear plaques, they infect cells, replicate, lyse the host and infect more cells and so on.
2. The temperate phage produce turbid plaques. This is because a temperate phage has a choice of two types of infection (lytic or lysogenic):
 - a. A virulent infection - a lytic infection (occurs in most cells in the population).
 - b. A lysogenic infection - does not result in the production of progeny phage and bacterial lysis. Rather, the phage DNA becomes integrated into the host chromosome. The phage produces a repressor (cI in the case of λ) that prevents expression of genes that lead to phage production cell lysis. The phage genome is then propagated as a prophage when the bacteria divide.


3. Bacteriophage lambda is a well-characterized temperate phage.
4. Following injection of its DNA, lambda makes a choice to undergo a lytic or lysogenic infection.
5. In a lysogenic infection the phage chromosome circularizes (in the virion the genome is linear double-stranded DNA) and integrates at a specific site in the bacterial chromosome.
6. The phage chromosome also has a similar site and recombination between the bacterial and phage chromosomes, called site-specific recombination, leads to integration (lysogeny).
7. The integrated phage DNA is called a prophage.
8. The prophage expresses a repressor of expression of all the genes required for lytic infection, and so the prophage is happily carried along as a passenger in the bacterial chromosome.
9. If the host bacterium undergoes a stress, such as UV-damage, the repressor protein is inactivated and the prophage excises and undergoes a lytic cycle.

Lysogeny of phage lambda



5. Mechanisms of gene transfer

A. In bacteria, three mechanisms are known:

1. Transformation - the transfer of genetic information to a bacterium following the uptake of naked DNA from outside the cell.
2. Transduction - the transfer of a gene from one bacterium to another by a phage that has mistakenly replaced part or all of its genome with some of its host's DNA.
3.  Conjugation – Transfer of genetic information that requires direct cell-cell contact. Examples include transfer of conjugal plasmids and transfer of portions of the bacterial chromosome by Hfr (high frequency of transfer) strains.
4. All three of these mechanisms occur in nature and are also used in the laboratory to genetically manipulate microorganisms.

B. Transformation

1. Transformation has played an important role in molecular genetics historically. Studying transformation in 1944 Avery, McCarty, and McClelland provided the first direct evidence that DNA is the genetic material.
2. Transformation has been observed in both gram positive and gram negative bacteria (including *Vibrio*, *Neisseria*, *Bacillus*, *Haemophilus*, *Streptococcus* and *Staphylococcus*).
3. In transformation DNA binds to the bacterial membrane and enters the cell. If it is chromosomal DNA, it undergoes homologous recombination with the host chromosome. If it is plasmid DNA, it replicates autonomously, independent of the chromosome.
4. Transformation can be used for linkage mapping, however, its main value is that recombinant DNA plasmids can be easily introduced into bacterial strains by transformation.
5. The ability of bacteria to be transformed is called competence. *E. coli*, which is normally not able to take up naked DNA, can be made competent (by treatment with CaCl_2 or by electroporation) and this is used widely in molecular cloning.

C. Two types of transduction:

Generalized transduction and specialized transduction. Generalized transduction involves transfer of a random region of DNA. Specialized transduction involves transfer of a specific region of DNA.

Generalized transduction

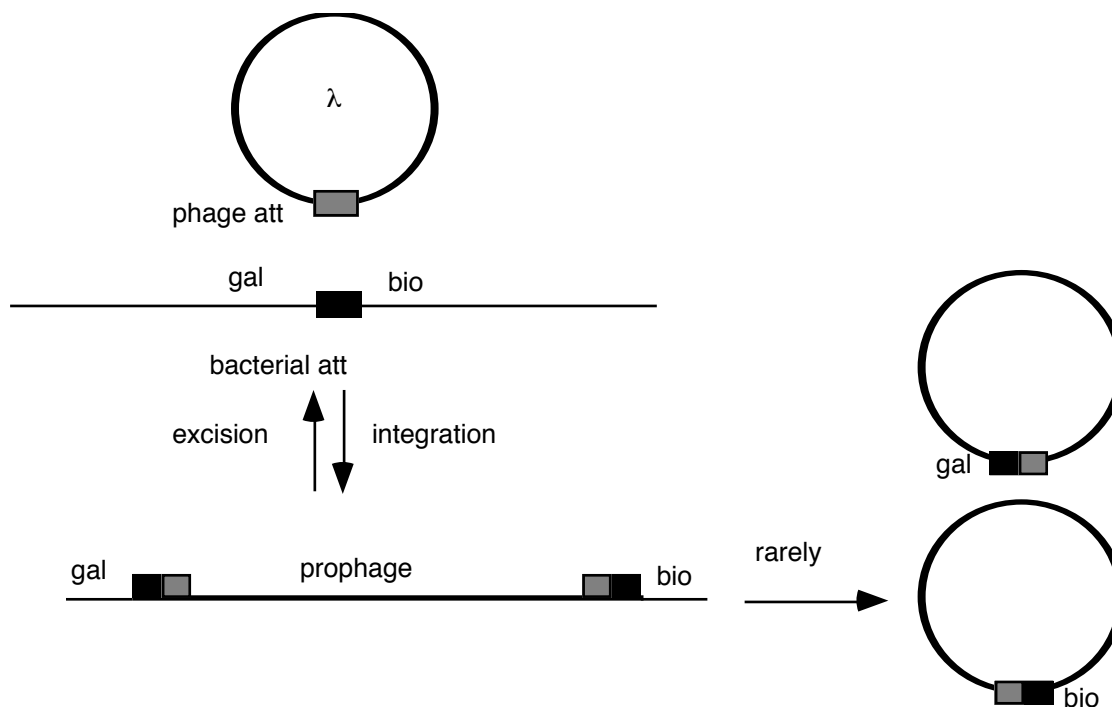
1. This is accomplished by a unique group of bacteriophage that produce some phage particles that contain only host DNA. Bacteriophage P1 is an example
2. Generalized transduction is used extensively for mapping studies.
3. It results from the mistaken encapsidation of host chromosomal DNA fragments into phage particles. The host DNA can be from any part of the bacterial genome.
4. A phage preparation grown on a donor strain of bacteria can be used to infect a recipient strain and recipient cells can be selected that have acquired genetic markers present in the donor strain.

5. Donor DNA that is present in transducing phage particles will recombine with the bacterial chromosome of the recipient strain by general recombination.
6. Only closely linked markers are cotransduced by an individual phage particle. This is because a phage particle can only hold a limited size fragment of DNA, generally 100 kilobases or less.

Specialized transduction

1. Temperate phage integrate into the host cell chromosome at a specific location.
2. Rarely, during the transition from lysogeny to lytic growth, the prophage excises incorrectly leaving behind some of its own genome and acquiring a limited set of bacterial genes from either side of the attachment site.
3. This phage is called a specialized transducing phage and can transfer these bacterial genes to another bacterium in a process called specialized transduction.
4. In contrast to a generalized transducing phage, a specialized transducing phage contains both DNA derived from the host bacterium and the phage.
5. Only genes near the site of integration in the bacterial chromosome (*gal* and *bio* in the case of lambda) will be transduced.
6. It is thought that this mechanism is how phage transfer toxin genes, such as the shiga-like toxin of enterohemorrhagic *E. coli*, diphtheria toxin, or cholera toxin. In these cases, the acquired genes have become a permanent portion of the phage genome.

Lambda as a specialized transducing phage



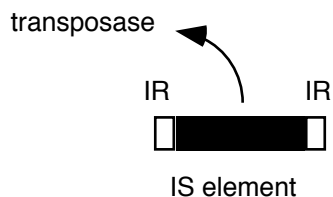
6. Plasmids

- a. Plasmids are extrachromosomal, circular DNA molecules that are capable of autonomous replication.
- b. The first type of plasmid discovered, the F factor, was identified because of its ability to transfer genes from a donor strain bearing an F factor (F+) to a recipient F- strain. Mating in bacteria is called conjugation.
- c. All plasmids have an origin of DNA replication and frequently carry optional genes that confer additional phenotypic properties on the host bacteria. Such as
 - Ability to donate DNA by conjugation
 - Resistance to: antibiotics, metals, UV-irradiation, bacteriophage
 - Production of: bacteriocins, proteases, toxins, antigens, hemolysins
- d. The most medically important class of plasmids are called R factors. R factors encode resistance to various antibiotics and represent an important problem in both hospital and community acquired infections. R plasmids are conjugative (R is a particular form of F).
- e. Another class of plasmids, called bacteriocidins, encode narrow spectrum antibiotics called bacteriocins that bacteria use against each other.
- f. Plasmids are widespread and have been found in all the genera of bacteria.
- g. Physical and genetic characteristics of plasmids
 1. Plasmids are circular, supercoiled, double-stranded DNA molecules.
 2. Plasmids range in size from small (1.5 kilobase pairs) to very large (400 kilobase pairs) encoding several hundred genes. The F plasmid and R plasmids are large plasmids.
 3. Plasmids can be physically separated from chromosomal DNA by density centrifugation or agarose gel electrophoresis.
 4. Plasmids are capable of autonomous replication because they have their own origin of DNA replication that is important in copy number control. Replication and transcription functions are provided by host enzymes.
- h. Plasmids and IS elements (transposons)
 1. Frequently plasmids contain IS elements, which have the ability to insert themselves randomly into any DNA molecule via transposition. A common side effect of transposition is genomic rearrangements such as insertions and deletions. Thus, they play an important role in plasmid evolution.
 2. IS elements in F plasmids allow integration into the host chromosome to form an Hfr strain (see below).
 3. IS elements flanking antibiotic resistance genes in R factors are responsible for generation of multiply resistant R factors since they permit single resistance genes to move onto plasmids that already carry other resistance genes.

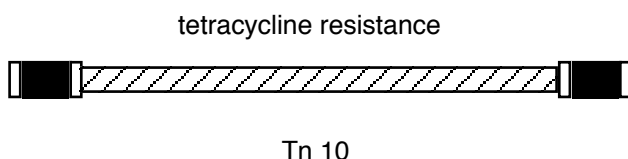
7. Transposable Genetic Elements

1. Transposons are mobile genetic elements that are integrated into bacterial chromosomes or plasmids and are capable of jumping from one location in DNA to another.

- Transposons have special sequences, called insertion sequences (IS), at their ends that are responsible for their ability to integrate at random locations. This is a nonhomologous (illegitimate) recombination.



- The simplest transposons are merely IS elements
- Transposition requires a transposase enzyme encoded by the IS element that allows it to jump by facilitating illegitimate recombination.
- Many transposons consist of a gene encoding an antibiotic resistance element flanked by IS elements. Some transposons carry toxin genes. So these types of transposons have specific medical relevance by permitting the movement of these genes.



- Many transposons that encode antibiotic resistance duplicate themselves during transposition so that they are retained at their original site. This can result in the movement of an antibiotic resistance gene from bacterial chromosome to a conjugal plasmid from which it can efficiently move to other bacteria as well as the reverse.
- Transposons can insert into bacterial genes disrupting them and causing mutations. These are typically null mutations since the genes with the transposon insertions are completely disrupted.

Although the bacterial chromosome is highly stable, transposable genetic elements are able to jump from one location to another on the genome. Transposition requires IS elements that control their own movement. Transposons encoding antibiotic resistance are important in the genesis of resistance transfer plasmids (see below).

8. Conjugation

- Conjugation is sexual reproduction in bacteria.
- The exchange of genetic information between the two parents is unequal.
- In conjugation DNA is transferred unidirectionally between a donor *E. coli* strain that contains, for example, an F plasmid, called an F⁺ strain, and a recipient F⁻ strain.
- Transfer of DNA requires cell-to-cell contact.

[conjugation was discovered by Lederberg in the mid-forties while a medical student.]

e. The F plasmid

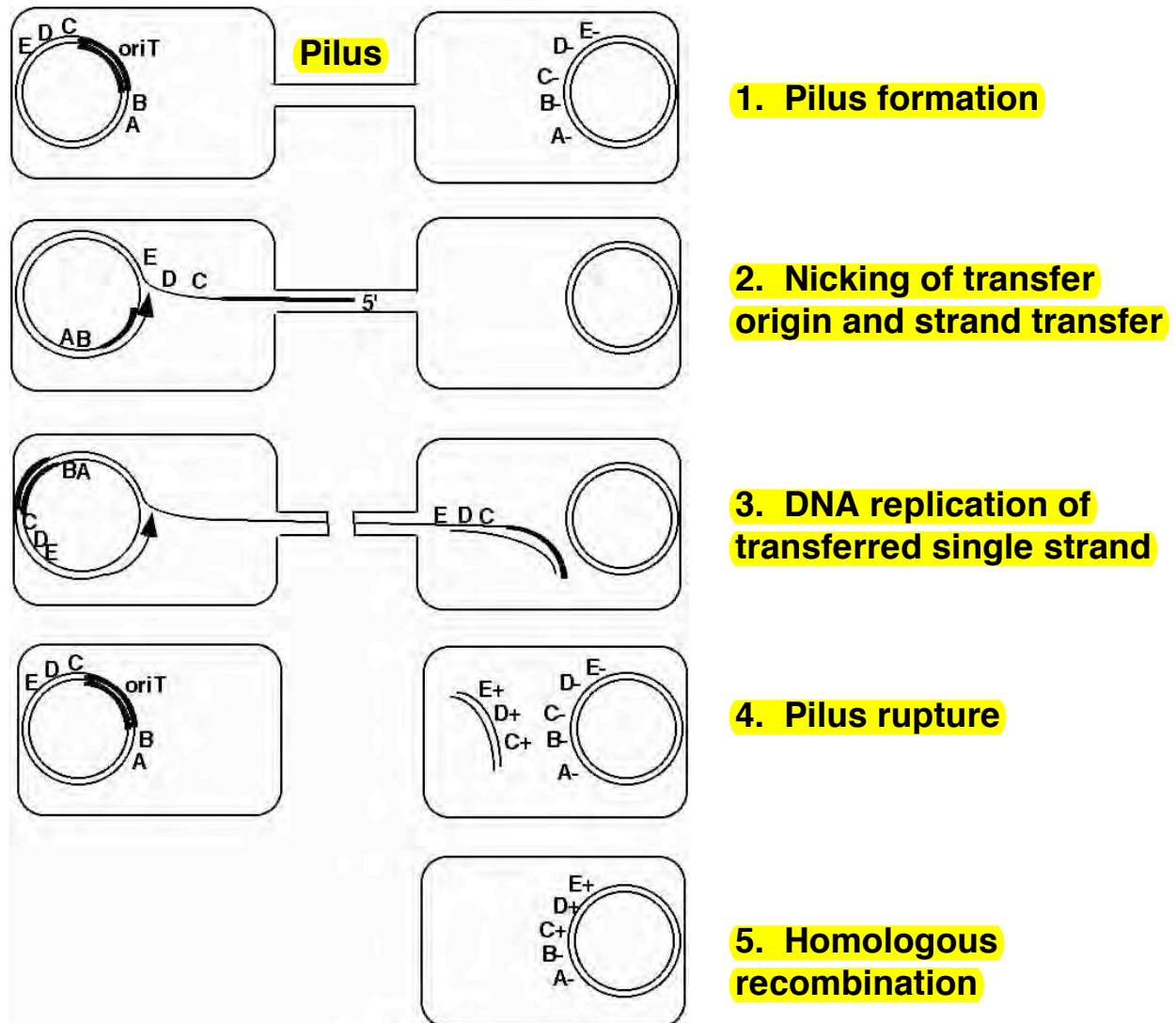
1. The F plasmid encodes proteins required for conjugal transfer.
2. Cell to cell contact is mediated through F plasmid encoded conjugation bridge, called a sex pilus [which consists of a protein called pilin].
3. The F plasmid encodes other proteins required for transfer called tra genes, and a transfer origin that is nicked to initiate conjugation. An F⁺ strain will only initiate pilus contact with an F⁻ strain.

f. Hfr (high frequency transfer) strain formation

1. An extrachromosomal F plasmid occasionally integrates into the bacterial chromosome [(approximately 1 in 10⁴ cells)].
2. Both plasmids and the bacterial chromosomes have multiple IS elements. Integration occurs via homologous recombination between IS elements or by IS-mediated transposition of the entire F plasmid.
3. A cell with an integrated F⁺ is called an Hfr cell. At a low frequency the F may excise from an Hfr.
4. Occasionally excision is imprecise and nearby chromosomal genes are carried on the F. This hybrid plasmid is called an F'.

g. Hfr transfer

1. When an Hfr mates with an F- cell, the Hfr can transfer donor chromosomal genes to the F- cell.

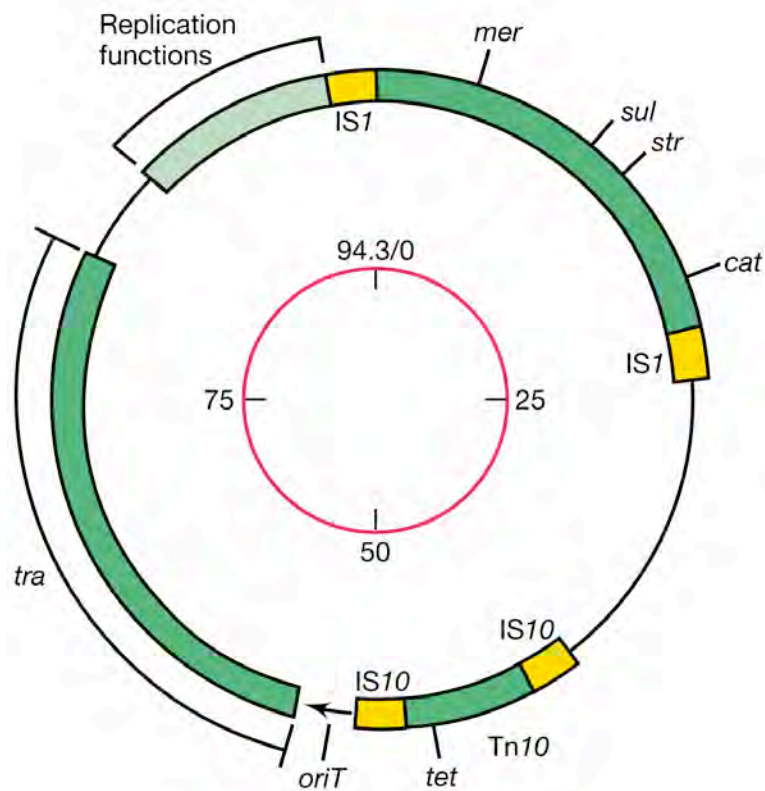


9. Resistance transfer factors (RTFs)

1. These were first discovered in a strain of enteric bacteria in Japan that was resistant to several different antibiotics.
2. The emergence of multiply resistant strains of pathogenic bacteria is of considerable medical significance.
3. The increase in appearance of multiply resistant bacteria has correlated with the increasing use of antibiotics in the treatment of infectious disease and as a supplement in livestock feed. When there is no selection for carrying a plasmid the plasmid is lost in most cells because it slows bacterial replication, so plasmid carrying cells are diluted in the population. However, selection with an antibiotic reverses that process.

4. RTFs are conjugal plasmids of about 90 kilobases in length. The R factor R100 carries resistance genes to sulfonamides, streptomycin, fusidic acid, chloramphenicol and tetracycline.
5. Many RTF's are capable of interspecies transfer. [R100 can transfer between genera of enteric bacteria including *Escherichia*, *Klebsiella*, *Proteus*, *Salmonella* and *Shigella*].
6. In most cases resistance genes are flanked by IS sequences. So genes from one R-factor may be transmitted to another R-factor by transposition. This is the mechanism by which multiple resistance arises.
7. Frequently, treatment with a subinhibitory dose of an antibiotic causes the emergence of cells with high resistance. In these cells the RTF contains multiple copies of the resistance determinant that have arisen by gene duplication, often by transposition. This is part of the basis of the important mandate that when a patient starts a course of antibiotics they should finish the full course.

R factor



DISINFECTION AND STERILIZATION

Learning Objectives:

To define the terms used to describe various practical procedures that partially or completely inhibit bacterial growth

To explain the advantages and disadvantages of physical and chemical methods of disinfection and sterilization

To explain the circumstances in which each method of disinfection and sterilization is best applied

To explain the basic mechanisms by which physical and chemical methods work

Reading assignment: Levinson, Chapter 13

The use of disinfectants and sterilizing agents has produced a striking reduction in food-and water-borne epidemics, and in infections after childbirth or surgery, exceeding, in its impact on public health and mortality, the curative effects of all the antibiotics.

Many different agents are used to kill bacteria in our environment. The list is long and is presented here under two categories: *physical* agents; and *chemical* agents.

The choice of a particular agent is determined by the particular setting in which bacteria are found. The questions asked are:

1. Where are the bacteria to be destroyed? Are they in the air? the water? the bedding? the food? on a body surface? or are they perhaps in contaminated tissues removed from a patient?
2. How much killing of bacteria is needed? Is it sufficient to reduce the bacterial count to some specified number per liter of air or water? Or is the goal to kill *all* bacteria in the specimen or area?
3. How does the presence of different kinds of bacteria influence the method of killing? For example, are there any pathogenic spores?
4. Are there undesirable effects from the killing of bacteria? For example, over-treatment of food may change its taste, and excessive heat (needed for sterilization) may destroy plastic materials.

5. DEFINITIONS:

- a. Sterilization = killing of *all* microorganisms (bacteria, fungi, viruses). In the sections below, only *bacteria* are discussed, but it should be understood that the same or similar statements may apply to fungi and viruses.
- b. Germicide (fungicide, viricide) = the agent used to kill bacteria, fungi, or viruses.
- c. Disinfection = use of a germicidal (fungicidal, virocidal) chemical agent to destroy the potential infectivity of an *inanimate* object. This does not imply killing of all types of bacteria, but only of those that are pathogens likely to be encountered in that setting. Examples: the application of lysol (containing phenol) to floors, or of alcohol to thermometers.
- d. Antisepsis = application of chemical agents to the surface of the *human body* to kill or inhibit pathogenic bacteria.
- e. Septic = characterized by the presence of pathogenic bacteria in living tissue. The opposite, aseptic, means *without* pathogenic bacteria.

- f. Sanitize = a word used to describe the lowering of the bacterial content of food utensils, or similar objects, without necessarily killing all bacteria. Dishes washed in hot soap and water are "sanitized."
- g. Preservative = an agent used in small (non-toxic) concentrations to inhibit the growth of organisms in, for example, food or vaccines.
- h. Phenol coefficient = a measure of the killing capacity of an agent, compared with phenol. The coefficient is the ratio of the minimal killing concentration of phenol (under standard test conditions) to that of the agent. Therefore an agent that works at concentrations *lower* than the killing concentration of phenol has a coefficient much *greater* than one.

For each of the many effective killing agents, both physical and chemical, it is useful to know its advantages and limitations, as well as an example of when one or another is used.

PHYSICAL AGENTS:

The three main agents, in order of use and importance are:

- a. Heat (wet or dry)
- b. Filtration
- c. Radiation (e.g., ultraviolet light -- UV; gamma rays).

HEAT:

Wet heat, particularly steam heat under pressure, is much more effective than dry heat. Water molecules speed the denaturation of proteins in bacteria by providing hydrogen bonds to replace those normally present in proteins. In other words, the heated water molecules disrupt the H-bonds.

An *autoclave* provides the standard conditions for complete killing of all bacteria (including spores): live steam under pressure (121°C, 15 pounds, for 15 minutes -- longer, perhaps an hour, when bulky material is in the autoclave). It is important to first evacuate the autoclave chamber to get rid of entrapped air and permit entry of steam. It is also important to be sure that the wrapping around materials is sufficiently loose so that steam can penetrate to the center.

Successful sterilization is monitored by test tape or spore strips.

In modern autoclaves, controls automatically regulate air evacuation, temperature, pressure, entry of steam, and the rapidity with which the pressure is released at the end of sterilization. For liquids, the pressure is released slowly to prevent boiling or overflow from the container; for solid materials, the pressure can be released rapidly.

Although autoclaving has the advantage of providing assurance of complete sterilization, it has the disadvantages of requiring complicated equipment and being unsuitable for heat-sensitive materials, such as most plastics.

When dry heat is used, the temperature and time needed for sterilization are increased. To eliminate spores, for example, typically 160°C for 1.5 to 2 hours is needed. Dry heat is used when materials would be damaged by water--e.g., some powders, or special surgical dressings.

Boiling at atmospheric pressure will kill most bacteria (but not spores) in five to ten minutes. It is often used when simple equipment is convenient or all that is available. For minor surgical procedures in a physician's office, instruments are commonly sterilized by boiling in water.

Of the various applications of heat, one of the most widely used is Pasteurization. Pasteur first used mild heating for treatment of wine but the common application now, of course, is for treatment of milk. Classical treatment is 63°C for 30 minutes kills common milk-borne pathogens (tubercle bacillus, *Salmonella*, *Streptococcus*, and *Brucella*), and reduces total bacterial count to 1 to 3% of the initial level. Another treatment, high temperature short time (HTST), also known as flash pasteurization has the conditions of 71-72°C for 15-17 seconds and yields similar results. Historically, the test organism to monitor pasteurization was *Coxiella burnetii*, agent of Q fever. This rickettsia was a good test organism because it is relatively heat-resistant. Today, milk is deemed pasteurized if it tests negative for alkaline phosphatase. This enzyme denatures under conditions similar to those that kill *C. burnetii* and its loss of activity is quicker and easier to assay than monitoring the presence of viable *C. burnetii*. NOTE: Pasteurization does not kill spores.

Flash pasteurization has been used for Tropicana's high end juices for the past 50 years. Odwella has been using it for its juices since 1996. It has scattered use for milk in North America and is the method of choice for milk in Europe and Asia.

FILTRATION:

This method is used when liquid material to be sterilized is sensitive to heat. It has the advantage of being non-destructive, but the disadvantage of being inconvenient, particularly if large volumes are involved. Pharmaceutical industries have automated this procedure, and nurses or physicians in hospital or office practice can use small, presterilized, disposable filters in a plastic holder that attaches readily to a syringe. Many vaccines, drugs, and other materials are commonly sterilized by filtration and kept in sealed, rubber-capped vials.

The types of filters vary. Some are rather small (1 to 5 cm diameter), thin, flat circles held between clamps, while others (used in industry) are large, three-dimensional structures, with pores throughout.

The idea is simple: hold back the bacteria and let the fluid through. Flow rate is determined by pore size, the pressure applied, and whether bacteria or other solids have begun to clog the pores. Sometimes filtration is done first through filters of large pore size, and then of smaller size. In some filters, the pore size given is the actual size of the holes (there may be many thousands on the surface of a single filter); in others, the pore size is not exact.

A few types of bacteria can squeeze through pores of 0.45 microns; most cannot. None pass through 0.22 microns, but viruses do. How big is *E. coli*? A red blood cell?

RADIATION:

The most common application involves ultraviolet light emitted by mercury vapor lamps. This is an example of nonionizing radiation. It is used, for example, to decrease the bacterial content of air in operating rooms, barracks, nurseries, restaurants, hospital wards, and animal rooms.

Nucleic acids and proteins absorb ultraviolet wavelengths. In the case of DNA, UV light produces cross-linked thymine dimers, and this interferes with DNA replication (ie. it is NOT lethal mutation that causes cell death). UV also causes toxic intracellular peroxide formation.

Sunlight provides some UV, but most is screened out by the ozone layer in the atmosphere.

The advantage of UV lies in the simplicity of setting it up. UV lights can be left on for long periods, and need little attention. The disadvantages are: poor capacity to penetrate anything but air and the thinnest layers of solids and liquids (UV will not penetrate glass); irritation and damage to human tissue (skin, eyes, and nasal mucosa if UV levels are too high). UV light should not be viewed directly and the light intensity has to be regulated.

X-rays and even more energetic radiation (gamma rays which is a form of ionizing radiation) have been used for food sterilization with questionable results. The quality of treated food is sometimes not well maintained over a long term nor is it yet generally accepted by consumers.

MISCELLANEOUS PHYSICAL METHODS:

Drying--lowers water levels below that needed for bacterial growth. High osmotic pressure--produced by high salt--acts as a preservative and sometimes kills by drying out microbes--or high sugar concentration (as in jams)--acts as a preservative, not as a killing agent. The following are of little or no practical use: ultrasonic devices; high atmospheric pressure (without heat); freezing and thawing (although repeated freezing and thawing will progressively lower bacterial count). Note: Ice cubes made from contaminated water may, after thawing, contain viable bacteria.

CHEMICAL METHODS:

Agents in this group interact with lipids, proteins, or DNA of bacteria. For many (not all), the rate of killing increases both with the *concentration* of the killing agent, and *length of exposure*.

- a. Gaseous sterilization: *Ethylene oxide* is used to sterilize heat-sensitive materials, particularly plastics. It is an alkylating agent, toxic to humans, and must be used with special equipment with particular precautions for proper venting. It is reliable, but suitable only for dry materials--not liquids. Because pure ethylene oxide is explosive, it is used only in the presence of a high concentration of carbon dioxide. This agent is used for sterilizing--besides plasticware--surgical equipment, hospital bedding, and materials handled by patients.

- b. Ethyl alcohol (*ethanol*): This agent has received the widest use. The optimum concentration is about 60 to 70 percent, with effectiveness falling off above and below (20% and 100% are almost ineffective). Ethanol denatures proteins. It is not reliably effective against all organisms. It is subject to legal restrictions and accountability for use. *Isopropyl alcohol* has no legal restrictions, is slightly more potent, less volatile, but more expensive than ethanol--although in small quantities the additional cost is not significant.

Ethanol is commonly used on skin before injections, in tinctures of antiseptics and germicides, or in jars where thermometers are stored. Ethanol does not kill spores.

- c. Halogens: Tincture of *iodine* (iodine in a water-ethanol solution) contains iodine and KI. This formulation oxidizes the hydroxyl group of reactive tyrosine residues. It is a reliable skin antiseptic, often used for minor wounds, but hurts when so used. Complexes of iodine and detergents are used for preparation of patient's skin before surgery. *Chlorine* is widely used as a gas, in water supplies and swimming pools, and as hypochlorite solutions, by the food and dairy industries, to clean surfaces. Chlorine as a sanitizing agent in the food industry has the advantage that traces are rapidly lost or destroyed, leaving no flavor or odor. Chlorine oxidizes cysteine sulfhydryl groups.
- d. Cationic detergents: These are active against all kinds of bacteria, disrupting cell membranes and dissolving lipid films that may protect bacteria. The most effective in this class are quaternary ammonium salts with three short chain alkyl groups as well as a long chain alkyl group. One commonly used cationic detergent has the trade name "Zephiran." Anionic detergents do not work as well since the molecules are repelled by the negative charge of the bacterial surface.

These agents leave a tenacious bactericidal film on surfaces of treated objects. They work at low concentrations and are usually non-irritating. Cationic detergents are not active against *Pseudomonas*, the most common agent in burn infections, and are but poorly active against *Mycobacterium tuberculosis*.

- e. Oxidizing agents: *Hydrogen peroxide*, as a 3% solution, is used, but infrequently, to treat some wound infections, particularly deep wounds that may contain anaerobes. Hydrogen peroxide is commonly used to control growth of the plethora of anaerobic bacteria that grow in the gingival crevices of the gums. Its action is somewhat antagonized by cells or tissues (or bacteria) that contain catalase. All *Staphylococci* are catalase⁺.

Potassium permanganate, in dilute solution, is another oxidizing agent, and is used as a urethral antiseptic.

- f. Phenols: Lister introduced the practice of spraying surgical operating rooms with phenol. It effectively denatures proteins, and therefore kills a wide variety of bacteria, but requires high concentrations. Substituted phenols--e.g., hexylresorcinol--are also used, primarily as skin antiseptics. Others such as Triclosan are used in antibacterial soaps and as a textile preservative.

- g. Soaps and detergents: These are surface-active agents (surfactants) often containing hydrophilic and hydrophobic portions. Common soaps are anionic detergents and are only weakly bacteriocidal, yet they do effectively *remove* bacteria and dirt from skin surfaces. Some fragile bacteria, however, are killed by soaps or detergents.

Antibacterial soaps - A number of soaps and other products such as skin lotions (and even cutting boards and entire bathrooms!) include the antibacterial agent, triclosan. Until recently, triclosan was thought to be a general bactericide with numerous cell targets of interaction. In fact, it has recently been determined that triclosan inhibits a specific step in lipid biosynthesis, and resistant mutants can be isolated. Should the public be concerned?

- h. Heavy metals: A common example is silver salts: a 1% solution of *silver nitrate*, placed in the eyes of newborns, kills gonococcal organisms. *Mercurial compounds* in organic form (e.g., merthiolate), are commonly used on minor skin wounds. Heavy metals bind to SH groups in proteins.
- i. Dyes: Gentian violet (the crystal violet of the Gram stain) in a 1% solution in saline is an antifungal agent used for *Candida* and *Tinea* (athlete's foot) infections. Mechanism of action is unknown.
- j. Aldehydes: *Formaldehyde* is used in vaccines. *Glutaraldehyde* is sometimes used to treat areas where blood samples and used syringes are present, and that may be contaminated with hepatitis virus. Cidex is a buffered 2% solution of glutaraldehyde.
- k. Preservatives: Short chain fatty acids and organic acids preserve food. Examples: lactic acid, in pickles and sauerkraut; acetic acid in pickles and relishes; propionic acid--as calcium propionate--in bread, to prevent mold growth; benzoic acid in pharmaceuticals and food; natural phenolics--such as thymol and eugenol--as mild antiseptics and preservatives. Smoked foods are covered and partially penetrated by phenolic compounds from wood smoke.

ANTIBIOTICS



Learning Objectives:

- To explain the overall concept of antibiotic function
- To explain how antibiotic effectiveness is determined
- To explain the effect of each antibiotic on the integrity of the cell
- To describe the individual classes of antibiotics and identify selected members of each
- To describe the general mechanisms of action of major classes of antibiotics
- To explain the mechanism of action (target) for selected example antibiotics
- To list the toxic side effects associated with selected example antibiotics
- To describe how antibiotics work (or don't work) in combination
- To list and describe the various mechanisms of antibiotic resistance

Reading assignment: Levinson, Chapters 10 and 11

INTRODUCTION

Agents useful in treating infectious disease must be *selective*; they must act against the offending organism at concentrations that have little or no toxic action against human cells. Some agents are of natural origin—for example, microorganisms in the soil produce organic compounds that inhibit bacterial growth—and these are called antibiotics. Others are produced by chemical synthesis—and hence the term "chemotherapy." Even others are partly of natural origin, and partly synthetic, for the organic chemist is now able to alter many structures at will. Terminological distinctions are no longer important, and we will group all agents in these lectures under the category *antibiotics*. A more general (and perhaps better) term would be *antimicrobial agents*.

In these lectures, we will deal almost entirely with agents effective against bacterial and fungal infections.

A general introduction to the major classes of antibiotics will be given. In the second year, very detailed presentations will be made of particular antibiotics within each category, along with case histories of various infectious diseases, and discussion of how best to carry out diagnosis and treatment.

Seventy years ago, a physician treating a patient with a serious bacterial infection might ask—is there an effective drug for my patient? More recently, the question has been, which antibiotic is best? However, with the alarming rate of arising antibiotic resistant bacterial strains, the question has, in part, reverted back to what it was seventy years ago.

Principles to consider:

1. Antibiotics are drugs. The essential feature of antimicrobial agents is *selective toxicity*. They interact not only with bacteria, but all too often with host human cells, producing toxic reactions. Moreover, the host interacts not only with the antibiotic by influencing absorption, distribution, and excretion, but also with the bacterial invader by mounting phagocytic and other immunological defenses. So there is a complex three-way interaction between bacterium, host, and antibiotic. Therefore antibiotics are not used indiscriminately.
2. Detailed thought must be given to optimal dose, route of administration, and duration of treatment.


In vitro determinations for selection of effective antibiotics: *These techniques require a pure culture of the organism.*

MIC and MBC - MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration. The clinical laboratory's report that an infecting organism is SENSITIVE or RESISTANT to an agent takes into account the MIC against that organism, as well as the agent's clinically achievable serum/tissue concentration.

Tube dilution method - MIC can be measured by a series of tube dilutions of the antibiotic in culture medium inoculated with the purified infectious agent to determine MIC. If MBC is required, aliquots are plated onto agar subsequent to culture tube growth. For example, knowledge of the MBC is particularly important for the treatment of bacterial endocarditis. Determination of MIC by the tube dilution method is a tedious procedure that can only be used with one antibiotic per tube dilution series.

Disc Diffusion (Kirby-Bauer) - The disc diffusion method provides multiple, simultaneous testing. An agar plate is spread with an inoculum of the purified infectious agent. Filter paper discs, impregnated with appropriate concentrations of each antibiotic to be tested, are placed onto the surface. After incubation the circular zones of inhibition of bacterial growth around each disc are measured. This technique has the advantage that multiple antibiotics are tested all at once, but the disadvantage that it cannot be used for determination of MBC.

β -lactamase production - Rapid test for β -lactamase production based on a chromogenic β -lactam substrate changing color within a short incubation time of several minutes after addition of a suspension of the infectious organism. Nitrocefin is a commonly used substrate for this test since it is chromogenic and has a β -lactam ring that is quite sensitive to hydrolysis. This rapid test is particularly important for certain acute infections for which β -lactam antibiotics would be the drug of choice.

3. Other forms of treatment for infectious disease--e.g., surgical drainage of an abscess--are sometimes needed in conjunction with antibiotic therapy.
4. The particular needs of a patient, as well as the route of administration and duration of treatment, influence the choice of an antibiotic. One considers (among many factors) age, sex, general health, presence of kidney or liver disease, history of allergic reactions, and immunological status.
5. Ideal antibiotics should have the following properties:
 - a. The drug should kill or inhibit one or more species of bacteria, with no toxic —including allergic reactions—to host (human) cells.
 - b. The drug should not be destroyed or eliminated by the host before the invading bacteria can be killed or inhibited.
 - c. The drug should not have lost its effectiveness because bacteria have become resistant to its action.
 - d. The drug should reach the sites that contain the bacteria—even if these bacteria are in pockets of pus, deep in bone, in brain tissue, or inside of cells.

Such ideal antibiotics have not yet been found. Some come close, for specific types of infectious organisms, but every antibiotic has its limitations.

We will discuss six major classes of antibiotics or antimicrobial compounds, with emphasis on their mechanisms of action, major uses, and common resistance mechanisms:

- I. Antimetabolites – particularly the sulfonamides.
 - II. Inhibitors of cell wall synthesis – particularly β -lactams and glycopeptides.
 - III. Agents that alter membrane permeability – polymyxins and polyenes.
 - IV. Inhibitors of protein synthesis – including the aminoglycosides (such as streptomycin), Macrolides (such as erythromycin), tetracycline, and chloramphenicol.
 - V. Inhibitors of nucleic acid synthesis – quinolone derivatives and rifampin.
 - VI. Miscellaneous antibiotics.
-

NOTE: You should become familiar with each of the underlined antibiotics described below. Summary tables and diagrams are provided at the end of this section of notes to help keep the antibiotics and antibiotic-resistance mechanisms organized.

ANTIBACTERIAL DRUGS (ANTIBIOTICS)

1. ANTIMETABOLITES

An antimetabolite interferes with the synthesis or function of a substance involved in normal cell metabolism. Often the antimetabolite is structurally similar (an "analog") to the natural substance.

Sulfonamides: This group includes antibacterial agents with structures similar to the simple compound para-amino-benzoic acid (PABA). One of the early members of this group was sulfanilamide, and this agent and its derivatives have the general name: sulfonamide. Although many hundreds have been synthesized, only a few are now in common use. For all, the mechanism of action is the same. The chemical structure of the sulfonamides will be shown in class. Sulfa drugs were packed into the wounds of wounded military personnel at Pearl Harbor, making this the first time that wound infections were not the leading cause of death for individuals wounded in combat.

Sulfonamides penetrate sensitive bacteria and inhibit the production of folic acid by competitively inhibiting one of the enzymatic steps required for its synthesis. Folic acid is necessary for bacterial DNA synthesis, and if DNA is not made, bacteria stop dividing. The action of sulfonamides is reversible; when the drug is removed, the bacteria resume growth. Thus the action is called bacteriostatic, not bactericidal.

Sulfonamides act against a wide range of bacteria, and even against some protozoa. Some are concentrated in the urine, and therefore find their greatest application in treatment of urinary tract infections caused by sensitive strains of bacteria. This exemplifies one aspect of antibacterial therapy that will be discussed in detail in the second year course on infectious diseases: i.e., the choice of one antibiotic instead of another depends on the capacity of the antibiotic to penetrate specific tissues, to be concentrated in fluids, or to penetrate cells containing intracellular bacteria.

The following questions will be discussed in class:

- a. Why is the drug effective against sensitive bacteria if it is only bacteriostatic?
- b. Why are host (human) cells not inhibited by sulfonamides?
- c. What is meant by a "competitive substrate" and what are the implications for antagonism of sulfonamide action by material released from dead host cells in an abscess.
- d. How does one tell if a drug is bacteriostatic or bacteriocidal, and what can we learn from measurements of the turbidity of a culture?
- e. If a sulfonamide is added to a rapidly growing bacterial culture, turbidity increases for a while, and only then remains constant; why is there a gap between the moment of addition of the sulfonamide and an obvious effect on growth of the culture?

Other antimetabolite drugs:

Trimethoprim:

A later step in bacterial metabolism of folic acid is the reduction of dihydrofolate to tetrahydrofolate, by the bacterial enzyme dihydrofolate reductase. This enzyme is inhibited by trimethoprim. When trimethoprim is used in conjunction with a sulfonamide, a synergistic action (that is, an action greater than the simple additive effect of the two drugs) is achieved. Therefore the two agents—trimethoprim and a sulfonamide—are sometimes jointly used in treatment of urinary tract infections. A common combination is trimethoprim and sulfamethoxazole (Bactrim). Although not intuitive, the action of trimethoprim is bacteriocidal as is that of Bactrim.

Isoniazid:

This drug is specific in its action against *M. tuberculosis* (narrow spectrum antibiotic), and probably acts by interfering with synthesis of *mycolic acid*, an organic material unique to the cell walls of mycobacteria and a small number of other types of bacteria. It functions, upon conversion to an active form inside the cell, by inhibiting an enzyme termed InhA that is essential for fatty acid elongation. It is bacteriocidal (unlike the bacteriostatic para-amino-salicylic acid) and penetrates well through the cytoplasmic membrane of human cells (an important feature for treating tuberculosis because in this disease, many of the bacteria are intracellular).

II. INHIBITORS OF CELL WALL SYNTHESIS

A. Penicillin

1. History

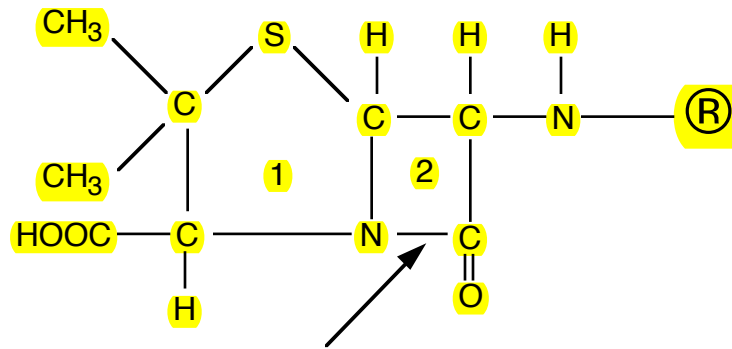
- (a) Fleming observed in 1928 that growth of staphylococci was inhibited by a mold (*Penicillium*). Extracts of the mold were unstable, and Fleming's work was largely ignored for some time.
- (b) More than a decade later, in 1941, Florey, Chain, and colleagues, demonstrated that dry powder (extracted from the mold) was stable. This led the way to commercial production with dramatic therapeutic results soon apparent.
- (c) Large-scale production began toward end of World War II.

2. Effectiveness

- (a) Enormous difference between sensitivity of susceptible bacteria and sensitivity of animal cells. Major toxic effect is an allergic reaction, sometimes serious but rarely fatal.
- (b) Reason for large difference between bacterial and animal cells—no cell wall in animal cells; only a cytoplasmic membrane. Synthesis of cell wall is essential (in almost all bacteria) for multiplication.
- (c) Growth conditions are *essential* for lethal action of penicillin. A period equivalent to one or two divisions is sufficient for killing. Penicillin causes bacteria to *lyse*. Can prevent or slow lethal action by providing unfavorable medium—true both for test tube experiments and in humans. For example, organisms *in vitro*, deprived of a nutrient essential for growth, will survive penicillin treatment.
- (d) Cell wall synthesis inhibitors are all bactericidal. All inhibit various steps of peptidoglycan synthesis. Inhibition of peptidoglycan synthesis is thought to uncouple the control of endogenous degradative activities (autolysins) which normally are synchronized with cell wall synthesis— cell lysis ensues in hypotonic media

3. Hydrolysis of penicillins

- A) Penicillin G is hydrolyzed by acidity of stomach. Penicillin is a 4-membered lactam (lactam: an anhydride link that forms a ring-structure in part of molecule). 4-membered ring is strained, and easily hydrolyzed.



1 = Thiazolidine ring

2 = β -lactam ring

B) Hydrolysis by bacterial enzymes—*penicillinases* (β -lactamases). Many bacterial strains produce this enzyme particularly notable for the staphylococci. Constitutive and inducible enzymes found. Penicillin is the inducer.

Gene for β -lactamase is often located on a plasmid—an extrachromosomal DNA replicon (analogous to the F) and often referred to as an R-factor.

Arrow in the preceding diagram shows the bond that is broken either by acid conditions or by penicillinase.

4. Table of properties of a few penicillins, representative of different classes:

	Side Chain on 6-amino-penicillanic acid	Sensitive to acid hydrolysis	Sensitive to penicillinase
penicillin G	Benzyl	yes	yes
penicillin V	Phenoxymethyl	no	yes
ampicillin	Aminobenzyl	no	yes

5. Biochemical mode of action


- β -lactam antibiotics are structural analogs of the peptidoglycan pentapeptide; they bind to and inactivate *penicillin-binding proteins* (PBPs)—transpeptidases that are responsible for the terminal stages of peptidoglycan synthesis and reshaping the cell wall during growth and division.
- Different β -lactams have different affinities for the various PBPs; inactivation of the transpeptidase(s) responsible for crosslinking (the final step in cell wall assembly) is the major bactericidal activity of the β -lactams; some PBPs are not essential for viability.
- Bacterial autolytic enzymes break down cell walls during normal growth; if the bacteria are not actively dividing penicillin will not cause lysis.

6. Spectrum of action

Not the same for all penicillins. Ampicillin, for example, has a broader spectrum of action than penicillin G.

7. Penicillin G, even though sensitive to acid hydrolysis and penicillinase, is the drug of choice for many gram-positive, sensitive cocci. It is also effective against *some* gram-negative cocci such as *Neisseria meningitidis* (causative agent of spinal meningitis) and against the spirochete *Treponema pallidum* (causative agent of syphilis).

Shortcomings of *penicillin G*: acid lability, thus no oral formulation; penicillinase (β -lactamase) sensitivity; development of allergic response; ineffective vs. G- enterics. All drawbacks except the allergic response have been improved with the development of semisynthetic penicillins.

8. Some semi-synthetic penicillins, resistant to penicillinase (e.g., oxacillin, nafcillin), are used for infections by bacteria that produce penicillinase. 

9. Penicillins in use:

- a. Sensitive to penicillinase – Limited spectrum – Highest activity against G+ bacteria and G-cocci; ineffective versus G- enterics; .
- Penicillin G (benzylpenicillin) – acid labile.
 - Penicillin V (phenoxymethyl penicillin) – relatively acid stable
 - Penicillin VK (Pencillin V Potassium) – increased solubility
- b. Sensitive to penicillinase – Broader spectrum (*active against G- enteric bacilli* as well as retaining most activity against G+) – Typically aminopenicillins.
- Ampicillin – acid stable.
 - Amoxicillin – like ampicillin; higher serum levels.
- c. Sensitive to penicillinase – Extended spectrum (*active against a wide variety of G- bacilli*, including *P. aeruginosa*, less active vs. G+ cocci).
- Tricarillin – b-lactam effective against *Pseudomonas aeruginosa*; it is a carboxypenicillin.
 - Piperacillin – An ureidopenicillin, it is the most active penicillin against G- enteric bacilli, including *P. aeruginosa* and anaerobes.
- d. Resistant to penicillinase – Slightly lower activity against G+ bacteria but still effective. Of little use against G-; (the antistaphylococcal penicillins)
- Methicillin – acid labile – use now avoided in adults because of high incidence of interstitial nephritis. But, remember the term with reference to MRSA (Methicillin resistant *Staphylococcus aureus*).
 - Nafcillin, dicloxacillin, oxacillin - Newer, more potent derivatives. Acid resistant and available orally.

B. Cephalosporins

Similar to penicillins in mechanism of action. They have a 4-membered β -lactam ring, like penicillins, but substitute a dihydrothiazine ring instead of the thiazolidine ring of the penicillins. As with penicillins, various side chain substitutions result in derivatives with different bacterial spectrums, host stabilities, and potencies.

Important points:

- a. Broad spectrum of action against both gram-positive bacteria and some gram-negative bacilli.
- b. As with penicillin, these agents are bactericidal.
- c. Cephalosporins differ from penicillins in having greater acid stability and being resistant to some penicillinases (but cephalosporinases exist).
- d. Useful when patients are allergic to penicillin because they are antigenically dissimilar. (5-15% cross-reactivity in penicillin-allergic patients)
- e. As new members of the class of cephalosporins were developed, they were called first, second, and third generation cephalosporins, differing in the spectrum of bacteria that could be treated by these agents. Important properties of newer agents: active against *Pseudomonas*; better penetration into cerebrospinal fluid.
 - 1) First Generation (e.g., cefazolin, cephalexin, cefaclor, cephalothin) – most active vs. G+ cocci; active vs. some G- enteric bacilli but not *P. aeruginosa*.
 - 2) Second Generation (e.g., cefuroxime, cefamandole, cefonocid, cefotetan, cefoxitin) – improved pharmacology and spectrum of activity: more effective vs. G- enterics, but less effective against G+; still ineffective vs. *P. aeruginosa*.
 - 3) Third Generation (e.g., ceftriaxone, ceftazidime, cefotaxime, cefoperazone) – improved β -lactamase stability and broader G- spectrum; ceftazidime is effective vs. *P. aeruginosa*. Ceftriaxone is notable for superior CNS penetration.

C. Antibiotics that have beta-lactam rings but are not otherwise similar in structure to penicillin:

1. Monobactams – (e.g., aztreonam). Resistant to most β -lactamases and minimal cross-immunogenicity with other β -lactams. Effective against aerobic G-, which includes *P. aeruginosa*. Ineffective against most G+ and anaerobic bacteria.
2. Carbapenems – (e.g., imipenem). Broadest antimicrobial spectrum and resistant to most β -lactamases but susceptibility among methicillin-resistant staphylococci is highly variable. Also, susceptible to renal dipeptidase so often used in combination with cilastatin (a renal dipeptidase inhibitor) e.g., Primaxin = imipenem + cilastatin.

D. β -lactamase Inhibitors – β -lactams which have minimal activity as antibiotics but extend the use of β -lactam antibiotics.

1. Clavulanic acid – presently available in fixed combinations with amoxicillin (Augmentin).
2. Sulbactam – available in fixed combination with ampicillin (Unasyn).
3. Tazobactam – available in fixed combination with piperacillin (Zosyn).

E. Glycopeptides – another class of cell wall synthesis inhibitors.

Vancomycin – activity restricted to Gram+ organisms.

1. Complex soluble glycopeptide
2. Binds to R-D-Ala-D-Ala structures, like the peptidoglycan precursors, blocking peptidoglycan precursor transfer. May also permeabilize protoplasts and inhibit RNA synthesis.
3. Somewhat toxic, but when less toxic drugs are ineffective or contraindicated, used against serious systemic staphylococcal or enterococcal infections or (because poorly absorbed from intestine) orally for *Clostridium difficile* enterocolitis. Side effects on hearing (8th cranial nerve) and kidneys.
4. Currently the only drug available for multiply resistant Enterococcus and MRSA (methicillin resistant *Staphylococcus aureus*).
5. VRE – vancomycin resistant Enterococcus, capable of transferring resistance to MRSA has been demonstrated under laboratory conditions. Vancomycin-resistant *S. aureus* (VRSA) HAVE NOW BEEN IDENTIFIED in a clinical setting. Vancomycin-resistant *Enterococcus faecalis* (VREF) represents a rapidly escalating serious clinical problem.

Teicoplanin – another glycopeptide antibiotic.

1. Chemically similar to vancomycin.
2. Greater lipophilicity resulting in excellent tissue and intracellular phagocytic penetration.
3. Potential advantages over vancomycin: Long elimination half-life, less toxic than vancomycin.
4. Disadvantages compared to vancomycin: No oral formulation, very expensive.
5. Not yet approved by the FDA. It is available from the manufacturer on a "compassionate use" basis, for treatment of vancomycin resistant enterococcus infections, mostly.

F. Cycloserine – secondary tuberculosis drug; toxic.


1. A D-Alanine analog which inhibits L-Ala → D-Ala, and D-Ala + D-Ala → D-Ala-D-Ala; inhibits cell wall synthesis.

G. Bacitracin – activity restricted to Gram+ organisms.

1. Inactivates the phosphatase responsible for regenerating the active form of the carrier lipid in murein precursor synthesis (Lipid PP → Lipid P + P).
2. Toxic and restricted to topical therapy, often in conjunction with polymyxin B and neomycin (in common antibiotic ointments such as Neosporin).

III. ANTIBIOTICS THAT AFFECT MEMBRANE PERMEABILITY

– cidal agents – *In contrast to b-lactams, cell growth is not required for activity.*

- A. Polymyxins – polymyxin B and polymyxin E (Colistin) – selective activity against G- enteric rods (especially important against *Pseudomonas*).
1. The positively charged polypeptide antibiotic binds first to the negatively charged LPS in the outer membrane, then to the cytoplasmic membrane phospholipids, causing membrane leakage.
 2. Commonly used as topical agents – systemic use largely supplanted by more ctive & and less toxic agents.
- B. Daptomycin – A lipopeptide that inserts into the cytoplasmic membrane of bacteria. Sold under the tradename of Cubicin (Cubist Pharmaceuticals) it is used specifically against multiply resistant gram positive organisms. It is administered intravenously once a day. The molecule is too large to penetrate the outer membrane of gram negative bacteria, and hence is ineffective against this group.

IV. INHIBITORS OF PROTEIN SYNTHESIS

- A. Biochemistry of protein synthesis in bacteria, in brief (and over-simplified):

mRNA binds to 30S. Then formylmethionyl tRNA and 50S subunit are added on to form an initiation complex. A second aminoacyl tRNA adds to ribosome and peptide bond is formed. Translocation, chain extension, and finally release of completed chain (protein). Special enzymes required for all steps.

Inhibitors are known to block some of these steps. Many of these inhibitors specifically inhibit protein synthesis in *bacteria* but not in *mammalian* cells-- hence they are of clinical use.

- B. Aminoglycosides – Streptomycin

Streptomycin, the first important agent of its class, is no longer widely used in therapy (except in the treatment of tuberculosis and in a few other special situations). It is useful, however, to consider it in some detail, as an example of how the mechanism of action was deduced. Other agents in this group—known collectively as the *aminoglycosides*—resemble streptomycin in many respects.

Streptomycin was discovered by Waksman (1944) in a deliberate search for antibiotics produced by soil bacteria. This discovery extended the range of antibiotic therapy to *Mycobacterium tuberculosis* and to many gram-negative organisms, for which there had not been an effective treatment.

Streptomycin is positively charged at physiological pH. It does not penetrate bacteria readily, and some metabolic activity by the bacterium is needed for streptomycin to enter. *Under anaerobic conditions, or acid conditions—as in urine—action is inhibited.*

Proposed cidal mechanism of aminoglycosides:

1. Rapidly bacteriocidal, but—in contrast to penicillin—not lytic. Can show that action is on 30S ribosomal subunit, by mixing sensitive and resistant subunits in a "criss-cross" experiment. Further proof now available by reconstitution of 30S subunits from individual RNA and protein molecules. Binds to a specific ribosomal protein.
2. A few molecules of streptomycin enter the cell through imperfections in the growing membrane—at low concentrations it binds specifically to a 30S ribosomal protein, distorting the acceptor site—causing misreading.

Misreading causes "bad" proteins to be made, membrane leakiness ensues and streptomycin uptake increases.
3. At higher concentrations inhibits formation of the initiation complex and of peptide bond formation.
4. Resistant mutants readily obtained. Mutation occurs before contact with streptomycin. Streptomycin "selects" for these mutants by providing an environment that favors their growth while inhibiting non-resistant bacteria.

C. Other aminoglycosides

This group includes: amikacin, gentamicin, kanamycin, tobramycin, neomycin, and paramomycin. All inhibit 30S ribosomal function, and are bacteriocidal. Aminoglycosides other than streptomycin interact with more than one ribosomal protein on the 30S subunit; hence one cannot obtain resistance to these agents in "one step," as with streptomycin. Some of these aminoglycosides have replaced streptomycin in clinical therapy.

In general, the aminoglycosides have toxic effects, damaging the 8th cranial nerve (auditory, vestibular), or renal function. Hence use of an aminoglycoside is limited to serious infections where the antibiotic must be used in spite of the risks of toxicity. In patients who have a compromised (i.e., defective) immune system, antibiotics that kill bacteria (bacteriocidal) are often preferable to those that only inhibit growth (bacteriostatic); hence aminoglycosides and other "cidal" antibiotics are often used for such patients.



As is true for streptomycin, other aminoglycosides require aerobic conditions to be effective.

D. Tetracyclines – tetracycline, doxycycline, minocycline

Important and widely used antibiotics. Block binding of aminoacyl-RNA to 30S ribosomal subunit. Bacteriostatic. Well absorbed orally and hence suited to out-patient treatment when therapy is needed over a week or two. Broad spectrum of action (including mycoplasma, rickettsia, and chlamydia). Not to be given in pregnancy because of possible adverse effects on fetus—some serious, some minor. Up to the age of about 8, children given tetracycline may develop mottled enamel—not a serious health hazard, but disfiguring.



A newer tetracycline derivative, introduced by Wyeth in June 2005, is tigecycline (trade name Tygacil). This is the most potent tetracycline derivative. It contains a chemical side-chain that makes it refractory to a common mechanism of tetracycline resistance that involves an efflux pump (more on the efflux pump later).

(Note: The broad spectrum of action may have drawbacks: after prolonged oral administration the composition of flora in the gastro-intestinal tract changes, sometimes resulting in diarrhea.)


E. Inhibitors of 50S ribosomal function

1. Erythromycin – (a macrolide) blocks chain elongation. Bacteriostatic. Very widely used therapeutic agent with spectrum of activity similar to penicillin G but includes mycoplasma and chlamydia.


Azithromycin and clarithromycin – new oral drugs related to erythromycin but with higher activity and a slightly broader spectrum. They give high and sustained tissue concentrations ($T_{1/2} = 70$ hr) which increase at a site of infection--attributable to uptake by phagocytes which migrate to the site.

2. Chloramphenicol – blocks polypeptide chain elongation. Simple structure. Bacteriostatic, although in some bacterial species (e.g., *Haemophilus influenzae*) cessation of growth leads to a cidal effect. Chloramphenicol rarely induces uncommon, but sometimes lethal, aplastic anemia. Hence *not* widely used. Use restricted to infections in which it is vital for therapy. Useful against some *anaerobes*, particularly bowel anaerobes such as *B. fragilis*. In the U.S., dispensing of chloramphenicol requires a prescription; in many other countries, it is sold over the counter.
3. Clindamycin – a derivative of lincomycin. Has activity against G+ and moderate activity against *anaerobes*. Bacteriostatic. Inhibits peptidyl transfer.
4. Sreptogramins – Another new class of antibiotics, binds to the 50S subunit. Bacteriostatic. The clinical prototype is a combination of dalfopristin and quinupristin (trade name Synergyn as the combination works synergistically being 16-fold more active than either alone). Potential for treating MRSA, VREF, and other multiply resistant bacteria.

F. Inhibition of translation by other interactions with the ribosome


Oxazolidinones - First new class of anti-ribosomal drugs to be developed in 35 years! Bacteriostatic. Highly active against G+ organisms. Potential for treating especially VRE  but also potentially of use against MRSA, VRSA, and other multiply resistant bacteria. The prototype is linezolid (trade name Zyvox), with excellent pharmacokinetics, equal bioavailability by both oral and intravenous routes and no need for dose adjustment in patients with renal impairment. Oxazolidinones probably work by inhibiting tRNA translocation. Interacts with the 16S RNA and the 23S rRNA of the 30S and 50S ribosomal subunits. Site of interaction determined by characterizing antibiotic resistant mutants. Drug was first used in US in April of 2000, first clinical isolates of linezolid-resistant VREF patients in April of 2001.

G. Inhibition of translation by other mechanisms

Mupirocin – An antibiotic that binds a specific tRNA synthetase (isoleucyl-tRNA synthetase) and prevents its function, resulting in no charged Ile-tRNA's available for protein synthesis. 

1. Bacteriostatic at low concentrations but bacteriocidal at high concentrations achieved by topical administration, perhaps due to lack of incorporation of isoleucine into protein chains in the cell wall.
2. Useful for treatment of MRSA, especially against nasal carrier state. Also for topical treatment of impetigo caused by *S. aureus* or *S. pyogenes*. Weaker effects against natural surface flora.

V. INHIBITORS OF DNA REPLICATION

- A. Quinolones – The most commonly used are the fluoroquinolones, ciprofloxacin and moxifloxacin. Active vs. G- enteric bacilli; some G+ cocci and *P. aeruginosa*; used for a variety of infections; notably UTI, respiratory and even anaerobic. Ciprofloxacin is the antibiotic of choice for therapeutic treatment of anthrax, and can even be used as a prophylactic when exposure to *B. anthracis* is a potential risk, such as in a first responder situation. 
1. Inhibits the enzyme DNA gyrase necessary for DNA synthesis. Bacteriocidal. The fluoroquinolones also target topoisomerase.
 2. However, despite two targets, resistance is emerging rapidly - in one study the ciprofloxacin resistance of MRSA went from <5% to >80% within 1 year! Therefore no longer recommended for treating MRSA.
 3. Quinolone derivatives should not be prescribed for pregnant women or children because they can damage growing bone. There are also reports of neurotoxicity.
- B. Nitroimidazoles – metronidazole (Anaerobic drug)
1. Activated form of the drug binds DNA and fragments it.
 2. Useful against anaerobic bacteria, especially *Bacteroides* species; bacteriocidal
 3. Also used for certain protozoal infections (trichomoniasis and amebiasis)
 4. Its action requires anaerobic conditions – In such cases the antibiotic is reduced and activated by an electron transport protein (Ferredoxin).

VI. INHIBITORS OF RNA SYNTHESIS

- A. Rifampin – broad spectrum, bacteriocidal—it inhibits transcription by binding to the β subunit of bacterial RNA polymerase, inhibiting specific binding to DNA.
1. Often used in combination with other antibiotics since resistance develops rapidly when used alone. For example, used in combination with isoniazid or pyrazinamide as major therapies against tuberculosis.
 2. Rifampin is efficiently secreted in saliva. This makes it very useful as a prophylactic against infectious bacteria that enter via the nasopharyngeal route. A notable example is for preventing spread of *N. meningitidis*.

VII. OTHER ANTIBIOTICS

- A. Ethambutol – Important antituberculosis drug; mechanism of action unknown. It is bacteriostatic against tubercle bacilli.
- B. Pyrazinamide - Important antituberculosis drug; mechanism of action unknown. It is a bacteriocidal drug that requires the activity of the Mycobacteria amidase to become activated.

VIII. COMBINED ANTIBIOTIC ACTION

- A. Possible consequences: indifference (additive), synergistic, antagonistic
1. Potential Antagonism – an agent which requires growth (e.g., penicillin) plus a bacteriostatic agent (e.g., tetracycline).
 2. Potential Synergism – an agent which damages the cell wall/membrane (penicillin/polymyxin) plus a cidal agent which is taken up poorly by the bacterium (aminoglycoside).
- B. Indications – in general, more than one agent should be used only when:
1. Synergistic antibacterial action can be expected.
 2. The susceptibility pattern of the most probable pathogens require use of more than one agent.
 3. The likelihood of development of bacterial resistance is reduced.
 4. The dosage of a toxic drug can be reduced.
 5. A polymicrobial infection requires use of more than one agent.
- C. Disadvantages – increased risk of the side effects and superinfections; possible drug antagonism; increased cost.

IX. MECHANISMS OF RESISTANCE TO ANTIBIOTICS

A. GENERAL REASONS FOR ANTIBIOTIC INACTIVITY

1. Antibiotic is inactivated, either extracellularly, intracellularly, or both.
2. Antibiotic cannot enter the cell, or is actively pumped out.
3. Bacterial cell contains an altered enzyme that resists action of antibiotic.
4. Antibiotic can enter the cell but the drug-binding target site is replaced.

B. ENZYMATIC INACTIVATION

1. β -lactamases cleave the β -lactam ring
penicillins, cephalosporins, etc.
2. Aminoglycoside modifying enzymes – generally modifies drug during transport across the cytoplasmic membrane
acetylation, adenylation and phosphorylation kanamycin, gentamicin, streptomycin
3. Chloramphenicol acetyltransferase – acetylation of chloramphenicol
4. Erythromycin esterase – hydrolyzes lactone ring

C. ALTERED MEMBRANE PERMEABILITY

1. Outer membrane porins decreased expression
 β -lactams, nalidixic acid, chloramphenicol
2. Inner membrane transporters altered
amino glycosides, rare since usually deleterious regarding PMF

D. ANTIBIOTIC EFFLUX FROM THE CELL

Tetracycline resistance

E. ALTERATION OF RIBOSOMAL TARGETS



1. Methylation of 23S rRNA (component of 50S ribosome)
Erythromycin resistance. There is also a methylation resistance mechanism for tet, the ribosomal target is not known.
2. Alter S12 of 30S subunit – streptomycin resistance
These mutations are the most common mechanism for Em^r and Sm^r . Don't occur so frequently for gentamicin, tobramycin, amikacin all of which have multiple ribosomal targets

F. ALTERATION OF CELL WALL PRECURSOR TARGETS

1. Important for vancomycin and teichoplanin resistance
Inducible gene that encodes an enzyme related to D-ala-D-ala ligases. However this enzyme makes D-ala-D-lactate which substitutes for D-ala-D-ala in PG precursors and is not recognized by vancomycin or teichoplanin.

G. ALTERATION OF TARGET ENZYMES

1. Altered PBP's – Lower affinity or change in amount
Methicillin resistance is associated with altered affinity PBP's.
Also common pen^r in *Neisseria*, *H. influenzae*, and *P. aeruginosa*
2. Altered dihydropteroate synthetase – sulfonamide resistance
Altered DHFR – trimethoprim resistance

H. BYPASS PATHWAYS

Trimethoprim resistance – other pathways to form DNA precursors



Antibiotics and common mechanisms of resistance

<u>Agent</u>	<u>Mechanism of Resistance</u>
1. Trimethoprim	Plasmid-coded, trimethoprim-resistant dihydrofolate reductase.
2. Penicillins	Hydrolysis of β -lactam ring by β -lactamase enzyme; decreased Cephalosporins permeability of bacteria to the antibiotic; altered penicillin binding proteins.
3. Methicillin	Altered penicillin binding proteins (not introduction of a β -lactamase). (methicillin is a β -lactamase-resistant penicillin).
4. Aminoglycosides	Enzymatic modification of drug by plasmid-coded enzyme; decreased permeability of bacteria to the antibiotic; mutation alters site to which antibiotic normally binds (e.g. S12 ribosomal protein for streptomycin).
5. Tetracyclines	Interference with transport of drug into cell.
6. Chloramphenicol	Detoxification of drug by acetylation of hydroxyl.
7. Erythromycin	Enzymatic methylation of 23S ribosomal RNA.
8. Ciprofloxacin Rifampin	Altered target enzymes (gyrase, RNAP)
9. Vancomycin	Alteration of cell wall precursor target.

SUMMARY OF MECHANISMS OF ACTION OF ANTIBIOTICS AND ANTIFUNGALS

<u>Agent</u>	<u>Mode of Action</u>
1. Antimetabolites sulfonamides, trimethoprim	Inhibit synthesis of dihydrofolate, ultimately nucleic acid synthesis.
2. Antimetabolite flucytosine	inhibits thymidylate synthetase and ultimately DNA synthesis
3. Isoniazid	Inhibits synthesis of mycolic acid (specific for Mycobacteria).
4. β -Lactams, penicillins cephalosporins	Interfere with cell wall biosynthesis. Inhibition of transpeptidation weakens cell wall. Autolytic enzymes of bacteria then promote lysis.
5. Cycloserine, bacitracin, glycopeptides, vancomycin, teichoplanin, caspofungin	Interfere with cell wall biosynthesis.
6. Polymyxins, polyenes nystatin, amphotericin B daptomycin	Disrupt membrane integrity.
7. Aminoglycosides streptomycin	Inhibit protein synthesis by binding to a specific protein of 30S ribosomal subunit.
8. Aminoglycosides gentamicin, kanamycin neomycin, amikacin tobramycin	Bind to 30S ribosomal subunit at multiple sites. Inhibit protein synthesis.
9. Tetracyclines	Block protein synthesis by inhibiting binding of amino-acyl t-RNA to 30S subunit.
10. Erythromycin, azithromycin, clindamycin chloramphenicol	Bind to 50S ribosomal subunit. Inhibit protein synthesis at chain elongation step.
11. Oxazolidinones linezolid	Bind rRNA on both ribosomal subunits (new)
12. Streptogramins dalfopristin, quinupristin	Bind to 50S ribosomal subunit (new)
13. Mupirocin	Binds isoleucyl tRNA synthetase, preventing charging of Ile-tRNA and thus affecting protein synthesis.
14. Fluoroquinolones ciprofloxacin	Inhibit bacterial DNA gyrase.
15. Nitroimidazoles	Fragment DNA
16. Rifampin	Binds to bacterial RNA polymerase and blocks transcription.
17. Azoles fluconazole, ketoconazole	Inhibit ergosterol synthesis.
18. Ethambutol	Unknown, anti-TB
19. Pyrazinamide	Unknown, anti-TB



DIAGRAM INSERTED HERE



Sites of action of various antibacterial agents; mRNA, messenger RNA; tRNA, transfer RNA; PABA, *p*-aminobenzoic acid; DHFA, dihydrofolic acid; THFA, tetrahydrofolic acid. From "The Crisis in Antibiotic Resistance" by H.C. Neu, *Science* (1992) **257**, 1064-1073.

Molecular Methods for the Diagnosis of Infectious Diseases

Learning Objectives:


- To describe the basis of the molecular techniques in clinical use.
- To describe the limitations of molecular diagnostics.
- To describe why false positives can occur.
- To describe the organisms best suited for molecular diagnostics.


1. Traditional techniques include Culture, Stains and Serology.
 - a. **Culture** relies on being able to grow and identify a pathogen, but this takes days to weeks, and there are agents that we cannot grow in the laboratory, or cannot identify once they are growing.
 - b. **Staining** relies on the reaction of dyes with various components of pathogens; most stains require more than 100,000 organisms per ml of specimen to detect any by microscopy. Some techniques, such as immunofluorescence, where a fluorescent dye is attached to a specific antibody, can detect less than this, but still are relatively insensitive.
 - c. **Serology** relies on the infected individual making an immune response to the pathogen. This usually takes weeks, and may not occur in immunosuppressed individuals.

2. Molecular detection of pathogens requires knowledge of specific DNA or RNA sequences of the organism, and a way to identify these in the specimen. Hybridization has been used for years, but is relatively insensitive. Amplification techniques allow million-fold multiplication of the target sequence or of a specific detector molecule. You still have to understand something about the organism and disease pathogenesis to be able to use the techniques wisely.
 - a. Advantages of molecular amplification methods
 - i. Can detect very small numbers of organisms (down to single numbers, especially where the target sequence is present in many copies per organism)
 - ii. Theoretically fast (minutes to hours)
 - iii. Can detect organisms that cannot be cultured
 - iv. Can be multiplexed to detect more than one target simultaneously

 - b. Disadvantages of molecular methods
 - i. Must know a specific nucleic acid sequence
 - ii. Must be able to interpret the meaning of detecting the specific sequence – eg. obligate pathogens, colonization vs. disease
 - iii. Must be able to exclude false positive tests, for instance from target contamination of environment of clinic or lab.
 - v. Present technology may not allow theoretical speed of test – many tests must be batched, may take days.

3. Amplification techniques in common clinical use
 - a. PCR (polymerase chain reaction)
 - b. TMA (transcription mediated amplification)
 - c. LCR (ligase chain reaction)
 - d. bDNA (branched chain DNA amplification)

4. **Formats in use** 
 - a. **Qualitative:** uses amplification to detect the smallest possible number of target molecules. Most sensitive, but may be useful only when detection of any evidence of the organism correlates with disease.
 - b. **Quantitative:** (“Pathogen Load”) Use of a technique that generates a graded signal that correlates with target number. Mostly used with agents that have a latent phase, such as herpes viruses. Useful in following therapy (e.g. HIV viral load). May also be useful in trying to sort out endogenous carriage from disease (CMV viral load in immunosuppressed patients)

5. Some bacterial pathogens currently detected by molecular techniques (not a complete list, examples of most used tests, viruses and protozoa not covered here.)
 - a. Bacteria
 - i. *Bordetella pertussis*
 - ii. *Neisseria gonorrhoea*
 - iii. *Chlamydia trachomatis*
 - iv. Group A and Group B Streptococci 
 - v. *Staphylococcus aureus*
 - vi. *Mycobacterium tuberculosis*
 - vii. *Mycoplasma pneumoniae*
 - viii. Lyme Spirochetes (*Borrelia burgdorferi*)
 - ix. Others
 - b. Fungi
 - i. *Histoplasma capsulatum*
 - ii. *Coccidioides immitis*

6. Example of the use of an amplified test for clinical diagnosis: Chlamydia/ GC by TMA method.


7. Other Molecular Techniques
 - a. Identification of microorganisms
 - i. Hybridization in solution for identification of isolated colonies
 - ii. PCR and sequencing for bacterial identification
 - iii. Multiplex PCR for rapid identification of bacterial pathogens
 - b. Strain comparison for epidemiology
 - i. PFGE (Pulsed Field Gel Electrophoresis)
 - ii. RFLP and other fragment identification schemes
 - iii. Example: bacteremia in dialysis patients
 - iv. Complete sequence of isolates “deep sequencing”

INTRODUCTION TO SPECIFIC PATHOGENS

Learning objectives

- To explain the relationships between species, strains and serotypes.
- To explain the use of flagellar and LPS antigens as the basis for strain identification.
- To explain the role of endotoxin (lipid A) in disease.
- To understand how the fermentation patterns and other characteristics of *Salmonella* and *Shigella* are used in diagnosis.

1. Species can be broken down in to serotypes and strains

- Bacteria are classified by family, genus, species, subspecies and strains.
 - A **strain** is a population of organisms within a species that descends from a single organism.
 1. Strains evolve within a species by mutation and/or by acquiring additional genes by horizontal gene transfer.
 2. Surface components often vary among strains within a species to decrease detection or killing by a host or predator
 - **A serotype is a stain that is differentiated by serological means**
 - Serotyping uses specific antisera that contains antibodies to specific bacterial antigens
 - Serotyping is based on antibody recognition of antigens
 - Specific antisera contain antibodies that recognize bacterial surface elements
 1. O antigens– the polysaccharide component of LPS 
 2. H antigens – the flagellar antigen
 3. K antigen - a polysaccharide capsule component
 - The O-antigen is made up of repeating oligosaccharide units. Up to twenty different sugars can be found, and some are unique to LPS.
 - O-side chains are easily recognized by the antibodies of the host, and thus they vary to aid in immune evasion.
 - Strains within a species can have different O-antigens (for example, *E. coli* O₁₅₇:H7 and O₁₀₄:H4)
 - Within a serotype, strains can vary due to mutations, gene acquisitions, and overall genome rearrangements.
2. **Infections within a person most often occur from a single strain. Epidemics are caused by a single strain.** In both cases, the strain can evolve different properties over short time periods (days and weeks).

3. **Infectious dose** is the approximate number of microbes that, on average, are required to cause an infection.

A person's susceptibility to infection is affected by many things including age, immune status, therapies, other infections, genetics (such as cell surface receptors), genetic diseases (cystic fibrosis, sickle cell anemia), or physical factors

4. A large part of diagnosis and prevention is understanding the risk factors for disease.
5. In an active infection, microbes are replicating. (Recall the differences in growth rates discussed in the Bacterial Growth Lecture.)

6. Some of the enzymes involved in nutrient acquisition directly cause damage to the host and thus are considered **virulence factors**. Examples include phospholipases, hemolysins, and proteases. Some of the enzymes are referred to as **toxins** or **exotoxins** because they are secreted from the cell.

The term "**virulence determinant**" refers to factors required for disease. Virulence factors are virulence determinants, but not all virulence determinants are virulence factors. For example, a metabolic pathway important for growth in the host is a virulence determinant but not a virulence factor.

7. Other secreted virulence factors or toxins modulate the immune system to decrease clearance. Secreted factors or toxins can also otherwise modulate the host in ways that increase microbial fitness (induction of a cough for an airborne pathogen or diarrhea for a GI pathogen). This can be detrimental to the host.

8. Some aspects of disease are caused by an overactive or systemic immune response. Gram-negative LPS in the outer membrane contains **endotoxin**, and endotoxin causes fever and shock.

- **The lipid A component of LPS is called endotoxin (in contrast to secreted exotoxins)**
- **The host innate immune system responds to low concentrations of endotoxin to enable rapid pathogen clearance.**

1. Macrophages activated by LPS to secrete $\text{TNF}\alpha$ in the tissue
2. Increased release of plasma proteins into tissue. Increased phagocyte and lymphocyte migration into tissue. Increased platelet adhesion to blood vessel wall.
3. Phagocytosis of bacteria.
4. Local blood vessel occlusion.
5. Plasma and cells drain to local lymph node.
6. Removal of infection.

- **Sufficient quantities of endotoxin induces a condition called "endotoxic shock"**
 1. Endotoxic shock is common and highly lethal (20-80% mortality) depending on patient status and the microbe
 2. More than 20,000 people die from toxic shock each year in the U.S.
 3. The net effects of host response to endotoxin in the blood stream is endotoxic shock
 - a. Fever
 - b. Inflammation
 - c. Hypotension
 - d. Disseminated Intravascular Coagulation (DIC) and bleeding
 - e. Organ failure due to lack of oxygen
- Many Gram-negative pathogens cause localized inflammation due to the response to LPS, and when Gram-negatives grow in the blood stream (sepsis), endotoxin can induce endotoxic shock.

9. Diagnosis can involve patient history, decisions about which samples to take, decisions about which tests to request. In the clinical labs, diagnoses rely on microbiological, microscopic, and molecular assays.

10. Treatment often, but not always, involves antibiotics. Supportive therapy can play a major role in treatment.

SALMONELLA AND SHIGELLA

Learning objectives

- To describe the properties of enterics
- To describe the syndromes cause by *Salmonella* and *Shigella* species.
- To describe how bacterial virulence determinants are thought to contribute to disease for each organism
- To explain the prevention and management of infections by *Salmonella* and *Shigella*

SALMONELLA

Salmonella is a member of the *Enterobacteriaceae* along with *Shigella* and *E. coli*.

Salmonella species, like *Shigella* and *E. coli*, are Gram-negative rods.

We will first focus on the species and strains of *Salmonella*, *Shigella* and *E. coli* that are pathogens of the human intestinal tract.

There are three clinically distinguishable syndromes related to Salmonellosis in man:

- 1) typhoid or enteric fever caused by *S. Typhi*
- 2) septicemias caused by *S. choleraesuis*
- 3) acute gastroenteritis caused by *S. enteritidis* or *S. typhimurium*

****In 2005, the nomenclature of Salmonella was modified to group different Salmonella “species” into the same species with different subspecies and serovars. However, the CDC recommends that for simplicity, the species are abbreviated. For example, *Salmonella enterica* serovar Typhi is often abbreviated *Salmonella typhi* or *Salmonella Typhi*.**

A. S. Typhi

1. Epidemiology:

22 million cases a year and more than 200,000 related deaths (WHO). *S. typhi* is rare in N. America, Europe and Australia, but common in the developing world.

2. Clinical presentation:

The disease caused by *S. Typhi* is referred to as *typhoid fever*.

Incubation period typically 7-14 days (early GI phase – may be subclinical with positive stool culture)


Episodic fever, bradycardia (slow heart rate), skin rash (called Rose spot) diagnostic, leukopenia (low WBC count) and enlarged liver and spleen (Bacteremic phase)

Intestinal hemorrhage or perforation during late stages (late GI phase)

3. Steps in Pathogenesis:

How does *S. Typhi* grow and survive? How do growth and survival of this microbe cause disease? *S. Typhi*:

- a. only infects humans (via contaminated food or water).
- b. Is somewhat resistant to killing by stomach acid.

- c. has adhesins that promote attachment to the intestinal epithelium.
- d. Salmonellae induce bacterially-mediated endocytosis after adherence to the apical membrane of epithelial cells. *The mechanism is discussed further below.*
- e. Macrophages take up extracellular bacterial cells. 
- f. Survival inside phagocytic vacuoles of macrophages (possibly enabled by Vi antigen which is a polysaccharide capsule.) The Vi antigen is only found in S. Typhi and not other *Salmonella*.)
- g. S. Typhi survives the acidic environment of lysosome in macrophages.
- h. It kills the macrophage and disseminates via the thoracic duct to blood, liver, spleen, and gall bladder.
- i. Bacterial factors in the blood stream cause fever and shock
- j. Reinvasion of the GI tract from the gall bladder
- k. GI bleed and sometimes diarrhea

4. Virulence determinants:

a. Invasion and intracellular survival are aided by the T3SS found on pathogenicity islands.

Specialized genes for *Salmonella typhi* virulence factors are encoded on two “pathogenicity islands”

Pathogenicity islands are acquired through horizontal gene transfer. A type of evolution!!

They have unexpected G+C content, presence of phage or transposon sequences, non-native adjacent sequences

SPI-1 (Salmonella Pathogenicity Island 1) encodes genes for invasion, and SPI-2 encodes genes for intracellular survival.

SPI-1 encodes a *type III secretion system* (T3SS). [See video.]

Type III secretion systems: A specialized form of secretion wherein a protein moves across the bacterial cytoplasmic and outer membrane AND across the host cell membrane through an injection needle.

Multiple pathogens have type III secretion systems

Effector proteins are virulence factors that are delivered into the host cell cytoplasm via the secretion apparatus. Effectors vary by species and strain.

In *Salmonella*, the T3SS delivers toxins that induce membrane ruffling by stimulating actin polymerization, and endocytosis.

b. Gram-negative LPS in the outer membrane contains endotoxin, and endotoxin causes fever and shock.

- Many Gram-negative pathogens cause localized inflammation due to the response to LPS, and when Gram-negatives grow in the blood stream (sepsis), endotoxin can induce endotoxic shock.

5. Diagnosis:

Isolation of the organism during typhoid fever varies depending on the stage of the disease.

- During the first, week of disease, the subclinical patient may have positive stool culture.
- Blood cultures are positive between 2nd and 3rd week and patients are symptomatic.
- Stool culture positive again after the 3rd week when the gall bladder is colonized during bacteremia
- Characterization of potential *Salmonella* infectious agents described in detail below.

Characterization of the isolated *S. typhi* is described at the end with the other *Salmonella* spp.

6. Treatment:

- *S. typhi* lives inside macrophages – consider tissue penetrations of antibiotics
- Fluoroquinolones (e.g. ciprofloxacin) or third generation cephalosporin (e.g. ceftriaxone).
- Chronic carrier states: 1) Ampicillin or Ciprofloxacin; 2) cholecystectomy (removal of the gall bladder)
- Relapse can occur in 10% of patients in endemic areas

7. Prevention:

- Control of water supplies and sewage disposal
High inoculum is required, and human are the exclusive host.
- Food safety
- Pasteurization of milk and screening for carriers among food handlers
- Two vaccines currently available
 - oral attenuated vaccine
 - Vi capsular polysaccharide vaccine
- Bacteria localized in a suppurative focus in gall bladder is secreted via the GI tract (3% of patients) and this is an important source for spreading infections under poor hygienic conditions
- The story of Typhoid Mary provides a classic example of this.

8. Vaccines:




Table 1: Typhoid Vaccines Available in the United States

Vaccine Name	How Given	Number of Doses Necessary	Time Between Doses	Time immunization should be completed by (before possible exposure)	Minimum Age For Vaccination	Booster Needed Every...
Ty21a (Vivotif Berna, Swiss Serum and Vaccine Institute)	1 capsule by mouth	4	2 days	1 week	6 years	5 years
VICPS (Typhim Vi, Pasteur Merieux)	Injection	1	N/A	2 weeks	2 years	2 years

Ty21a is a live attenuated vaccine, ViCPS is a capsular polysaccharide vaccine that targets the Vi antigen.

B. S. cholerae


- A rare disease. 
- Source: swine
- Oral ingestion of contaminated food
- Infectious dose: 1000 organisms
- Short incubation period (6-72 hrs)
- High fever and bacteremia after onset of gastroenteritis
- Microabscesses can develop in any body tissues
- Young age, malaria, immune dysfunction due to AIDS, steroid use, or immune suppressive therapy, and sickle cell anemia are associated with increased susceptibility.

C. S. enteritidis and S. typhimurium


1. General properties

- Most common Salmonella infections in the US
- *S. typhimurium* and *S. enteritidis* are associated with similar risk factors and their relative prevalence is similar in recent studies.
- Diarrheal disease mostly confined to the GI tract
 - positive blood cultures occur, but are infrequent (~5%). Non-typhoidal bacteremia associated with decreased immune system: young age, old age, immune suppression, etc.
- Used in one of two US bioterrorism events (1984 Oregon, Rajneeshee Attack)

2. Epidemiology:

- ~ 30,000 confirmed cases of *Salmonella* gastroenteritis in the US per year
- Estimates of 1.4m cases per year with 600 deaths 
- Over 2,200 different serotypes have been identified.

3. Sources:

- Poultry, pork, dog food, and eggs are reported sources for infection.
- Contamination of eggs is particularly common, and egg contamination can be both outside and inside the shell.
- Fruits and vegetables can carry the pathogens due to contamination. 
- Pets such as turtles and other reptiles can carry these pathogens.
- Playing in a sandbox is associated with disease.

4. Clinical syndrome:

- Symptoms begin 8-48h after consumption of contaminated food.
- Sudden onset of headache, chills, abdominal pain, vomiting, then diarrhea with fever.
- Lasts 1-4 days

5. Pathogenesis and virulence determinants:


- LPS release during invasion of epithelial cells of the small and large intestines are responsible for many symptoms.
- T3SS mediates invasion of epithelial cells
- Extracellular cells produce toxins (including a pertussis-like toxin) that promote inflammation and secretion.

6. Treatment:

- Mostly self-limiting, though complications can arise. Death uncommon.
- Fluid and electrolyte replacement might be needed
- Treat patients with predisposing conditions with appropriate antibiotics
- Antibiotic resistance testing can be useful as resistance is possible.

7. Discussion of the outbreak in 1984

8. Diagnosis of *Salmonella* infections (relevant for all three classes):

- Isolation of *Salmonella* in feces, or blood in the case of typhoid fever caused by *S. Typhi*.
- *Salmonella*, like all members of Enterobacteriaceae family, ferment glucose, are oxidase negative, and can reduce nitrate.
- *Salmonella* does not ferment lactose identified and non-lactose fermenters can be identified on MacConkey or EMB Agar 
- *Salmonella* strains are motile (in contrast to *Shigella*) and produce H₂S (which can form a black precipitate)
- Urease negative (in contrast to *Proteus*, which is urease positive)
- Indole negative (*E. coli* is indole positive-indole is the byproduct of tryptophan hydrolysis)
- Serotyping with O and H antigens and PCR tests allow species identification to trace outbreaks.

SHIGELLA

1. General properties:

- Hippocrates provided the first description of dysentery (bloody diarrhea)
- Epidemics of bacillary dysentery (shigellosis) described as early as 5th century B.C.
- ~14,000 cases reported annually in the US

2. Epidemiology:

- *Shigella dysenteriae*-most common species in the developing world
- *Shigella flexneri* (18% of the cases in the US)– also common in developing countries
- *Shigella sonnei* – most common species found in the US (75%)
- *Shigella boydii* (common in the Indian subcontinent)

3. Sources:

- Spread by Food, Fingers, Feces and Flies (4Fs)
- Children under 10 more susceptible, also infects adults
- No animal reservoir

4. Clinical Syndrome:

- Fever (due to LPS), diarrhea and bloody diarrhea with mucus, and abdominal cramps (due to Shiga toxin)
- Usually self-limiting, rarely fatal
- Bacteremia rare
- Hemolytic uremic syndrome can occur in association with *Shigella dysenteriae*
- Can detect organisms in feces up to 1-4 weeks after recovery (think about dissemination)

5. Virulence determinants and pathogenesis:

- Low inoculum (100 bugs) – acid tolerance
- Incubation period of 1-4 days
- Invades intestinal cells in the terminal ileum and colon
- Uptake by macrophages into phagocytic vacuoles (induced by T3SS-secreted *Shigella* protein)

- Escape from the phagocytic vacuole into the cytoplasm and cell-to-cell spread (T3SS dependent)
- Induces apoptosis (programmed self death) of macrophages, re-infect new cells
- IL-1 and TNF from monocytic cells leads to fever and systemic symptoms
- Intestinal ulceration due to Shiga toxin in *S. dysenteriae*
- Shiga toxin is an exotoxin with two subunits (A and B); subunit B binds to receptor on intestinal cells, and subunit A interferes with the function of 60S ribosomal RNA, inhibiting protein synthesis (also discussed in the *E. coli* lecture)
- May lead to apoptosis of mucosal cells and ulceration
- Diarrhea ensues due to fluid malabsorption

6. Treatment:

- Fluid and electrolyte replacement, especially in young children
- Antibiotics in severe cases
- Antibiotic susceptibility testing may be important as resistance is increasing

7. Diagnosis:

- Clinical symptoms not diagnostic
- Isolation of microorganism from feces
- Detection of PMNs in stool indicative of invasive disease
- Important diagnostic traits:
 - no gas with glucose fermentation
 - Shigella are lactose non-fermenters (like Salmonella)
 - no H₂S (Salmonella produces H₂S)
 - Shigella is nonmotile (no flagella), whereas Salmonella is motile.
- Contains O antigens only, no H antigen
- Indole and Urease negative

[Kligler Iron Agar demonstrates analysis of sugar fermentation, gas production, and H₂S production]

8. Prevention:

- Improve sanitation (human is the only host)
- No effective vaccine (live attenuated vaccine not effective)
- Recombinant O-antigen vaccine conjugated to inactivated Shiga toxin is a promising vaccine candidate – not approved yet

ESCHERICHIA COLI

Learning Objectives:

- To differentiate between commensal *E. coli* and enteropathogenic *E. coli*
- To understand the processes involved in the “evolution” of a pathogen
- To describe the basic properties of the enteropathogenic *E. coli* with respect to adhesins, toxins, and the diseases they cause, and their identification
- To explain how infections by *E. coli* are prevented and treated

Reading assignment: Levinson, pp. 150-153

E. coli is an enteric like *Salmonella* and *Shigella*.

E. coli are part of the normal microflora, and a major member of the microflora in newborns
E. coli synthesizes vitamin K and protects against other pathogens

However, specific strains of *E. coli* have evolved to become serious gastrointestinal pathogens by acquiring virulence genes as in *Salmonella* and *Shigella*.

There are a variety of *E. coli* intestinal pathogens:

- Enterohemorrhagic and Shiga Toxin *E. coli* (EHEC and STEC)
- Enteropathogenic *E. coli* (EPEC)
- Enteroaggregative *E. coli* (EAEC)
- Enteroinvasive *E. coli* (EIEC)
- Enterotoxigenic *E. coli* (ETEC)

Different enteropathogenic *E. coli* have acquired different sets of virulence factors.

Both endemic diarrhea and epidemic outbreaks associated with *E. coli*.

Strains are categorized as being either Shiga-toxin producing (STEC) or diarrheagenic (the rest).

Overview of *E. coli* epidemiology relating to intestinal disease:

- 2-4 billion episodes of diarrhea in developing countries
- 3-5 million deaths
- 25-50% of cases are due to *E. coli*
- Important agents of food poisoning as discussed below.

A. Enterohemorrhagic *E. coli* (STEC)

-Main serogroups is O157:H7. other EHEC serogroups include: O111, O26, O157:H⁻ (it is important to understand the “O” and “H” nomenclature for serogroups)

-In 2012, O104:H4 caused a major outbreak in Germany. (Discussed further below.)


1. Sources of infection

- Food (undercooked, contaminated ground beef, leafy vegetables, unpasteurized apple cider/juice, raw milk/dairy products, vegetables)
- Petting zoos
- Person-to-person contact is also an important mode of transmission.
- Very low infectious dose

2. Clinical Syndrome:

- a) Abdominal pain
- b) Bloody diarrhea
- c) The toxins can spread leading to hemolytic uremic syndrome (HUS).
 - i. HUS results in about 7% of cases and is due to destruction of red blood cells & damage to the lining of blood vessel walls
- d) Acute renal failure in severe cases (usually in children under 10; the elderly are also susceptible.)
- e) Cells remain largely extracellular (some strains can invade mucosal cells), but does not become systemic

3. Virulence Determinants and Pathogenicity:

- a) Pili-mediate attachment (relatively weak)
 - a. *E. coli* common pilus
- b) Type III secretion system (T3SS) that induces formation of attaching and effacing lesions [see Salmonella lecture for description of the T3SS]
- c) Genes for the T3SS are encoded on a pathogenicity island called the LEE pathogenicity island. The LEE encodes intimin, and tir. LEE stands for the locus for enterocyte effacement.
- d) **Tir**: a T3SS-secreted bacterial protein that is delivered to surface of epithelial cells to allow for *E. coli* attachment
- e) **Intimin**: Tir binding protein on surface of *E. coli*
- f) Other *E. coli* proteins can **recruit host cell actin**, causing altered morphology, and impact signal transduction pathways in the host cell to form A/E lesions
- g) Lesions leads to effacement (destruction of host cell microvilli)
- h) Shiga-like toxin
 - a. Similar to Shiga toxin (produced by *Shigella*)
 - b. Gene found on phage
 - c. Shiga-like toxin disrupts eukaryotic protein synthesis and is cytotoxic.
 - d. -Interferes with protein synthesis via its RNA cleavage activity (subunit A) and may impact cytoskeleton (subunit B)
 - e. HUS (Hemolytic uremic syndrome) is due to the activity of Shiga-like toxin.
 1. Why is the cow not susceptible to EHEC? Shiga toxin binds to Gb3/CD77 host glycolipid likely by the B subunit unit and this host receptor is not present in cattle.
- i) Hemolysin
 - a. Pore forming protein that inserts into host cell membranes
 - b. Common in *E. coli* strains that cause meningitis (next lecture)
 - c. Present in other Gram-negative pathogens
 - d. Encoded by a plasmid
- j) Capsule (K-antigen), LPS, and nutrient acquisitions pathways

B. Diarrheagenic *E. coli*

- **Enteropathogenic *E. coli* (EPEC)**
 - Overt human pathogen that is transmitted by person-to-person contact
 - Leading cause of childhood diarrhea in developing countries; very common in developing countries with over 100 million cases per year.
 - Forms A/E lesions
 - Localized adherence by bundle forming pili
 - Toxins **not** detected in stool of infected human volunteers

- **Enterotoxigenic *E. coli* (ETEC)**
 - Traveler's Diarrhea and problematic in infants in the developing world.
 - Fimbriae adhere to specific receptors on enterocytes in the **small intestine**.
 - **Heat-labile toxin (LT)** which targets adenylate cyclase leading to increased cAMP levels that result in excess chloride ion secretion and blocked sodium ion uptake. Leads to net loss of fluid and electrolytes into lumen of the gut and watery diarrhea.
 - **Heat stable toxin (ST)** which alters cGMP levels with similar outcome to the heat-labile enterotoxin.

- **Enteroaggregative *E. coli* (EAEC)**
 - Childhood diarrhea similar to EPEC; common in developing countries.
 - Can cause a persistent diarrhea that can lead to weight loss.
 - Somewhat more aggressive than EPEC due to different colonization factors that lead to more aggressive epithelial cell attachment
 - No A/E lesions and non-invasive
 - A heat stable-like toxin (called EnteroAggregative stable toxin or EAST), a poorly characterized plasmid encoded toxin (Pet), and hemolysin produced.

- **Enteroinvasive *E. coli* (EIEC)**
 - Less common in industrialized nations
 - Attaches to cells within the colon by non-fimbrial adhesins
 - Organism invades mucosal cells, multiplies within these cells, but does not become systemic
 - Clinical syndrome is similar to *Shigella* dysentery with a watery diarrhea that can contain blood and mucus
 - Genes for invasion, replication within host and survival are encoded by a plasmid that contains genes very similar to those found in *Shigella*
 - EIEC strains do **not** produce ST and LT

- **Diffuse adhering *E. coli* (DAEC)**
 - Diarrhea in older children in developing countries
 - Poorly characterized, may be a heterogenous collection of strains

D. *E. coli* strains continue to evolve.

The combination of the ability to form aggregates on cells (as in EAEC) and the ability to produce Shiga-like toxin in one strain led to deadly outbreak in Germany in 2012.

4. Diagnostics :

- *E. coli* is Lac⁺, easily discerned on EMB or MacConkey agar, whereas *Salmonella* and *Shigella* are Lac⁻.
- Main diagnostic tools for O157:H7
 - O157:H7 cannot grow on sorbitol. Sorbitol MacConkey Agar O157:H7 is sorbitol-negative (colorless), while commensal *E. coli* strains are sorbitol-positive
 - Serology: Direct or latex agglutination tests (to identify O157 antigen); H7 serology and toxin analysis (Reference Laboratory)
- Immunoassay for Shiga-like toxin. (CDC recommends assays for detection of Shiga-like toxins early in disease to catch outbreaks and start early fluid therapy to fight HUS.

- PCR or DNA probe analysis of virulence genes is an important way to identify strains.
- Tissue culture assays
- Strain typing (Pulsed Field Electrophoresis) is important in tracing an outbreak
 - Description of an outbreak of EHEC infections
 -

5. Prevention:

- 2010 FDA report finds 28% of cattle shed EHEC.
- Grain fed cattle harbor more *E. coli* and the *E. coli* are more resistant to acid shock. Hay feeding leads to a higher pH and thus lack of acid adaption of pathogenic *E. coli*
- Change in practices may reduce carriage (hay feeding and probiotics)
- Hygiene is key.

Summary of the *E. coli* intestinal pathogens

	Acquisition and Symptoms	Toxins and vir factors*	Invasion	Diagnosis	Treatment
STEC (EHEC)	Food and water, person to person	A/E, H, SL	Mod.	PCR/immunoassay for Shiga toxin, Nucleic acid detection of virulence genes EHEC-sorbitol ferm.	Supportive care. Antibiotics and antimotility agents contraindicated
ETEC	Food and water, person to person traveler's diarrhea; childhood diarrhea (developing countries)	ST, LT	No	Nucleic acid detection of virulence genes	Loperamide (may be combined with fluoroquinolones), azithromycin, or rifaximin for travelers
EPEC	Person to person Infantile diarrhea in developing countries	A/E, Bfp	Moderate	Nucleic acid detection of virulence genes or tissue culture assay for localized adherence	Antibiotics guided by susceptibility testing for severe or protracted cases
EAEC	Chronic diarrhea Emerging cause of other diseases	H, EAST	No	Nucleic acid detection of virulence genes or tissue culture assay for aggregative adherence	Fluoroquinolones may be used for travelers and HIV patients
EIEC	Contaminated food	?	High	Nucleic acid detection of virulence genes	
DAEC	Children in developing countries	?		Tissue culture assay for diffuse adherence	

*Toxins: Attaching and effacing lesions (A/E), Shiga-like toxin (SL), heat stable toxin (ST), heat labile toxin (LT), EnteroAggregative stable toxin (EAST), and hemolysin (H)
Bfp=bundle forming pili

GRAM-NEGATIVE OPPORTUNISTIC INFECTIONS

Key organisms: *Escherichia coli*, *Klebsiella pneumonia*, *Serratia marcescens*, *Enterobacter cloacae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*

Learning Objectives

- To describe some commonly isolated gram negative bacteria that often grow as opportunists in debilitated patients.
- To explain how specific properties of each organism can contribute to its ability to cause infections.
- To describe the characteristics of gram negative opportunistic pathogens that aid in identification and diagnosis.
- To explain how infections by gram negative opportunists are prevented and the role of antibiotics in controlling infections.

Reading assignment: Levinson, pp. 160-164; pp. 209-210 (*Acinetobacter*)

Opportunistic pathogens-An introduction:

- Pathogens that are capable of causing disease only in *compromised* people
 - Decreased or altered immune system function
 - Alterations in innate protective mechanisms
 - Breach in physical barriers
- These pathogens show a less-specialized adaptation to the host than typical pathogens.
- Cause 90,000 deaths and \$5 b/year in US (NEJM 348:651)
- Many of these infections should be preventable
- Need for surveillance to identify sources of contamination

GENERAL POINTS ABOUT GRAM-NEGATIVE OPPORTUNISTIC INFECTIONS

1. Opportunistic infections are increasing for the following reasons:

- a. The increased scope of surgical treatments, implants, and transplants.
- b. Increased use of indwelling devices such as catheters.
- c. The increased capacity to sustain the chronically ill.
- d. The increased use of medical interventions that directly and substantially affect the immune systems such as chemotherapies and immunosuppressive therapies.
- e. Immunosuppression due to a primary infection (i.e. AIDS) has resulted in increased clinical cases of opportunistic infections.

2. Gram-negative opportunistic pathogens are found in numerous environments and survive under diverse conditions

3. LPS contributes to the symptoms of infections.

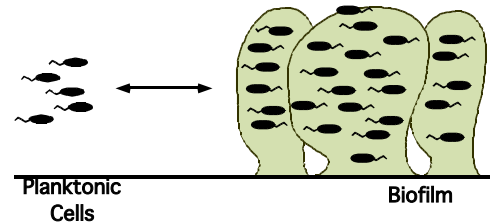
Other virulence determinants can include:

- Cell-surface structures that aid in the colonization of host surfaces.
- Factors involved in nutrient acquisition from the host
- Toxins and secreted enzymes

4. Opportunistic infections that occur in the hospital are referred to as hospital-acquired infections or nosocomial infections.

A major contributor to these infections is a simple lack of washing hands!

5. Most opportunistic bacteria can form biofilms which are dense microbial communities surrounded by an extracellular matrix.



- Associated with implant and catheter-related infections
- Implicated in chronic lung infections, endocarditis, and UTIs
- ~65% of nosocomial infections are thought to be biofilm-related
- Biofilm bacteria have increased resistance to a range of antimicrobial agents compared to planktonic cells

SPECIFIC OPPORTUNISTIC PATHOGENS

A. *ESCHERICHIA COLI*

E. coli is the most common cause of Gram-negative infections. *E. coli* can cause:

1. Gastrointestinal infections (previous lecture)
2. Urinary tract infections (UTI)
3. Bacteremia
4. Meningitis

E. coli is part of our normal flora and the most common opportunistic pathogen. Normal flora *E. coli* can cause UTIs, bacteremia and meningitis. Gastrointestinal infections are caused by specialized strains.

Uropathogenic *E. coli* (UPEC)

1. Epidemiology:

E. coli cause 95% of all non-hospital acquired urinary tract infections. About 60% of women in the United States will have at least one UTI during their lifetime, and 11% experience at least one per year.

The infecting organisms are associated with only a few serogroups (e.g., 01, 02, 04, 06, 07, 075, 0150).

Generally, women are more susceptible to UTI than men. Sexual intercourse in women can contribute to infection by bacteria. This is due to the presence of bacteria on the vulvar and perirectal areas and the short length of the female urethra.

Catheters are a source of infection.

2. Clinical Syndrome:

A bacteriuria infection is an infection in which at least 10^5 bacteria/ml are present in urine.

Cystitis is the term used to describe the syndrome involving dysuria (burning feeling during urination), frequency, urgency, and occasionally suprapubic tenderness. It typically involves infection of the lower urinary tract but may include upper urinary tract infection as well.

Acute pyelonephritis results from a urinary tract infection that has disseminated to the kidney. Results in a clinical syndrome characterized by flank pain, tenderness and fever, dysuria, frequency and urgency

These infections are typically thought to be acute and self-limiting, however 27–44% of women with an initial UTI will experience at least one recurrence of symptoms within six months, despite antibiotic therapy.

3. Pathogenicity:

Invasion and spread of bacteria into the urinary tract is almost always associated with an ascending route of infection. The urethra is usually colonized with bacteria.

Not all UPEC strains are equivalent. Different *E. coli* strains have different types can elaborate different types of surface adhesions. The type of adhesin elaborated by a particular *E. coli* strain can influence the locations that it is capable of colonizing and thus the type of infection caused.

<u>Uropathogenic <i>E. coli</i> adhesins</u>	
<u>Adhesin</u>	<u>Disease</u>
<u>pili/fimbriae</u>	
P pili	Pyelonephritis/cystitis
Prs pili	Cystitis
Type 1 pili	Cystitis
S pili	Cystitis
<u>nonfimbrial adhesin</u>	
F adhesin	Pyelonephritis
Dr adhesin	Cystitis

Not all humans are equivalent (in terms of disease susceptibility). P pili are present on strains of *E. coli* that are associated with pyelonephritis. P pili mediate attachment of bacteria to the P blood group globoseries Gal-Gal constituent of glycolipids present on uroepithelial cells and erythrocytes.

The formation of “pods” or biofilm-like structures that form within epithelial cells of the urinary tract can contribute to persistent infections. UPEC is able to invade epithelial cells and replicate within the cells.

Surface adhesins can also play a role in the colonization of urinary catheters. Almost 100% of these catheters are colonized with bacteria within 3 days.

Additional UPEC virulence factors that are LPS, capsule, motility, and a variety of exotoxins (including hemolysin).

4. Bacteremia caused by *E.coli*

- *E. coli* is a major cause of bacteremia, and is the leading cause of nosocomial bacteremia. Although the majority of *E. coli* isolates are noninvasive, two routes of entry are UTI and the use of indwelling devices such as intravenous catheters. The mechanism by which *E. coli* crosses from the UT to the blood is not yet been elucidated but perhaps its ability to invade host cells allows crossing into the blood stream.
- Bacteremia is most often associated with UTI in instances where urinary flow is obstructed. As expected, given the source of infection, most *E. coli* associated with bacteremia have features similar to those associated with UTI. Serum resistance is a critical trait of such strains and is correlated with production of K1 capsule. Bacteremia stemming from intestinal infection occurs less frequently.
- The K1 capsule is a polysialic acid capsule nearly identical to that of *Neisseria meningitidis*. Sialic acids are constituents of most host glycoproteins and glycolipids, which may explain the lack of an effective immune response against *E. coli* K1.
- The hallmark of Gram-negative bacteremia is the systemic reaction to endotoxin or LPS. This is perhaps the most toxic component of most bacteremia isolates of *E. coli* and is life-threatening. The management of bacterial sepsis and accompanying endotoxic shock is difficult.

5. Neonatal meningitis caused by *E. coli* K1

- *E. coli* remains the most common neonatal pathogen, and one of the leading causes of neonatal meningitis.
- *E. coli* K1 is isolated from 20-40% of healthy individuals including newborns. Generally can colonize newborns within hours of birth via vertical transmission from the mother or from nursery staff.
- The mechanisms by which *E. coli* translocates into the bloodstream or into the CSF are not known. The K1 polysialic acid capsule is the major determinant because it allows the organism to escape phagocytosis. Siderophores contribute to the ability of an organism to cause disease.
- Proliferation in the CSF causes inflammation and tissue damage

B. PSEUDOMONAS AERUGINOSA

P. aeruginosa is a major pathogen associated with:

- Burns
- Catheters
- Implanted medical devices
- Ventilator associated pneumonia
- Eye wounds
- Can cause bacteremia in immunocompromised patients.
- **Chronic infections by *P. aeruginosa*** generally occur in the lung in association with respiratory diseases, such as those associated with Cystic Fibrosis (CF) or COPD (chronic obstructive pulmonary disorder).
 - Individuals with the genetic disease CF are susceptible to a chronic bacterial infection that cannot be cleared by any current antimicrobial therapies. Patients usually die of respiratory failure in early adulthood.
 - In the CF lung, *P. aeruginosa* clearance is impeded host factors and bacterial factors.

P. aeruginosa secretes a number of toxins that contribute to its pathogenicity:

- Endotoxin, as it is a Gram-negative organism
- Exotoxins, proteases (elastases) and phospholipases contribute to disease.
 - Elastases break down elastin, a connective tissue protein in the lung and can lead to tissue damage in the lung. Breaks down phospholipids in lung surfactant and in host cell membranes.
 - Some toxins are secreted by a T3SS.

P. aeruginosa produces a characteristic blue-pigment called pyocyanin (which is also a virulence factor that generates ROS).

Since it cannot ferment sugars, detection in blood culture requires aerobic incubation. While *P. aeruginosa* can grow via anaerobic respiration with nitrate as an electron acceptor, or ferment the amino acid arginine, **it is considered an obligate aerobe in clinical laboratories.**

C. KLEBSIELLA PNEUMONIAE

Clinical syndromes include:

- Primary pneumonia when underlying medical problems are present (e.g., alcoholism, diabetes, lung disease)
 - “Red current jelly” sputum
- Urinary tract and wound infections
- Bacteremia and meningitis
- Diarrhea by enterotoxigenic strains

Capsule is the main virulence factor:

- Reduced phagocytosis
- Reduced complement susceptibility
- Capsule production also help identify *K. pneumoniae* based on a mucoid colony morphology

D ENTEROBACTER CLOACAE

- Associated with burns, wounds, respiratory, and urinary infections.
- Infection occurs in the hospital setting secondary to antibiotic therapy.
- Formerly lumped in with *Aerobacter* and *Klebsiella*, but differs from *Klebsiella* in that it is motile and generally less heavily encapsulated.
- *Enterobacter cloacae* accounts for many hospital acquired infections. *E. cloacae* and *E. agglomerans* were associated with an intravenous tubing contamination affecting hundreds of patients in 25 hospitals.

E. SERRATIA MARCESENS

- *Serratia* are common in the environment.
- Prodigiosins produced by *Serratia* give rise to a characteristic red color.
- Infections are seen secondary to broad spectrum antibiotic therapy or secondary to instrumentation (tracheostomy, indwelling catheters, renal dialysis, etc.). They can involve most tissues. Pneumonia is contracted after use of a contaminated respirator.
- *Serratia* is different from the other enterobacteriaceae in that it is less likely to colonize the GI tract, and is more associated with the respiratory and urinary tract. The GI tract is, however, an important reservoir among neonates.
- Outside the hospital setting, *Serratia* has been associated with heroin addicts. In out-patient setting *Serratia* is associated with septic arthritis.
- *Serratia* produces MS-fimbriae, proteases, siderophores that may be virulence factors. It exhibits a swarming motility that may aid in colonization of the urinary tract.

F. PROTEUS VULGARIS; PROTEUS MIRABILIS

A frequent cause of urinary tract infection. There are two characteristics that are known to contribute to its pathogenicity.

1. Flagella (which mediate swarming motility)
2. Urease synthesis

urease
Urea $\xrightarrow{\quad}$ NH₃ + CO₂ $\xrightarrow{\quad}$ alkaline urine $\xrightarrow{\quad}$ salt crystalization and stone formation $\xrightarrow{\quad}$
chronic infection

G. ACINETOBACTER (ACINETOBACTER BAUMANII):

- Oxidase negative non-fermenter; short rod.
- *Acinetobacter* spp. are second only to *Pseudomonas aeruginosa* in frequency of recovery of nonfermentative gram-negative bacilli from human sources.
- Opportunistic infections in hospitalized patients (one's likelihood of colonization increases in the hospital) and infection is often associated with use of indwelling medical devices.
- Reservoirs within hospitals may exist.
- Resistant to multiple classes of antibiotics-multidrug resistant strains are increasingly described.
- Virulence factors include capsular polysaccharides, protein adhesins, proteolytic and lipolytic enzymes, and lipopolysaccharide.

H. A few other Gram-negative pathogens:

- *Morganella* causes infections similar to *Proteus*
- *Providencia* causes nosocomial infections of urinary tract, blood, respiratory tract and wounds
- *Citrobacter*: neonatal meningitis and brain abscesses by *Citrobacter diversus*; *Citrobacter freundii* can be enterotoxigenic
- *Edwardsiella*: gastroenteritis

DIAGNOSIS OF GRAM-NEGATIVE PATHOGENS:

- Colony morphology
- Selective medium
- Biochemical tests

Most Gram-negative opportunistic pathogens are facultative anaerobes. Their ability to ferment lactose is often used to differentiate between different pathogens in the clinical laboratory.

Growth is detected by use of a pH indicator to detect acids produced via fermentation and via gas production.

	<u>Energy Metabolism</u>	<u>Lactose Fermenting</u>
<i>Escherichia coli</i>	facultative anaerobe	+
<i>Klebsiella pneumonia</i>	"	+
<i>Serratia marcescens</i>	"	-
<i>Enterobacter cloacae</i>	"	+
<i>Proteus mirabilis</i>	"	-
<i>Acinetobacter baumannii</i>	non-fermenter	-
<i>Pseudomonas aeruginosa</i>	obligate aerobe*	-

- As discussed above, *P. aeruginosa* is described in the text as being an obligate aerobe because it does not ferment sugars.

TREATMENTS:

Antibiotics used will vary based on the infecting microbe (species and strain) and the site of infection.

Many opportunistic pathogens are resistant to antibiotics.

- Nosocomial infections can be particularly problematic because these bacteria often carry multiple antibiotic resistance genes on plasmids making them difficult to treat.
- Biofilm bacteria have increased resistance to a range of antimicrobial agents compared to planktonic cells
- Innate resistance to antimicrobial compounds can be an issue. (*Pseudomonas aeruginosa* and *Acinetobacter baumannii* are two important examples that we will discuss.)
- CRE stands for carbapenem-resistant Enterobacteriaceae, and they are members of the Enterobacteriaceae family (*Klebsiella* species and *Escherichia coli* (*E. coli*)) can become carbapenem-resistant.

PREVENTION:

- Control of underlying compromising factors.
- Reduced catheter usage and increased catheter hygiene. Development of "colonization-resistant coatings" underway.
- Decontamination of medical equipment and cleaning of hospital spaces (such as operating rooms).
- Handwashing and glove use!
- Much remains to be learned about how to control these infections.

Cholera, Campylobacter, Helicobacter

Learning Objectives:

To explain the historical importance of cholera

To explain the pathophysiology of diarrhea in cholera, the morphology of cholera bacteria and how cholera is diagnosed

To describe the basic epidemiology of cholera

To describe the virulence factors expressed by *V. cholerae* and their mechanisms of action

To explain the treatment and recognize the lack of a good vaccine

Reading assignment: Levinson, pp. 157-158 (Cholera), 158-159 (Campylobacter), 159-160 (Helicobacter), 639-640.

Vibrio cholerae and Cholera

I. General Properties

- Motile gram negative fermenter, comma shape, polar flagellum
- Yellow opaque colonies on special TCBS medium (usual media for enteric pathogens, such as EMB, may inhibit growth)
- Produces O, H antigens, endotoxin and potent enterotoxin (cholera toxin, an exotoxin)
- All epidemic cholera is produced by *V. cholerae* O1 (with a blip of O139 in mid-90s).
- There are 2 O1 biotypes: classical and El Tor (hemolytic). El Tor is responsible for all epidemic cholera in the last decade.
- Major colonization factor is the toxin coregulated pilus (TCP)

II. Pathogenesis

- Gastric acid inactivates ingested organisms, but some survive
- Organisms enter small bowel and bind to epithelium by multiple mechanisms
- TCP is expressed and bacteria form microcolonies in intestinal crypts
- Cholera Toxin is expressed and secreted, binds to GM₁ gangliosides of cell membrane
- Neuraminidase of *V. cholerae* converts other gangliosides to GM₁ and thereby increases amount of toxin binding sites
- Cholera Toxin enters the intestinal cells by endocytosis and stimulates adenylate cyclase and cAMP production; results in massive intestinal fluid loss via 2 mechanisms:
 1. villus cells: decreased NaCl absorption from gut
 2. secretory cells: increased Cl and increased HCO₃ secretion into gut, along with H₂O (secretory diarrhea)

III. Clinical Features

- Painless, odorless profuse watery diarrhea (rice water stool)
- Isotonic volume loss (10-15 liters/day) and dehydration, low blood pressure ("shock"), potential death
- [Stool electrolyte concentrations: Na 135, K 15, Cl 100, CO₂ 45 mEq/L]

IV. Treatment

- Replace fluid using same concentrations of electrolyte
- Oral or IV rehydration solutions both acceptable
- Cholera cot facilitates measurement of stool volume to be replaced
- Antibiotics reduce duration of diarrhea from 5-10 days to 1-3 days; Doxycycline for adults or azithromycin for children and pregnant women.

V. Epidemiology and Patient Populations of Special Interest

- Accounts of cholera since BC
- Disease limited to humans and associated with poverty and inadequate sanitation. Endemic in some regions (south-central and southeast Asia), and recently associated with refugee camps mainly in Africa where it is now endemic. Since 1992, has become endemic in South America.
- Seven pandemics documented since 1800 originating from endemic focus in common delta of Ganges and Brahmaputra rivers
- Seventh pandemic (El Tor O1) began in 1961 in Sulawesi, Indonesia (formerly known as Celebes) and in 1992 spread to Latin America. This represented the first cholera outbreak in the Western world in more than a century. Spread occurred rapidly with hundreds of thousands of cases and mortality rate of about 1%. Cholera is more of a world-wide problem now than it was prior to 1992. The 7th pandemic is global.
- Haiti – the newest large epidemic began in fall of 2010. *V. cholerae* was introduced into Haiti by UN aid workers who unknowingly brought it from Nepal. Haiti had never experienced cholera and this contributed to quick initial spread and a high case/fatality rate. There have been over 8000 deaths and 1 in every 16 people has been sickened.
- John Snow, father of epidemiology, determined mode of spread of cholera in London in 1855
- Broad Street pump outbreak
- Outbreaks related to contaminated water, shellfish, other seafood
- Infectious dose influences mode of transmission of enteric pathogens
- For *V. cholerae*, the infectious dose is source dependent. Contaminated rice is highly infectious.

<u>Organism</u>	<u>Infectious Dose</u>	<u>Transmission</u>
<i>Vibrio cholerae</i>	$10^2 - 10^9$ (source dependent)	water (food)
<i>Salmonella typhi</i>	10^5	water (food)
<i>S. typhimurium</i>	10^{3-4}	contact, water or food (esp. poultry)
Campylobacter spp.	5×10^2	uncooked food (esp. poultry), unpasteurized milk
<i>Shigella spp.</i>	$<10^2$	contact, water or food

VI. Prevention

- Water precautions: bottled and carbonated, chlorinated, boiled (beware of ice, unsealed bottled water)
- Food precautions: dry, steaming hot, special attention to shellfish
- Cholera vaccine
 1. current inactivated vaccine: two oral doses of crude bacterial suspension, 70% effective, not recommended even for travel. Currently being used in Haiti.
 2. live attenuated vaccine: effective in North American clinical trials but unproven in field studies, mild side effects common, licensed but not manufactured

[VII. Related pathogens]

- *Vibrio parahaemolyticus*: invasive gastroenteritis from contaminated shellfish
- *Vibrio vulnificus*: infections in wounds contaminated by seawater or shellfish. Overall, limited to individuals with underlying liver disorder.

Campylobacter species

Learning Objectives:

To describe basic epidemiology

To describe pathogenesis and accompanying clinical features

To explain the common source, prevention and treatment

To recognize the high incidence

To describe the bacterial structure, explain how infections are diagnosed and the growth conditions used to identify campylobacter in the clinical laboratory

1. General properties. Curved (campylo = curved) or comma-shaped microaerophilic gram negative rods present in numerous animal species. The most common causative agent is *C jejuni*, followed by *C. coli*.
2. Pathogenesis. Organisms are ingested in contaminated food or water, (and, if they survive gastric barrier) arrive in small and large bowel to cause invasive inflammatory process. Rarely, organisms enter the bloodstream (bacteremia). Recovery and specific antibody-mediated immunity are characteristic.
3. Epidemiology and Patient Populations of Special Interest. Zoonotic disease transmitted to humans from animals, especially poultry. More than half of the raw chicken in US grocery stores contains Campylobacter. Cases are typically sporadic, rather than epidemic and tend to peak in summer and early fall. Multiple simultaneous cases usually point to a common contaminated source such as unpasteurized milk. Campylobacter does not make animals ill. Human to human transmission is rare.
4. History. Campylobacter has probably caused disease for centuries, but it was not isolated until 1968 and not recognized as a cause of diarrheal illness until the 1970s. In part, this was due to the fact that Campylobacter has to grown under microaerophilic conditions on special medium.
5. Occurrence. It is now recognized that Campylobacter is the most common etiologic agent of diarrhea in the world, far surpassing Salmonella and Shigella. More than a million reported cases in the US per year. Infants through young adults are most often affected in developed countries and infants are the major focal group in developing countries.
6. Clinical features. Incubation period of 3-5 days, prodrome of fever, malaise, headache, then fever, abdominal pain and diarrhea lasting a few days - week. One in a thousand people with Campylobacter infection develop autoimmunity to their own nerves. This can lead to paralysis that lasts for several weeks (Guillain-Barre Syndrome). Such cases typically require intensive care. [Immunocompromised patients (especially humoral) have more severe illness with *C. jejuni* and are also the risk group for the rare and sometimes fatal syndrome of *C. fetus* systemic infection and bacteremia.]
7. Infectious dose. As few as 500 organisms can cause disease. One drop of raw chicken juice is sufficient.
8. Diagnosis. Established by culture of stool on selective media [e.g. campy-BAP with cephalothin] incubated in 5-10% O₂ at 37° or 42°C [latter is optimal]. Interestingly, 42°C is the body temperature of poultry.
9. Treatment. Supportive therapy for all (fluids, etc.) with antimicrobial therapy for some (erythromycin; alt: ciprofloxacin).
10. Prevention. Pasteurization of milk, avoid undercooked meats (esp. poultry).

Helicobacter

Learning Objectives:

- To discuss the link between the microorganism and chronic disease
- To explain the pathogenesis underlying gastritis, peptic ulcers, gastric cancers and mucosal lymphomas
- To describe the enormous global load of Helicobacter
- To describe the special diagnostic tests for Helicobacter
- To explain the basic treatment and recognize the lack of a vaccine

1. General Properties. *Helicobacter pylori* is a spiral shaped, gram-negative bacterium that lives in the stomach and duodenum. The stomach is a very acidic environment (pH~2). The stomach is protected from its own gastric juice by a thick layer of mucus that covers the stomach lining. Helicobacter lives in this mucus lining. Once there, it secretes an enzyme, **urease**, which converts urea to bicarbonate and ammonia, which are both quite basic. Similarly shaped *Campylobacter sp.* are urease-negative.

2. Additional virulence factors. The bacterium produces **adhesins**, which bind to membrane-associated **lipids** and **carbohydrates** and help it adhere to epithelial cells. For example, the adhesin **BabA** binds to the **Lewis b antigen** displayed on the surface of stomach epithelial cells. The bacterium also injects the *cag* PAI-encoded protein, **CagA**, into the stomach's epithelial cells, where it is phosphorylated and disrupts the **cytoskeleton**, adherence to adjacent cells, intracellular signaling, **cell polarity**, and other cellular activities. Finally it injects vacuolating cytotoxin A (**VacA**), which further damages the epithelial cell lining.

3. Pathogenesis. The immune system responds to the presence of *H. pylori* by sending white cells, killer T cells, and others. Since these cells cannot readily penetrate the mucus lining, the response continues unabated. As these cells die, they release superoxide radicals that destroy stomach cells. Within a few days gastritis and perhaps at later times a peptic ulcer develops. In addition, the presence of *H. pylori* confers an approximately six-fold increased risk of gastric cancer, accounting for about half of all gastric cancers. This is thought to be the result of chronic gastritis leading to intestinal metaplasia, which then undergoes malignant change. A minority of cancers associated with *H. pylori* are mucosal-associated lymphoid tissue (MALT) lymphomas.

4. Epidemiology and Patient Populations of Special Interest. *H. pylori* is believed to be transmitted orally. This might be via fecal matter present in tainted food and water. In addition, it is thought that *H. pylori* can be transmitted from the stomach to the mouth by gastro-esophageal reflux due to the symptoms of gastritis. The bacteria could then be transmitted by oral contact (kissing).

5. History. Infection by *H. pylori* was proven to be the etiologic agent of stomach ulcers in 1984 by Barry Marshall and J. Robin Warren. They received the Nobel Prize for Medicine and Physiology in 2005 for their discovery.

6. Occurrence. The organism is present in about 50% of the world's population. Presence is highest in developing countries and correlates with a greater incidence of gastric cancer in these countries.

7. Clinical features. Gastritis and peptic ulcer are characterized by recurrent pain in the upper abdomen, frequently accompanied by bleeding into the gastrointestinal track.

8. Infectious dose. The infectious dose is not known for humans. It has been determined to be greater than 10,000 organisms in nonhuman primates.

9. Diagnosis.

A. Radiolabeled urea test (Breath Test) – The patient swallows a capsule containing 1 micro-Curie of C14 urea. If the urea is cleaved by urease the C14 is released as aerosol and can be monitored by any of a variety of methods that analyze the patient's breath.

B. Stool culture – The same medium is used as for Campylobacter. A rapid urease test distinguishes between Campylobacter and Helicobacter.

C. Seroconversion – Blood samples often show a positive response against Helicobacter antigens.

D. PCR – Detection can be made using samples from stool or dental scrapings.

E. Endoscopy – Endoscopy followed by histology of the biopsy provides a definitive diagnosis.

10. Treatment. A variety of antibiotic regimens are recommended. Treatment is generally routine and not prolonged. Antibiotic therapy is often supplemented with bismuth salts. Occasionally relapses occur and require further treatment. Antibiotic treatment is contra-indicated for asymptomatic infected individuals except in special circumstances. A common triple treatment for symptomatic cases is comprised of bismuth, metronidazole, and tetracycline.

11. Prevention. There is no vaccine.

HAEMOPHILUS AND BORDETELLA

Learning objectives:

- To explain the role of the capsule in *Haemophilus* infection.
- To explain the meaning of non-typeable *H. influenzae* and describe clinical syndromes and manifestations of *Haemophilus* bacteria.
- To explain importance of toxins in disease and adhesins in disease.
- To memorize the particular growth conditions needed by *H. influenzae* and *B. pertussis* and explain methods for laboratory identification.
- To recognize the effects of vaccination on disease prevalence.

Reading assignment: Levinson, pp. 167-170, 642

Haemophilus influenzae

- a) Small, Gram-negative, non-motile, non-spore-forming bacillus or coccobacillus (pleiomorphic).
- b) Name is a misnomer. Discovered in 1892 during influenza pandemic. Was thought to be the cause of flu epidemics (which in fact were viral in origin; *H. influenzae* was likely a secondary invader)
- c) *Haemophilus influenzae* is categorized based on whether or not it has a capsule. The two categories are: Encapsulated (typeable) and unencapsulated (non-typeable).
 - i. Encapsulated strains cause bacterial meningitis in young children. Until the introduction of a vaccine in 1988, 20,000 children per year had serious *H.i.* infections; mortality rate of 3-6%.
 - ii. Unencapsulated strains cause ear aches, respiratory disease along with other infections.
- d) Up to 75-80% of the population carries *H.i.*, ~5% of these isolates are encapsulated.

A. H. INFLUENZAE TYPE B (HIB) AND OTHER ENCAPSULATED STRAINS

- The capsule is made from carbohydrates.
- The capsule is required for virulence and is antiphagocytic
- The capsule is the basis for serotyping. Six types (a,b,c,d,e,f) have been identified by agglutination, precipitation, or "quellung" (capsular swelling) tests with specific antisera. The need for this assay is less common today because of effectiveness of vaccine.
- *Type b* most important pathogen. Unlike a, c, d, e, f, which have hexose in their polysaccharide capsular structure, b has ribose (5-carbon sugar). It is *not* known if or how this unusual ring structure relates to the pathogenicity of the type b bacterium. Type B strains are the most common among encapsulated strains.

1. Epidemiology:

Approximately ~3-5% of individuals tested carry type b strains. Decreasing with increased vaccine use.

Until the early 90s, this organism was the most common cause of bacterial meningitis in children under 4 years. Meningitis due to *H. influenzae* type b (Hib) affected 41 per 100,000 children under 5 in 1987, and only 1.3 per 100,000 in 1998. Approximately 60% of children infected with Hib developed

meningitis and the death rate due to this disease is 3-6%. In 2004, the annual incidence of Hib disease was 0.15 cases per 100,000 with a fatality rate of 5-10% even with early antibiotic therapy.

Hib remains important in developing countries where the vaccine is not available routinely. Hib is currently still responsible for 500,000 deaths of children under 5 worldwide.

Disease by non-type b encapsulated strains is not common, but can occur.

2. Clinical Syndrome:

1. Disease starts as a nasopharyngitis (with otitis media—infection of the middle ear—or sinusitis). *H.i.* can invade the epithelium and enter into blood stream. Bacteremia may follow with involvement of the meninges.
2. Less common, but equally serious: *epiglottitis* and obstructive laryngitis. Sudden onset. *May be fatal* within 24 hours. Treatment must be prompt and airway ensured, if necessary, by tracheotomy.
3. Sometimes bacteria spread to face (causing *cellulitis*), or to joints, causing childhood pyarthrosis (pus in a joint). Or bacteria may cause pneumonia.

3. Pathogenesis:

1. Portal into humans is via the respiratory tract generally through aerosols between children. Colonization can persist for weeks or months.
2. Pathogenic mechanism is not well understood.
 - a. Produces an IgA protease that may aid in immune evasion.
 - b. Can induce ciliary stasis through decoration of LPS with host choline.

4. Immunity:

1. Passive immunity acquired from mother lasts only a few months. Most susceptible period is between 6-12 months and levels of resistance remain low for the first few years.
2. After 3 to 4 years or so, most children have acquired active immunity from asymptomatic infections, and incidence of disease falls off—although some cases are found in older children and, uncommonly, in adults.
3. A conjugate capsular vaccine that immunizes against type b *H. influenzae* introduced in 1988 has been very effective.
 - a. The vaccine is capsule (PRP = polyribosyl phosphate) linked to diphtheria toxoid so as to have increased dependence of T cells and memory, as compared to the previous formulation composed only of capsule. It is also more immunogenic in very young children than the previous versions.
4. Complement mediated resistance is an important part of immunity and patients with defects in complement function are more susceptible to Hib disease.

5. Treatment-encapsulated *H.i.*:

For meningitis: Ampicillin was the drug of choice. However, since a significant number of isolates are now ampicillin-resistant, a third generation cephalosporin is used immediately. Also, recently

developed combination therapy (e.g., Augmentin) combines ampicillin with clavulanate (a β -lactamase inhibitor) to render ampicillin-resistant strains sensitive to this antibiotic. If the organism, after isolation, proves to be β -lactamase-negative, treatment is switched to ampicillin.

If treated early, mortality rate is low. Previously, one-third of those who recovered had residual neurological damage. This has been decreased with the use of corticosteroids during antibiotic treatment to reduce inflammation and cytokine production due to LPS released as a result of lysis of the bacteria.

B. NON-TYPEABLE *H. INFLUENZAE*

1. General properties:

Does not have a polysaccharide capsule.

2. Clinical manifestations:

- a) As opposed to typeable strains, which become systemic, non-typeable strains are generally restricted to the respiratory tract and infections of the ear.
- b) Colonization starts in the nasopharynx with preferential adhesion to respiratory mucus, non-ciliated cells and damaged epithelium.
- c) Second most common cause of otitis media (middle ear infections) after *S. pneumoniae*. Most common in children - why?
 - a. Immune system of children does not fight the infections of the respiratory tract as effectively.
 - b. The structure of the eustachian tube, in young children, is felt to make fluid and infections more likely due to a straighter angle and a shorter length.
- d) Can cause conjunctivitis
- e) Typically causes respiratory tract infections in patients with underlying respiratory issues (COPD, chronic bronchitis, CF) and acute sinusitis.
- f) In cases of meningitis, there is usually a predisposing factor (trauma, sinusitis, CSF leak). A important pathogen in neonates with a mortality rate of 50%.

3. Virulence determinants and Pathogenesis:

- a) A variety of surface structures contribute to adhesion (*these do not need to be memorized*):
 - a. peritrichous pili
 - b. Hap adhesin: found among typeable and non-typeable
 - c. HMW1/2 adhesin: expressed by most non-typeable (but not typeable) strains
 - d. Hia adhesin: expressed in most non-typeable strains lacking HMW1/2
 - e. Hsf adhesin: homologue of Hia found in typeable strains
 - f. LPS: may bind to the platelet activating factor (PAF) receptor
- b) Invasion via 3 routes. *Haemophilus* was thought to be primarily an extracellular pathogen, but recent data examining clinical samples suggests that this organism can also invade mammalian cells.

4. Immunity:

- Any passive immunity acquired from mother lasts only a few months.
- Adults are susceptible to infection – not clear that long term immunity develops to non-typeable strains as is case for Hib.
- Immunity appears to be strain specific – variation in OM proteins.
- No vaccine currently.

5. Treatment:

- For otitis media and sinusitis: amoxicillin, orally, for out-patients. One of top reasons antibiotics are prescribed in the US.
- ~20-35% of isolates are resistant to amoxicillin
- Alternative: use amoxicillin with β -lactamase inhibitor (i.e., clavulanate) or ceftriaxone
- Infections can be persistent and recurrent due to its ability to form an antibiotic resistant biofilms and its ability to invade host cells and thus avoid antibiotics?

6. Diagnosis of both encapsulated and unencapsulated strains :

- a) Facultative anaerobe. Requires two growth factors, both present in blood. X is heat-stable; V is heat-labile.
 - a. X is hemin (a precursor of the heme groups of respiratory enzymes).
 - b. V is NAD (nicotinamide adenine dinucleotide) or NADP.
 - c. Some organisms, such as staphylococci, secrete sufficient V factor so that they can promote the growth of *H. influenzae*; on agar plates with no other source of V factor, such as blood agar plates, staphylococcal colonies will be surrounded by small "satellite" colonies of *H. influenzae*. X and V factors are also released from red blood cells by the action of hemolysins secreted by some species of Staphylococci and Streptococci.
 - i. Fresh blood agar does not support growth. Therefore chocolate agar is used for culture. The mild heat used in preparation of this medium *releases* both X and V from red blood cells.
 - ii. Sugar fermentation reactions are *not* of diagnostic use.
- b) Very susceptible to disinfectants and drying.
- c) Encapsulated virulent strains grow as smooth colonies. (Spontaneously give rise to "rough" unencapsulated variants.)
- d) **Additional diagnostic points for encapsulated strains:**
 - i. History and age of patient most important.
 - ii. In suspected meningitis, blood and spinal fluid are cultured (spinal fluid is centrifuged and the sediment examined by gram stain).
 - iii. Spinal fluid specimen streaked on chocolate agar and incubated in a CO₂ incubator, and the requirement for X and V factors demonstrated as described above.

- iv. Detection of type b capsular Ag in spinal fluid by immunofluorescence or immunoelectrophoresis (sensitive and accurate measure) is 90% effective in detecting Hib in culture-proven cases. Some laboratories use a latex-agglutination test.
- v. Strains can also be characterized biochemically using urease, ornithine decarboxylase and indole production tests. Hib isolates are predominantly type I strains that are positive in all of these tests.

7. Prevention:

Vaccination. Vaccines against Hib are made from capsule linked to a protein carrier. The length of the capsule and the protein carrier varies by brand.

Prophylaxis may be necessary in households with young children.

C. Other Haemophilus pathogens

- a) *H. ducreyi* - An emerging sexually transmitted disease in the U.S. Cause of *chancroid* ("soft chancre" appearing as a ragged ulcer on genitalia). Venereal disease. Uncommon in the United States but beginning to increase especially around cities that serve as major seaports; occurs more commonly in parts of Africa and Asia.
- b) *H. aegypticus* (*H. influenzae* biogroup *aegyptius* and Koch-Weeks bacillus): Produces a purulent conjunctivitis. Common in hot climates and one of the major causes of conjunctivitis in the southern United States.
- c) *H. parainfluenzae*: Occasional cause of pharyngitis and bacterial endocarditis. Major component of the microflora of the oral and upper respiratory tract.

BORDETELLA

A. *Bordetella pertussis*

Bordetella pertussis (isolated by Bordet and Gengou 1906) is the cause of *whooping cough* a severe childhood disease.

Small, gram-negative coccobacillus. Very similar in appearance to *H. influenzae*.

Obligate aerobe.

1. Epidemiology

- a) Found only in humans. No natural animal reservoir known.
- b) Pertussis was a major childhood killer pre-1940
- c) Immunization in the latter half of the 20th century produced a dramatic decline in whooping cough. In the United States there were 120,000 cases by 1950, but only about 2,000 by 1977. The incidence of pertussis has been rising since the mid 1980's with nearly 9,000 cases reported in 1999.
- d) Highest fatality rates among children. Caused more infant deaths than measles, diphtheria, polio & scarlet fever combined in the early 20th century.

2. Clinical features:

- a) *B. pertussis* causes whooping cough.
- b) Begins with mild upper respiratory symptoms.
- c) The incubation period is ~7-10 days. The disease is divided into two stages. A runny nose, sneezing, low-grade fever and a mild occasional cough (symptoms similar to a cold) characterize the **catarrhal stage**.
- d) Next, the **paroxysmal stage** is characterized by bursts of coughing (probably to dislodge mucus), followed by a high-pitched “whoop” and occasional vomiting. Following prolonged series of coughs, inspiration through narrowed glottis produces a *whoop*. Patients can become cyanotic (turn blue) during the coughing attacks. Coughing occurs more frequently at night. Infants often do not make the whooping sound characteristic of this disease.
- e) Infants are more likely to succumb to the infection.

3. Pathogenesis:

a) Steps in infection:

1. Introduction via water droplets
2. Interactions with ciliated epithelial cells in the trachea and nasopharynx
3. Adherence
4. Multiplication and toxin production [Described in detail below]
Acute local inflammation, increased mucous secretions, and patchy ulceration of respiratory epithelium follow. May result in diminished oxygen supply or pneumonia. Organisms do not invade the blood stream, but remain in the respiratory tract.
5. Evasion of host defenses
6. Spread of disease.

b) Specific toxins:

1. *Pertussis toxin*: ADP-ribosylating toxin that catalyzes transfer of ADP-ribose from NAD to certain members of the family of guanine nucleotide binding regulatory (G) proteins in target cells. Perhaps most notable is ADP ribosylation of the G protein involved in inhibition of adenylate cyclase, thus causing increased accumulation of cAMP. This can be contrasted to cholera toxin which ADP ribosylates the stimulatory G protein also resulting in increased adenylate cyclase activity and cAMP level. Pertussis toxin also affects the control of phospholipase C and ion channels. These combined actions are responsible for lymphocytosis, sensitization to histamine, and enhancement of insulin secretion.
2. Calmodulin-stimulated *adenylate cyclase toxin*: This toxin catalyzes the production of cAMP from ATP and is activated by endogenous calmodulin. This results in supraphysiologic concentrations of cAMP that may impair leukocyte functions and even cause cell death.
3. *Dermonecrotic toxin*: Also known as mouse lethal toxin or heat-labile toxin. First discovered by Bordet and Gengon when necrotic lesions developed following intradermal injection of *B.*

pertussis into suckling mice. Causes vascular smooth muscle contraction resulting in ischemic necrosis of lung tissue.

4. *Tracheal cytotoxin*: Causes ciliostasis, inhibits DNA synthesis and ultimately kills tracheal epithelial cells.

c) Physical interactions with host:

1. *Pili*, mediate attachment to ciliated epithelial cells of upper respiratory tract and cause diminished ciliary activity.
2. Another structure participating in colonization is the pilus-like filamentous hemagglutinin (*FHA*).
3. A surface molecule called *pertactin* plays a role in colonization.

4. Immunity:

- a) The first vaccine was introduced in the UK in 1937, and routine use in the US by 1944
- b) Vaccination has decreased the number of pertussis cases by ≥ 100 -fold
- c) Neither immunization nor the disease, however, produces lifelong immunity.
- d) Until the mid-90's, immunization was through one component of the trivalent DTP (diphtheria toxoid + tetanus toxoid + heat killed pertussis organisms). Potential side effects including encephalopathy and permanent neurologic sequelae led to fewer infants receiving the vaccine from the late 80's into the 90's, and so pertussis cases actually began increasing until the acellular pertussis component was introduced in the mid-90's (DTaP).
- e) Much research in the area of developing lower risk component vaccines containing the specific pertussis antigens described above has resulted in formulations that have proven to be safe and effective.
- f) Several such acellular vaccines have been made.
- g) Each is incorporated into one of several DTaP trivalent vaccines that are now the recommended vaccines. DTP is essentially phased out.

5. Diagnosis and Treatment:

- a) *B. pertussis* requires *very fresh* media for growth, so most clinical labs have switched to a PCR based method to detect this organism from washes of the nasal cavity. Specific kits are available to test for this organism.
- b) History of contact, classic cough, marked lymphocytosis.
- c) Isolation of organism by nasopharyngeal swab or cough plate. Colonies grow very slowly. Identify by appearance of colony, gram stain, biochemical reactions.
- d) Rapid direct fluorescein-labeled antibody test for nasopharyngeal specimens.
- e) PCR-based tests
- f) Erythromycin is the drug of choice (alternatives: tetracycline, chloramphenicol). Not sensitive to penicillin or ampicillin.

Other species of Bordetella:

B. parapertussis can cause sporadic cases of whooping cough. *B. bronchiseptica* causes respiratory illness in animals and only occasionally in humans. Both of these organisms produce a number of virulence factors similar to those of *B. pertussis*, but do not carry the genes for pertussis toxin.

Link to video of children with whooping cough: <http://www.nevdgp.org.au/video/immune/pertussis.html>

Similarities and differences for *H. influenzae* and *B. pertussis*

Property	<i>H. influenzae</i> (type B)	<i>B. pertussis</i>
Major infection	Meningitis, epiglottitis, pneumonia, otitis media	Pertussis (whooping cough)
Less common infect.	Arthritis, osteomyelitis	Pneumonia
Reservoir	Humans	Humans
Transmission	Droplet	Droplet
Morphology	Gm-, coccobacilli	Gm-, coccobacilli
Growth	Requires X (hemin) and V (NAD) factors	Bordet-Gengou complex medium
Virulence	Capsule, pili, IgA protease	FHA, pili, pertactin numerous toxins
Toxins	No exotoxins	Pertussis toxin, adenylate cyclase toxin, tracheal cytotoxin, dermonecrotic toxin
Diagnostics	Gram stain blood and spinal fluid; culture chocolate agar demonstrate XV req's confirm type b antigen	Gram stain nasopharyngeal swab; direct anti-body test; culture on B-G
Treatment	3rd generation cephalosporin, then amoxicillin if sensitive; Contacts-rifampin, ciprofloxacin, or ceftriaxone	Erythromycin and treat household contacts
Vaccine	Hib vaccine	Acellular vaccine with PT, FHA, pertactin, pili

6. Links between clinic and basic research in treatment and prevention

LEGIONELLA, MYCOPLASMA, CORYNEBACTERIA AND DIPHTHERIA

Learning objectives:

To describe the pathogenesis of legionellosis, the symptoms, the unusual reservoir for Legionella and how infections are prevented.

Describe methods for direct detection of Legionella in clinical specimens and media used for culturing the organism from clinical specimens

To describe the peculiar morphological features of the Mycoplasma as a group.

To describe the limitations in growing and visualizing *Mycoplasma pneumoniae* bacteria and how infections are diagnosed.

To describe the epidemiology of illness and clinical symptoms caused by *Mycoplasma pneumoniae*.

To explain the reason for choosing or avoiding particular antibiotics in treatment.

To explain the method for definitive diagnosis of diphtheria.

To describe the mechanism of action of diphtheria toxin.

To explain how diphtheria is treated.

Reading assignment: Levinson, pp. 171-172, 642 (*Legionella pneumophila*); pp. 39-44 (exotoxins); 141-143, 637 (*Corynebacterium*), Chapter 23, 645 (*Mycoplasma*)

1. LEGIONELLA PNEUMOPHILA

General:

1. A Gram-negative pleomorphic rod.
2. This organism is a common but only recently identified (1976) pathogen. Legionnaires' disease acquired its name in 1976 when an outbreak of pneumonia occurred among persons attending a convention of the American Legion in Philadelphia. Later, the bacterium causing the illness was named *Legionella*. The organism provides a model for the study of a "new" disease ("Legionaire's disease").
 - The first recorded outbreak of pneumonia associated with *Legionella*:
 - A large population (at least 189) was affected.
 - Geographically circumscribed outbreak.
 - Significant mortality (15%).
 - Large at-risk population, generally elderly or immune compromised individuals.
 - Well publicized.
3. It exhibits intracellular growth.
4. In nature, it is most frequently found in the water of cooling towers. It resides within a free-living amoeba (*Acanthamoeba* sp, *Naegleria* sp) or free-living in biofilms.
5. An estimated 8,000 to 18,000 people get Legionnaires' disease in the United States each year.

Symptoms:

1. Pneumonia is the principal clinical symptom in serious cases. Fever, chills, and a cough, which may be dry or may produce sputum. Some patients also have muscle aches, headache, tiredness, loss of appetite, and, occasionally, diarrhea. It is difficult to distinguish Legionnaires' disease from other types of pneumonia by symptoms.
 - Agents which cause pneumonia are traditionally divided into 'typical' and 'atypical', each dictating a distinct antibiotic treatment. Atypical agents refer to certain bacteria - namely, *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*. Typical pneumonia refers to that caused by *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. Differences between typical and atypical include antibiotic sensitivity, localization within the lung, and symptoms.
2. In severe cases, Legionella can disseminate from the lung causing systemic damage largely due to the presence of LPS.
3. Renal transplant patients, immunocompromised and immunosuppressed patients are predisposed.
4. The more mild form of the disease is called "Pontiac Fever."

Legionella pneumophila: Pathogenicity:

1. Infection is acquired by the airborne route from environmental contamination (in warm and cold water sources) *not* from infected people. Reservoirs include stagnant water in air conditioning and heating units. Growth in amoebae might prime infectivity.
2. An incubation period of 2-10 days is seen.
3. Most infections are probably clinically insignificant ("cold symptoms") as suggested by serological studies of large populations (4% of all Americans).
4. When outbreaks do occur, they are recognized in the summer and early fall, but cases may occur year-round.
5. In macrophages and alveolar epithelial cells, *L. pneumophila* induces apoptosis during the early stages of infection. A second phase of necrosis induced by a pore-forming activity takes place in infected human phagocytes. Both of these processes lead to death of host cells in the respiratory tract.
6. Legionella can disseminate from the lung and cause invasive disease.
7. A vigorous immune response is required to control Legionella.

Diagnosis:

- Legionella is nutritionally "fastidious". Is grown on charcoal yeast extract with iron and cysteine.
- Direct fluorescent antibody test to identify the organism is the test of choice.
- Detection of *Legionella* antigens in urine samples
- Analysis of antibody levels to *Legionella* in two blood samples obtained 3 to 6 weeks apart.

Prevention is largely achieved through proper water handling.

2. MYCOPLASMA PNEUMONIAE

General:

1. Smallest replicating bacteria (0.15-0.3 µm)
2. No cell wall (and thus resistant to antibiotics that target the cell wall such as penicillins and cephalosporin's)
3. Growth on cell-free media only if sterols (cholesterol) and other nutrients are provided by yeast extract and animal serum. It will not grow on standard laboratory medium. Cholesterol provides membrane rigidity.
4. Variable lipoproteins increase immune evasion and provide rigidity.
5. Colonies on solid media are small and grow very slowly; may take a week or two for a colony to appear--colonies have a "fried egg" appearance.
6. Pleomorphic and Gram variable
7. Pathogen only of humans

Epidemiology:

1. Studies suggest that it causes 15-50% of pneumonias.
2. It is responsible for 50% of "summer pneumonias" generally occurring from the late summer into autumn. Epidemics arise at 4-8 year intervals.
3. This is a disease primarily of school-aged kids and teenagers through young adults. Severity is generally correlated with age.
4. Outbreaks can occur in groups (families, military, college students). Close contact a factor...playmates more than classmates.
5. Commonly associated with exacerbations of childhood asthma, chronic lung disease, and immunodeficiency.
6. Only one serotype known. Immunity, after one has had pneumonia, is not lifelong. May acquire the disease again in 5 to 10 years.

Symptoms:

1. Causes atypical pneumonia with (symptoms similar to viral infections) with a gradual onset, nonproductive cough and small amounts of non-bloody sputum. Symptoms and signs of mycoplasmal pneumonia; weakness, fever, cough, headache, diffuse changes seen on X-ray of chest; slow onset; fatalities rare.
2. Considered atypical because patients with this infection do not respond to penicillin or sulfonamide therapy which treats pneumococcal pneumonia. Atypical pneumonia does not reveal the normal lobar arrangement in Xray, and includes spotty points of infection. *M. pneumoniae* is one of a number of organisms that cause atypical pneumonia.
3. Extrapulmonary infections can occur with the central nervous system as complication of pneumonia.

Pathogenesis:

1. Transmitted by aerosols
2. Attaches to epithelium via adhesins including a P1 protein complex at the tip of the bacterium, and induces cilliotosis resulting in necrosis.
3. Production of hydrogen peroxide causes oxidative damage and inflammation.
4. Inflammation can contribute to disease. Increasing severity with age may suggest that prior exposure enhances inflammation.

Diagnosis:

1. Sputum gram stain may reveal monocytes and PMNs, but often no predominant organism. Sometimes there is CNS involvement however the organism is rarely isolated from these sites. Diagnosis in early stages made by clinical picture.
2. Throat swab from pneumonia, pharyngitis, meningoencephalitis samples can take 3 weeks due to slow growth. Samples are susceptible to desiccation.
3. PCR detection can give earlier results and can be use with less well-preserved samples.
4. Cold agglutination with serum IgM antibodies and other serological tests (difficulties associated with repeat infections). "cold hemagglutinins" (agglutinate human type O RBCs at 0 to 4°C, but *not* 37°C). (Bedside capillary test can be done in a few hours; routine test results can be back in a day.) This test may be positive in only about half the patients. A rise in titer is an important indicator of active infection.

Treatment:

1. Because cells do not have a cell wall, it is resistant to antibiotics that target the cell wall such as penicillins and cephalosporins
2. Antibiotics that interfere with protein synthesis, such as macrolides and tetracyclines, are effective in vitro and in vivo in eradicating most mycoplasmas.
3. No vaccine is available.

3. OTHER MYCOPLASMA ORGANISMS

A. *M. hominis*

- commonly found in the genital tract, especially females.
- highly correlated to having a role in various infections of upper female gen. tract--Pelvic Inflammatory Disease (PID), endometritis, low birth weights, postpartum fever.
- resistant to erythromycin; sensitive to tetracycline

B. *M. arthritidis*

- produces a super antigen and *may* be responsible for some infectious arthritis
- this or other mycoplasma implicated as a factor in rheumatoid arthritis--role of super antigens

C. *Ureaplasma urealyticum*

- is a mycoplasma
- common inhabitant of genital tract
- responsible for perhaps 20% of nongonococcal urethritis (NGU) not attributable to chlamydia; mild; also associated with infertility in males (reversible with doxycycline); hydrolyzes urea (hence the name; basis of lab ID).

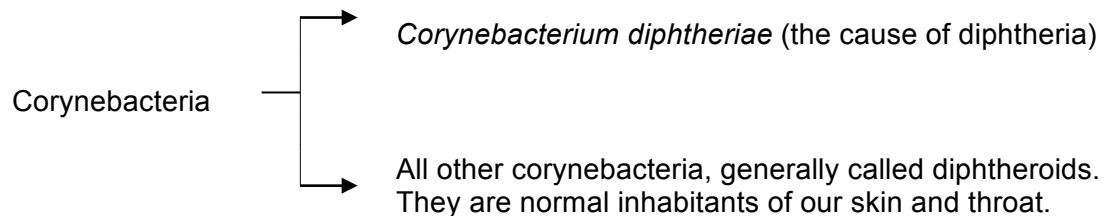
D. *M. genitalium* Recent reports suggest a link to some cases of NGU in males

E. *M. fermentans*; *M. incognitus* Some evidence that they are cofactors for HIV infection

4. CORYNEBACTERIUM DIPHTHERIAE

General:

1. The corynebacteria are pleomorphic (varying in size and shape) **Gram-positive** rods. They are aerobes and do not form spores. The rods are often club shaped.
2. For our purposes, all corynebacteria are divided into two groups:



3. On gram stain of material from a colony, *C. diphtheriae* rods will often be arranged in a non-orderly array suggestive of Chinese characters.

Epidemiology:

1. Diphtheria was once a major cause of death in the USA. Before immunization a significant fraction of the population were carriers of *Corynebacterium diphtheriae*. Immunization has drastically reduced the carrier rate.
2. From 1980 through 2002, 54 US cases of diphtheria were reported in the United States in total.
3. Epidemics are now seen in poorly immunized populations with inadequate medical care. Diphtheria remains a major problem in those developing countries with inadequate pediatric immunization. The vaccine provides reliable immunity for only 10 years. Travelers to developing countries are at risk and should be immunized before departure [using the combination tetanus-diphtheria vaccine].

Disease and Pathogenesis:

1. Diphtheria is one of the best understood and (in the developed countries) one of the best-controlled bacterial diseases of humans.
2. Humans are the only natural hosts for *Corynebacterium diphtheriae*. Transmission is usually by respiratory droplets.
3. After an incubation period of 2-5 days bacterial growth in the throat results in a local inflammatory response with fever, cough, sore throat that progresses to the formation of a gray *pseudomembrane* composed of fibrin, necrotic epithelium, and white cells. In young patients this pseudomembrane plus edema can cause death by blocking respiration.
4. In the tropics (and sometimes elsewhere) diphtheria is also seen as a necrotizing skin infection spread by contact.
4. *Corynebacterium diphtheriae* does *not* produce a systemic infection. The bacteria are confined to the initial focus in the throat (or skin, in the tropics). Systemic symptoms are produced by an secreted *exotoxin*.
 - a. Diphtheria toxin is lethal to eukaryotic cells. In fatal cases the heart (myocarditis and congestive heart failure), kidney (tubular necrosis) and nervous system (weakness or paralysis) are the primary targets.
 - b. Note that the toxin that damages these target tissues comes from bacteria in the throat. The bacteria are NOT in the damaged tissues.
 - c. All toxogenic strains of *C. diphtheriae* are lysogenic for a specific temperate bacteriophage called beta. The gene for the toxin is part of the bacteriophage genome and thus this is an example of lysogenic *conversion*. [Reviewed in microbial genetics lecture.]
 - d. the diphtheria exotoxin has two domains. The cell surface receptor (B or binding domain) promotes endocytosis. Within endosomes, the bound diphtheria toxin dissociates into its two domains and the toxin domain (A or active domain) enters the cytoplasm in a process promoted by the B domain.
 - e. The toxin domain of diphtheria toxin BLOCKS EUKARYOTIC PROTEIN SYNTHESIS by inactivating elongation factor two (EF2), an enzyme involved in peptide bond formation on ribosomes. The mechanism of inactivation involves the adenosine diphosphate ribosylation of EF2 by the toxic A-domain diphtheria toxin which acts here as an enzyme.

Diagnosis and Treatment:

1. Severe cases are relatively easy to spot. Milder cases in a non-epidemic context may be difficult to detect.
2. *Corynebacterium diphtheriae* is tentatively identified through characteristic growth on special media tellurite agar, Tinsdale's medium and Leoffler media where metachromatic granules can be visualized.

3. Definitive diagnosis depends on the demonstration of toxin production
 - a. Identification of the toxin (ELEK test).
 - b. PCR test
 - c. Immunoassay
4. These laboratory tests will be carried out by diagnostic microbiology laboratory only upon request. You must indicate suspicion of diphtheria when you submit the throat swab.
4. If you suspect diphtheria, treatment must begin at once. You cannot wait for laboratory confirmation. Diphtheria is treated with horse antitoxin (beware of hypersensitivity and anaphylactic shock) or human antitoxin. The antitoxin will neutralize free toxin and toxin bound to cells but intracellular toxin is not affected. Penicillin is also used to eliminate bacteria that would otherwise continue to produce toxin. But antitoxin treatment is the essential component.
5. The Great Race of Mercy <http://www.cdc.gov/24-7/savinglives/diphtheria/index.html>
6. Persons who have had contact with the patient should be reimmunized and treated with penicillin.

Immunization:

1. Since the disease is caused by a toxin, immunity depends on the presence of antibody against the toxin (antitoxin).
2. Gaston Ramon (1886-1963) discovered that formaldehyde treatment would abolish the toxic activity but retain the immunogenicity of diphtheria toxin. The formaldehyde-treated product is called TOXOID. This is the vaccine used today. In the USA this immunization is generally coupled with those for pertussis (whooping cough) and tetanus (DTaP).

OBLIGATE INTRACELLULAR BACTERIA: RICKETTSIA AND CHLAMYDIA

Session objectives:

- To explain the chlamydial life cycle, how different species are distinguished from one another and how infections are diagnosed.
- To describe the vectors for rickettsial infections.
- To compare the route of human infection with *Coxiella burnetii* (Q fever) with that of other rickettsia.
- To explain how chlamydial and rickettsial infections are treated.

Common characteristics of intracellular pathogens:

1. Small size, about 300 nm.
2. Obligate intracellular growth.
3. They have *bacteria-like cell walls*. They are most closely related to gram-negative bacteria.
4. The chlamydia, rickettsia and *Mycobacterium leprae* are examples of obligate intracellular bacterial pathogens.

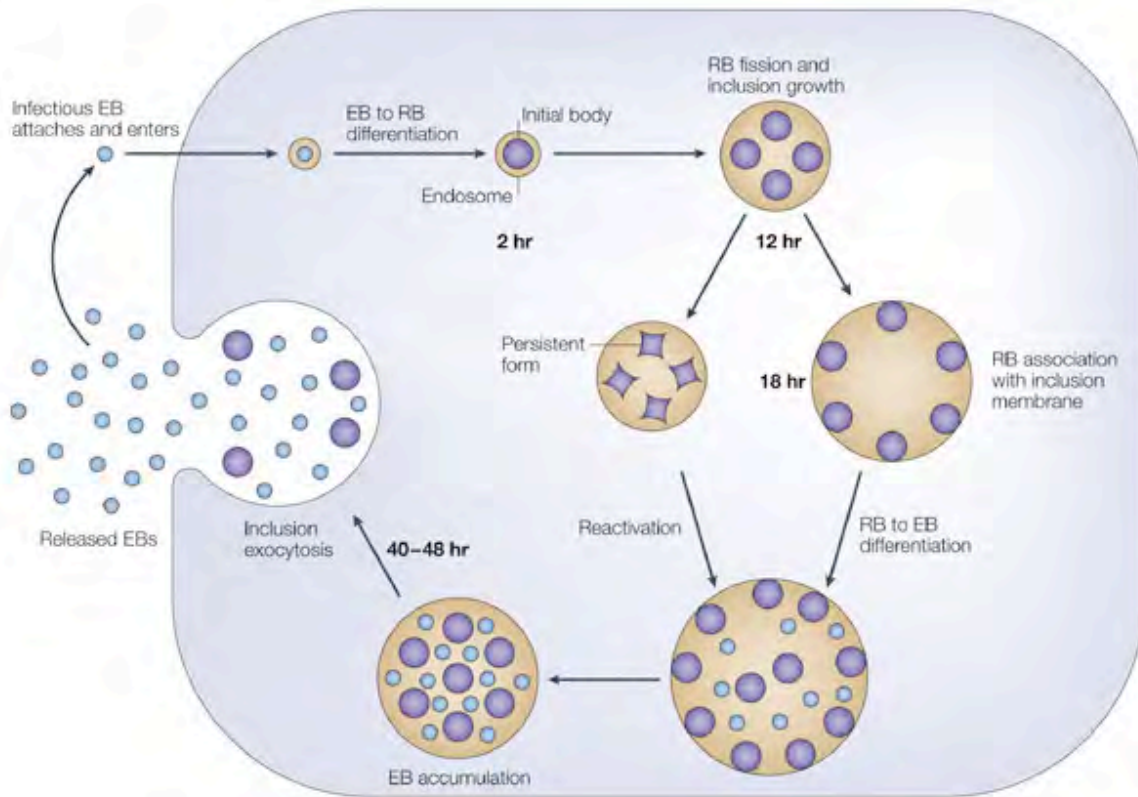
Other bacteria are *facultative intracellular bacteria*. These bacteria are often intracellular during infection but can grow extracellularly both *in vivo* and *in vitro*.

1. CHLAMYDIA

A. General information:

1. Chlamydia and Chlamydophilia that cause human disease are normally parasites of humans, other mammals, or birds. Arthropods are not involved (compare Rickettsia, below).
2. *Chlamydia psittacosis* and *Chlamydia pneumoniae* have recently been reassigned to a new genus, *Chlamydophila*. In some places, you will still see them referred to as *Chlamydia*.
3. Explanation for obligate intracellular growth: Chlamydia cannot make ATP and thus depends on host cell ATP.
4. There are two stages in the developmental cycle:
 - a. ELEMENTARY BODIES (EB): small, non-multiplying, with a rigid bacterial-like cell wall. This form transmits the infection from cell to cell and from person to person.
 - b. INITIAL BODIES (IB): [also called reticulate bodies (RB)]: larger, actively multiplying, lacks rigid wall, noninfectious.
5. Intracellular growth cycle:
 - a. Elementary bodies enter by inducing the host cell to phagocytose them. Thus they can enter cells that are not normally phagocytic.
 - b. During the first 24 hours the infecting elementary bodies lose their cell wall, double in diameter, and synthesize RNA to yield initial bodies.
 - c. The initial bodies divide by binary fission and some of the progeny are converted back to the original smaller, highly infectious elementary bodies.

From Brunham & José Rey-Ladino (Nature Reviews in Immunology (2005) 5:149



Nature Reviews | Immunology

6. Classification: Three are known human pathogens in the Chlamydiaciae:

- a. *Chlamydophila psittaci*: causes psittacosis (one serotype)
- b. *Chlamydophila pneumoniae*: pneumonia, mostly in adults (a single serotype).
- c. *Chlamydia trachomatis*: different serotypes cause trachoma, lymphogranuloma venereum and a complex array of different clinical presentations including inclusion conjunctivitis, newborn infant pneumonia, and urethritis.

A. CHLAMYDOPHILIA PSITTACI: PSITTACOSIS [A ZONOSIS]

1. *Chlamydophila psittaci* is naturally a parasite of birds. In birds it can produce a chronic subclinical infection with *constant fecal excretion*. This subclinical avian infection can become overt under conditions of malnutrition, crowding, etc., and excretion of elementary bodies markedly increases.
2. Human infection is most commonly acquired by inhaling bird feces. Most cases arise from contact with parrots, parakeets, and pigeons. History of bird contact is important, including occupational exposure to chickens or turkeys.

3. Pathogenesis: Psittacosis is a generalized infection, the agent can be recovered from the blood or sputum. Most patients have fever and headache. The most distinctive and severe sign of infection is extensive INTERSTITIAL PNEUMONIA.

B. CHLAMYDOPHILIA PNEUMONIAE

1. *Chlamydophila pneumoniae* causes a significant fraction (about 10%) of pneumonia in adults. [Before *Chlamydia pneumoniae* was discovered most of its infections were misdiagnosed as mycoplasma pneumonia].
2. This disease is spread directly from person to person as respiratory aerosols *without* involvement of birds (compare *C. psittaci*).
3. [Epidemiological evidence suggests that *Chlamydia pyneumoniae* may play a role in coronary atherosclerosis. But clinical trials of antibiotics have been negative.]

C. DIFFERENT CHLAMYDIA TRACHOMATIS SEROTYPES:

1. NONGONOCOCCAL URETHRITIS (SEROTYPES D-K)

- a) A common venereal disease (possibly the most common). Worldwide, an estimated 90 million sexually transmitted *Chlamydia trachomatis* infections occur each year with the majority in the developing world. Estimated 15 million new cases occurring in Africa and 45 million new cases in southern Asia every year. In a random sample of young adults (18–26 years) in the United States, 4.2% were positive for *C. trachomatis*.
- b) Many males are asymptomatic. Those who are, usually present with a purulent urethral discharge. *Chlamydia trachomatis* causes about half of the cases of nongonococcal urethritis..
- c) Females are frequently asymptomatic although *C. trachomatis* is growing in the cervical cells and elsewhere.
- d) Infection increases the likelihood of HIV transmission.
- e) Both sexes will sometimes have more severe disease involving the epididymus or Fallopian tubes. [Infection with *Chlymidia trachomatis* is one of the causes of Reiter's Syndrome (to be covered in SBM).] Damage to the Fallopian tubes can result in sterility (50,000 infections result in sterility every year) or ectopic pregnancy. Asymptomatic at-risk women of child-bearing years should be screened by PCR of urine specimens. Those infections detected are best treated with a single high dose of azithromycin to avoid problems of poor compliance.
- f) Treatment must involve both sexual partners.

2. INCLUSION CONJUNCTIVITIS AND INFANT PNEUMONIA (SEROTYPES D-K)

- a) Symptomatic or asymptomatic woman infected with the chlamydial serotypes that cause nongonococcal urethritis are at risk of transmitting *C. trachomatis* to neonates at birth.
- b) The most common neonatal disease acquired in this way is INCLUSION CONJUNCTIVITIS (the intracellular chlamydia stain as an inclusion body, as in virology). The usual measures to protect against neonatal gonococcal conjunctivitis [erythromycin or sulfonamides] do not prevent inclusion conjunctivitis. Inclusion conjunctivitis is occasionally seen beyond the neonatal age where environmental contamination (swimming pools, etc.) is involved.

- c) Some neonates also get a chlamydial pneumonia, usually as an extension of the ocular disease.
- d) In adolescents and adults, inclusion conjunctivitis can be an STD.

3. TRACHOMA (SEROTYPES A, B, C)

- a) The serotypes that cause trachoma are more invasive.
- b) Transmission is mechanical (finger to eye) and by flies under conditions of poor hygiene. Trachoma is prevalent in tropical Africa and Asia particularly in arid regions.
- c) Chronic reinfection of the conjunctiva causes an infolding of the eyelashes that results in corneal scarring and blindness.
- d) Annual universal treatment with azithromycin is very effective.

4. LYMPHOGRANULOMA VENEREUM (LGV) (SEROTYPES L1, L2, L3)

- a) Caused by serotypes of *C. trachomatis* that are transmitted venerally but are more invasive than those that cause nongonococcal urethritis.
- b) The characteristic symptom is a painless papule progressing to an ulcerating vesicle first appears two weeks after exposure.
- c) Four weeks after exposure the infection will sometimes further progress to a painful suppurating disease of regional lymph nodes.
- d) Delayed hypersensitivity to heat-killed LGV injected intradermally (FREI TEST) indicates prior or current disease.

D. CHLAMYDIAL TREATMENT

Tetracyclines work well in all chlamydial infections. But azithromycin may be the preferred treatment. An antibiotic that can enter cells is required.

E. LABORATORY DIAGNOSIS OF CHLAMYDIAL INFECTIONS

1. All chlamydia share a group antigen but they are distinguished from one another by specific antigens.
2. Remember that the various diseases caused by *C. trachomatis* are each associated with a series of specific serotypes. *C. trachomatis* is diagnosed based on detection from intraurethral or endocervical smear by direct immunofluorescence test, enzyme immunoassay, DNA probe, and PCR-based test. *C. pneumoniae* or *C. psittaci* have antibody assays.
3. For laboratory diagnosis, compare acute and convalescent antibody titers (as in virology).
4. Chlamydia can be isolated by inoculation of cultured cells and detecting characteristic cytoplasmic inclusion bodies by staining or, more specifically, by fluorescent antibody. Growth is slow.
5. Identification of intracellular forms and extracellular elemental bodies by fluorescent antibody allows an immediate diagnosis by examination of appropriate clinical specimens.

SUMMARY TABLE

<u>Species</u>	<u>Disease</u>	<u>Route of Infection</u>
<i>C. psittaci</i>	interstitial pneumonia	respiratory (from birds)
<i>C. pneumoniae</i>	interstitial pneumonia	respiratory (from other humans)
<i>C. trachomatis</i> (various serotypes are specifically associated with different diseases)	nongonococcal urethritis cervicitis, epididymitis, salpingitis, proctitis	STD
	inclusion conjunctivitis and neonatal pneumonia	perinatal
	trachoma	mechanical contact (finger to eye or flies to eye)
	lymphogranuloma venereum	STD

2. RICKETTSIA

A. Basic properties:

1. Most rickettsia that cause human disease are normally parasites of arthropods. Most human infections are acquired from arthropods.
2. Intracellular growth cycle in human or arthropod cells:
 - a. Enter by phagocytosis. The phagocytosis is promoted by the rickettsia and requires energy expended by the rickettsia. They readily enter non-phagocytic cells.
 - b. Multiply slowly by simple binary fission (division time about one day).
 - c. The progeny are released by lysis of the host cell.
3. There is no good explanation for the obligate intracellular parasitism of the rickettsia. They are well adapted to this biochemically rich habitat in that they can take up ATP, NAD, and other phosphorylated metabolites. But they can also make their own ATP.

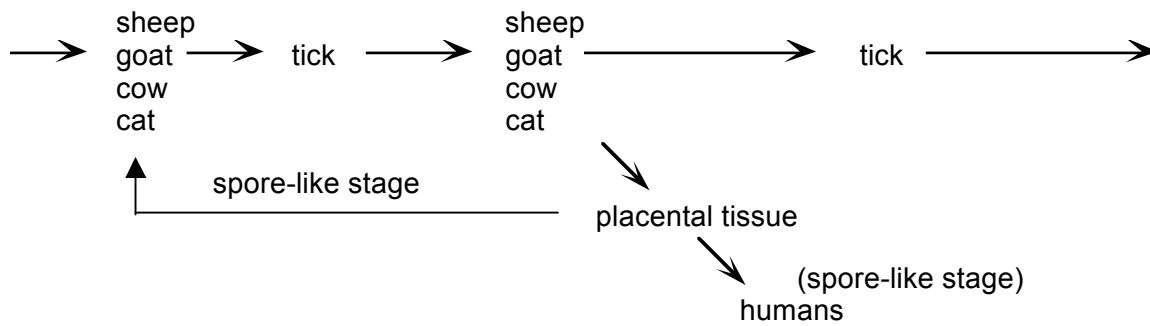
A. *Rickettsia prowazekii* -PRIMARY EPIDEMIC TYPHUS

1. Primary epidemic typhus is caused by *Rickettsia prowazekii*. The rickettsia multiply first in the capillary endothelial cells. After an incubation period of about 10 days there is an abrupt onset of fever and severe intractable headache. A rash follows 4 to 7 days later. The untreated disease is frequently fatal except in children. A toxin may be responsible.
2. Charles Nicolle discovered the mode of transmission.
3. Humans have three kinds of lice (they will be considered in SBM).
 - a. head lice
 - b. crab lice
 - c. body lice: The body lice live in folds and seams of the clothing since they prefer a temperature of about 30°C.
4. Transmission of *R. prowazekii* in primary epidemic typhus:

————> human ———> body louse ———> human ———> body louse

[Both humans and their lice are killed by *R. prowazekii* suggesting that they are not the natural hosts that maintain the rickettsia.]
5. The louse punctures the skin to get a blood meal and defecates at the same time. Scratching drives the fecal material and rickettsia into the bite wound.
6. Primary epidemic typhus is a disease of human misery, famine, and war.
7. Treatment with tetracyclines is very effective.

3. Pattern of transmission:



Think of animal, especially occupational, exposure.

4. The disease is characterized by interstitial pneumonia, fever, headache, and elevated liver function tests (LFTs). Some patients have a rash.

4. EHRLICHIOSES

1. The ehrlichioses are newly emerging tick-borne diseases caused by two different obligate intracellular bacteria. The natural vertebrate hosts are under investigation.
2. Two kinds of ehrlichiosis are known.
 - a. MONOCYTIC EHRLICHIOSIS infects monocytes [and is caused by *Ehrlichia chaffeensis*].
 - b. GRANULOCYTIC EHRLICHIOSIS infects granulocytes [and is caused by *Anaplasma phagocytophilum* and is transmitted by the same tick species that transmits Lyme disease.]
3. Infections are characterized by fever, lymphocytopenia (think of white cells destroyed through being infected), and elevated liver function tests due to liver damage
4. The best treatment is tetracycline

SUMMARY TABLE

<u>Pathogen</u>	<u>Disease</u>	<u>Vector</u>
<i>Rickettsia prowazekii</i>	primary epidemic typhus	human louse
<i>Rickettsia prowazekii</i>	endemic typhus	flying squirrel louse
<i>Rickettsia prowazekii</i>	Brill-Zinsser disease	none directly involved because it is a recrudescent disease.
<i>Rickettsia typhi</i>	endemic murine typhus	flea (squirrel, rat)
<i>Rickettsia rickettsii</i>	Rocky Mountain spotted fever	tick (dog or other)
<i>Rickettsia akari</i>	rickettsial pox	mouse mite
<i>Coxiella burnetii</i>	Q fever	NONE (for human disease)
two ehrlichioses (you do not need to know the genus and species names)	ehrlichiosis	ticks

NEISSERIA
(discovered in 1885 by Neisser)

Learning Objectives:

To describe the common bacteriological features of the Neisseria, methods used for diagnosis and role of vaccines

To describe the geographic distribution and target populations for Neisseria infections

To explain the major manifestations of illness due to *N. gonorrhoeae* and *N. meningitidis*

To describe which antibiotics are preferred in treatment of illness caused by *N. gonorrhoeae* and *N. meningitidis*

Reading assignment: Levinson, Chapter 16; pp 635-636

Two important pathogens:

Neisseria meningitidis, the agent of *meningococcal* meningitis, also called *meningococcus*: (other bacteria can also cause meningitis).

Neisseria gonorrhoeae, the agent of gonorrhea. Also called *gonococcus*.

I. COMMON FEATURES OF MENINGOCOCCUS AND GONOCOCCUS:

1. Both are gram-negative diplococci, with adjacent sides slightly flattened.
2. Require rich medium for isolation; grow better in 5 to 10% CO₂. Thayer-Martin selective medium permits recognition of *N. meningitidis* and *N. gonorrhoeae* from materials contaminated with other bacteria; contains chocolate agar, with vancomycin (to inhibit gram-positives) and colistin (to inhibit gram-negative enterics) and nystatin (anti-fungal). Most non-pathogenic Neisseria will also fail to grow on Thayer-Martin. The pathogenic species of Neisseria require blood products in the medium, while nonpathogenic species do not.
3. Readily killed by drying, heat, disinfectants.
4. No animal reservoir known. Pathogenic strains confined to humans.
5. Oxidase-positive. Addition of a drop of 1% solution of dye (dimethyl- or tetramethyl-p-phenylenediamine) to colony causes a pink and then a black color to appear within seconds. (However, non-pathogenic Neisseria also react positively). This reaction is part of your laboratory exercise.
6. Sugar fermentation tests distinguish between meningococcus and gonococcus, as well as non-pathogenic Neisserial species. Four sugars are used; glucose, maltose, lactose, and sucrose. *N. gonorrhoeae* ferments glucose only, not maltose, lactose or sucrose; *N. meningitidis* ferments glucose AND maltose, but not lactose or sucrose; and non-pathogenic *Neisseria* ferment all the sugars except sucrose.
7. Secrete a protease that splits secretory IgA1. This protease may contribute to the capacity of these bacteria to cause disease.
8. Pili and OMP's may contribute to colonization and affect colony morphology on agar medium.
9. LOS (lipid-oligosaccharide). In contrast to LPS, it contains no O side chains. However, it is still endotoxic and mediates resistance to serum bactericidal activity.

II. MENINGOCOCCUS (*Neisseria meningitidis*)

A. General Properties:

1. Multiplies *outside* of cells, but *not* once phagocytized (although bacteria can be seen within leukocytes).
2. Endotoxin from organism damages walls of small vessels.
3. Lives in nasopharynx. Carrier rate varies; it may be about 5% in the general population, but much higher in households where there has been a case of meningococcal meningitis.
4. Differences in composition of polysaccharide capsule are the basis for classification into serogroups: bacteria of groups A, B, C, Y, and W-135 are the most important. Meningitidis, but not gonorrhoeae, are encapsulated.
5. Within each serogroup, there may be serotypes (e.g., group B has a dozen serotypes) based on differences between outer membrane proteins.

B. Pathogenicity

1. Transmitted from person-to-person in airborne respiratory droplets. Carrier state may last from days to many months. In some, a mild pharyngitis and fever are all that occur. Transmission most often in crowded conditions (ex. day care centers, military barracks, college dormitories).
2. In a small percent of infected humans, bacteria progress from nasopharynx to blood stream. Incidence of severe disease--about 1 in 50,000, per year. Incubation period is a matter of days to a week.
3. Capsule has antiphagocytic properties. This contributes to virulence.
4. An uncommon occurrence: meningococemia may cause adrenal failure circulatory collapse, and shock, with rapid death. Known as the Waterhouse-Friderichsen syndrome.
5. Most adults have antibodies that confer immunity. Evoked within a week by the carrier state.

C. Epidemiology and Patient Populations of Special Interest

1. Generally, meningitis caused by *N. meningitidis* occurs sporadically and with no particular geographic distribution except for the following instances.
2. The Sub-Saharan Meningitis Belt - Largest burden of meningococcal meningitis in the world. Caused by *N. meningitidis* type A, which is virtually non-existent in the western world.
3. Stretches from Senegal in the west to Ethiopia in the east.

4. During the dry season between December and June dust winds, cold nights and upper respiratory infections combine to damage the nasopharyngeal mucosa, increasing the risk of meningococcal disease.
5. When diverse groups are brought together--as in college dormitories--there is exposure to serotypes of bacteria not encountered before. Hence outbreaks may occur.
6. Transmission of *N. meningitidis* is facilitated by overcrowded housing and large population displacements at the regional level due to pilgrimages and traditional markets.

D. Diagnosis

1. Clinical picture of upper respiratory infection followed by high fever and signs of meningitis. Patechiae (small purplish-reddish spots caused by minute hemorrhages) may appear in skin, followed by large areas of ecchymosis (black and blue spots caused by leakage of blood from small vessels).
2. Specimens from blood, spinal fluid, nasopharyngeal secretions are cultured and examined for gram-negative diplococci. Can further identify organism by sugar fermentation test (takes 4 hours) and/or latex agglutination test (10 minutes) based on capsule antigen in CSF.

E. Treatment

Treatment must be prompt. Intravenous penicillin is the drug of choice (unless there is hypersensitivity to penicillin) or third generation cephalosporins (ceftriaxone). For prophylaxis in household contacts, rifampin is used, because this antibiotic is efficiently secreted into the saliva, or increasingly ciprofloxacin is used because it is so potent.

F. Prevention and immunity

Vaccines have been tested in several countries, and are moderately successful. A quadrivalent vaccine is now available for groups A, C, Y, and W-135. Mainly for epidemics or military. Type B capsule is composed of sialic acid which is not recognized by the C3b component of complement, and thus does not stimulate recruitment of phagocytes.

G. New vaccine designed specifically for Africa

Massive immunization in the meningitis belt began in December, 2010. MenAfriVac is produced by nonprofit PATH, of Seattle, WA. It costs a mere 44¢ per dose. It is a conjugate vaccine.

H. Lumbar puncture and meningitis

It is essential to test CSF when meningitis is indicated. These circumstances include any fever in a neonate (suspect group B Streptococcus, or *E. coli* K1). Otherwise, clinical picture is critical. The presence of a patechial rash in the case of an adult or child with neurological symptoms is indicative (*N. meningitidis*). Meningitis caused by *H. influenzae* and *S. pneumoniae* in children may be the most difficult to diagnose.

III. GONOCOCCUS (*Neisseria gonorrhoeae*)

A. General Properties:

1. Can be seen by Gram stain in fluid of purulent exudate (pus). Can also be seen inside of epithelial and phagocytic cells.
2. Endotoxin activity (unlike meningococcus) is not a major factor in disease state.
3. Lives primarily in genitourinary tract. Other tissues are sometimes involved (see below).
4. More than 100 *serotypes* based on antigenicity of pilus protein. Gonococcus, unlike meningococcus, has no capsule.

B. Pathogenicity:

1. Common mode of transmission: sexually transmitted disease (STD), direct genital contact, resulting in genitourinary tract infection. Additional portals of entry for bacteria: rectal and pharyngeal mucosa (*non-pathogenic Neisseria* also found in pharynx) and the conjunctiva of the eye of newborns. In the latter case, transmission occurs during passage of the newborn through the birth canal and may result in serious disease: ophthalmia neonatorum (discussed further below).
2. Infection is established within an hour of exposure. Bacteria anchor to the surface of epithelial cells using their pili. The bacteria then penetrate through the surface cells (intracellularly) and reach sub-epithelial connective tissue in a few days. This is followed by inflammation and yellow purulent urethral discharge (with burning sensation on urination). Symptoms in males typically appear in 2 to 3 days. The incubation period for gonorrhea is more variable and less well defined in females. Most females who will become symptomatic do so within 10 days. However, a high percentage of infected females remain asymptomatic, in contrast with males. The infection may occasionally spread to the epididymis or prostate in males and, more often, to Fallopian tubes in females.

Pili of gonococcus are associated with virulence. They promote sticking to epithelial cells, and impair phagocytosis by polymorphonuclear leukocytes. Non-piliated strains are not virulent.

3. Incidence: gonorrhea--and infection with venereally-transmitted *Chlamydia trachomatis*--are probably the two most prevalent communicable bacterial diseases of humans. There is a worldwide pandemic of gonorrhea, not appreciably lessened by the introduction of chemotherapy.
4. *Asymptomatic* carrier states are common. Carriers can transmit infection. For males it is estimated that 1 to 10% of those infected and able to transmit the disease are asymptomatic. This percentage is greater for females.
5. Complications of disseminated disease:
 - a. arthritis-dermatitis syndrome, with spread to one or more joints, and with skin lesions; joint infection may occur in the *absence* of prior overt genito-urinary symptoms.
 - b. chronic pelvic inflammatory disease, in females.

C. Epidemiology and Patient Populations of Special Interest

1. The incidence of infection is greatest in young, sexually active adults, typically with multiple partners.

D. Diagnosis of acute disease

1. Clinical picture of discharge, plus history of exposure.
2. Stained smears of fresh exudate often show gram-negative diplococci *within* polymorphonuclear leukocytes.
3. Culture should be done *before* treatment is started, if possible. Colonies show oxidase-positive, gram-negative diplococci.

E. Treatment

1. Drug resistance is a major problem in treating gonorrhea. Recommended regimens have changed several times in the past 10 years. There are three major forms of gonococcal resistance:
 - a. plasmid-mediated penicillinase producing *N. gon* (PPNG).
 - b. plasmid-mediated tetracycline resistant *N. gon* (TRNG)--Tet^R plasmid is conjugative and can mobilize Pen^R plasmid.
 - c. chromosome-mediated resistant *N. gon* (CMRNG). CMRNG is a broad-based resistance--includes Pen, Tet, and some cephalosporins--related to mutations affecting permeability; level of resistance low; treatment failure rare.

Because of widespread resistance, single-dose penicillin therapy is no longer recommended. Rather--IM Ceftriaxone and 10 day oral tetracycline, doxycycline or azythromycin for chlamydia. (Since *Chlamydia trachomatis* is the STD of highest incidence, one assumes chlamydial infection is present in cases of gonorrhea.) Unfortunately, cephalosporin resistant *N. gonorrhoeae* are currently emerging.

2. Ophthalmia neonatorum: This disease was once the cause of 50% of blindness in children. Prophylactic treatment of the newborn is required by law: Options for this treatment--tetracycline or erythromycin ointment, or dilute (1%) silver nitrate (the latter is taken from standardized ampules to prevent damaging effects of overdosing with silver nitrate).

F. Prevention and immunity

1. Immune response, although it can be demonstrated after infection, is weak, for unknown reasons. Hence repeated attacks are common. A high degree of antigenic variation of surface components (for example, 100's of antigenic pilus variants) may contribute to this.
2. No vaccine is available.

Similarities and differences for the pathogenic *Neisseria*

Property	<i>meningitidis</i>	<i>gonorrhoeae</i>
Major infection	Meningitis, septicemia	Gonorrhea, PID
Less common infect.	Arthritis	Arthritis, conjunctivitis
Reservoir	Humans	Humans
Transmission	Droplet	Direct, STD
Morphology	Gm-, diplococci	Gm-, diplococci
Oxidase	+	+
Fermentation ^a	Glu ⁺ Mal ⁺ Lac ⁻ Suc ⁻	Glu ⁺ Mal ⁻ Lac ⁻ Suc ⁻
Virulence	Capsule, meningitis usually assoc. w/ type B; IgA protease; pili	Pili, IgA protease, Omps?; Opa?
Toxins	LOS (LPS); No exotoxins	LOS (LPS); No exotoxins
Clinical	Meningitis-fever, stiff neck vomiting, lethargy, petechial rash Septicemia-fever, petechial rash, hypotension, W-F syndrome	Men-urethritis Women-cervical gonorrhea, PID Neonates-Ophthalmia neonatorum
Diagnostics	Gram stain, spinal fluid, blood, nasopharyngeal or free-living and internalized bacteria; culture on chocolate agar and Thayer Martin.	Gram stain urethral pus, fresh exudate for bacteria w/in WBC's.
Treatment	Penicillin G, ceftriaxone; rifampin prophylactically for close contacts	Ceftriaxone and doxycycline for <i>chlamydia trachomatis</i>
Vaccine	Available for capsule types A,C,Y and W-135; but not for type B	No vaccine

^a nonpathogenic *Neisseria* ferment all three sugars

EXOTOXIN PRODUCING CLOSTRIDIA

Learning objectives:

To describe shape and location of the spore in the medically important species.

To describe the epidemiology of tetanus and botulism.

To describe different types of toxin and the mechanism of action of botulism and tetanus toxins.

To describe the most prominent features used for clinical and laboratory diagnosis.

To explain the treatment and prevention of clostridial diseases; Describe when toxoid or antitoxin is indicated and explain the role for antibiotic treatment.

Reading assignment: Levinson, pp. 39-44 (Tables), pp. 136-141; pp. 636-637

Bacteria cause disease by two major mechanisms:

1. Toxin-mediated disease -

a) toxin - clostridial toxin such as botulism toxin (no organisms required in ingestion)

Other example: *Staphylococcus aureus* enterotoxin - the most common food-borne diarrheal disease in the US

2. Invasion and Inflammation

a) disease can occur via direct tissue invasion and then tissue damage from over-reactive immune response from the host (e.g. *Clostridium perfringens*)

Pathogenic steps in establishing bacterial infections by ingesting toxin

1. Toxins preformed in the food -

a) *Clostridium botulism*, preformed in food, is ingested

b) *S. aureus* enterotoxin preformed in contaminated food
(Disease can occur in the absence of the organism here)

2. Ingestion of microorganisms with adherence, colonization and toxin formed in the gut

a) Infant botulism- organisms in the honey, toxin formed when it is in the gut (remember the intestinal lumen is anaerobic)

b) Another example: *Vibrio cholerae* – intestinal colonization followed by secretion of cholera toxin

Clostridium

General:

- a. obligate anaerobic Gram positive rods, "Spore-forming"
- b. Location of spore in the bacterium may aid in species identification (e.g. terminal spores=*Clostridium tetani*)
- c. catalase negative
- d. oxidase negative

Species:

- a. *Clostridium botulinum* - noninvasive, causing **botulism** (can be agent of bioterrorism)
- b. *Clostridium tetani* - generally noninvasive (very limited invasion potential), causative agent of **tetanus**
- c. *Clostridium difficile* - noninvasive, secreted toxin causing pseudomembranous enterocolitis (a cause of antibiotic-mediated diarrhea)
- d.
 - i. *Clostridium perfringens* - **very invasive pathogen, gas gangrene**
(The following are not required for exam, for your interest only)
 - ii. *C. septicum* - invasive in malignancy
 - iii. *C. Ramosum*
 - iv. other invasive clostridial species

Pathogenesis: Ability of these organisms to synthesize a variety of extracellular toxins

1. botulism toxin - cause of flaccid paralysis
2. tetanus toxin - cause of tetanus (locked jaw)
3. exotoxins A and B - cause of diarrhea in **pseudomembranous colitis** due to *Clostridium difficile*
4. Alpha toxin - lecithinase which lysed host cell membrane
- in combination with other degradative enzymes as the cause for "**Gas Gangrene**" due to *Clostridium perfringens*

A.

Tetanus

Bacteriology

- *Clostridium tetani*, Gram + rods, the only one with “**terminal spore**” (looks like a tennis racket – this is highly diagnostic), all other species have subterminal spores

Epidemiology

- soil (I have seen only one case with a landscaper working in a Rose bush)
- very rare disease in US, less than 40 cases per year mostly among non-immunized individuals (immigrants)
- more common in developing countries (e.g. infected umbilical stump)
- many cases occur via puncture wounds contaminated with soil containing *C. tetani*; spores germinated in wound under anaerobic conditions (dirty and deep wound)

Pathogenesis

- non invasive, **a toxin mediated disease**
- only **one serological type** (remember botulism toxins, in contrast, have several serotypes)
- disease caused by the tetanus toxin, which is a **neurotoxin** transported from the site of infection to peripheral and CNS nerves (affect anterior horn cells of spinal cord and on brain stem)
- toxin composed of two subunits (A and B), one subunit binds to neuronal ganglioside, the other subunit has the neurotoxin activity
- inhibit release of neurotransmitters (glycine and GABA) - resulting in spastic paralysis, “**convulsive contractions** – locked jaw)

Clinical syndromes

- incubation period of 4 days to weeks (history may be vague)
- violent muscle **spasm**, with the flexor muscle predominant
- clenched teeth, neck and back arched
- “**No fever**”
- “**No sensory deficit**”
- involvement of respiratory muscles leads to respiratory failure
- aspiration, dysphagia with oral pharyngeal involvement
- complications may be pulmonary infections and respiratory problem

Diagnosis

- **clinical** mostly
- organism rarely seen in wound site (may occur weeks ago)
- culture positive in 39%

Treatment

- human tetanus immunoglobulin (only one anti-toxin)
- penicillin plus wound debridement (metronidazole if penicillin allergic)
- respiratory support
- immunization with tetanus toxoid

Outcome

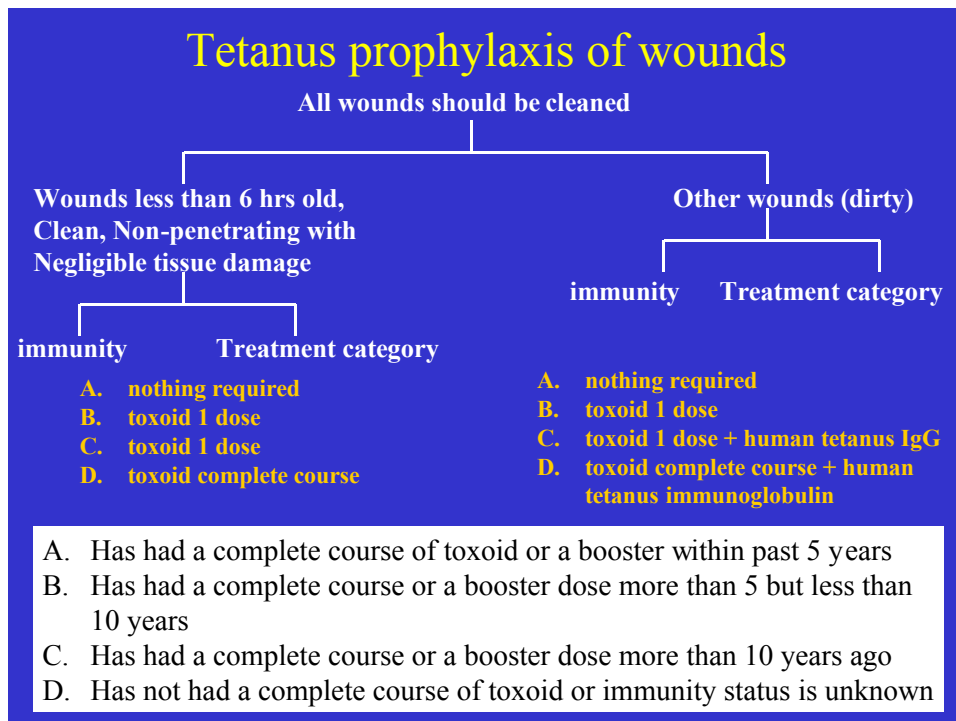
- high fatality rate (60%) - due to pulmonary complications and secondary infections

Prevention

- immunization with three doses of tetanus toxoid in the first 6 months of life (toxoid is toxin inactivated by formaldehyde);
- given as DPT (diphtheria, pertussis and tetanus vaccines) booster at 1 year and prior to school entry.
booster every 10 years

Tetanus prophylaxis

- clean wound in fully immunized individuals - booster every 10 y
- dirty wound in uncertain immunization - anti-tetanus immunoglobulin + complete immunization (3 shots)
- unimmunized - complete immunization



B.

Botulism

Bacteriology:

Clostridium botulinum, Gram + rod with subterminal oval spores (spore location not diagnostic for this species)

Epidemiology

- very low incidence (123 cases documented from 1976-1984)
- Soil organism, spores very resistant to physical and chemical agents; **Contrary to the C. botulinum spores, botulism toxin is very heat-labile (cook your food from a can).**
- *C. botulinum* spores found in contaminated food, under anaerobic conditions (canned goods), germinate, grow and produce botulism toxin in 2-3 days.
 - A. Food botulism found in canned vegetables (e.g. green beans, peppers and mushrooms), smoked fish and preserved fruits
 - B. wound associated botulism – requires spore germination in the wound
 - C. infant botulism (honey as a source) – this requires ingestion of spore, germination and intestinal colonization in the infant

Pathogenesis

Botulism toxin - extremely potent (1 mg killed 200,000 mice)

- **heat-labile** (inactivated by boiling for 10 min)
- Not destroyed by stomach acid
- 7 types (toxins A to G), with toxins A, B and E being most common
- two subunit toxins (A and B subunits)

Mechanism of action

- upon absorption in intestine, carried via blood to peripheral nerve synapses, acting as a neurotoxin,
- binding of toxin to a receptor on the nerve synapse, toxin enters cell, blocking release of acetylcholine from the cell by interfering with proteolytic processing (i.e. the toxin is a protease) and hence release of the acetylcholine.
- flaccid paralysis, **“No fever”**, **“Normal mental status”**

Clinical manifestations

- incubation period 18-36 h
- weakness or flaccid paralysis of peripheral nerves including cranial nerves, symmetrical in distribution
- dysphagia, diplopia, dry throat, dilated pupils
- **“no sensory deficit”**
- may affect respiratory muscles

Diagnosis

- usually a clinical diagnosis
- Do not rely on culturing the microorganisms (preformed toxin is the culprit)
- detecting botulism toxin in the serum, vomitus or feces
- detecting bacterial toxin in food with serological tests
- EMG (electromyography) - diminished action potential of the peripheral nerves, results suggestive not diagnostic

Differential Diagnosis

- Myasthenia gravis
- Guillain-Barre syndrome (an ascending paralysis syndrome that can also be distinguished with history and toxin detection)

Therapy

- removal of toxin from stomach (if detected early) with lavage
- treatment with antitoxin, remember the antitoxin (from horse serum) is toxin specific (types A, B, E)
- supportive care, may require respiratory support
- 12% fatality rate

Prevention

- Canned food must be **cooked** (100°C for 10 min to inactivate the toxin)

There are two clinical variants of botulism (also due to *Clostridium botulinum* and its toxin) besides food botulism

a. Infant botulism

- infants 1- 8 months of age
- honey is a common source of contamination
- food contaminated with *C. botulinum*, toxin formed as a result of bacterial proliferation in the gut (does not occur in adults - ? immune system immaturity)

- **symptom is more subtle**; constipation, weak head control (flaccid), cranial nerve deficit common
- diagnosis by demonstrating toxin and/or organism in stool; or organism in food
- treatment used to be supportive (due to allergic reaction to old antitoxin), but new human antitoxin antibodies (against serotypes A and B) are helpful

b. Wound-associated botulism

- Spores in soil contaminate wound, germinate and produce toxin.
- diagnosis by wound culture and demonstrated of toxin in serum.

C. Pseudomembranous colitis due to *Clostridium difficile* enterotoxin

Bacteriology

- *C. difficile*, anaerobic Gram + rod

Epidemiology

- a common cause of **antibiotic associated diarrhea** due to toxin-mediated inflammation of the colon
- found in GI tract of 3% of the general population
- 30% of hospitalized colonized with *C. difficile*, probably via fecal oral route from hospital personnel
- recent emergence of a more virulent strain 027 in Canada and England, presumably due to increased production of toxins (toxins A and B), which cause the diarrheal syndrome

Pathogenesis

- antibiotic-induced suppression of normal flora - *C. difficile* would proliferate and synthesize two distinct toxins (exotoxins A and B)
- exotoxin A is an enterotoxin that bind to the gut receptor
- exotoxin B is a cytotoxin, damaging colonic mucosa (bloody diarrhea), by ADP-ribosylating Rho, a GTP-binding protein
- damage to the colon by exotoxin B leads to pseudomembrane formation

Clinical syndromes

- diarrhea with pseudomembranes (yellow-white plaque) on colonoscopy or sigmoidoscopy
- usually not bloody but can be

Diagnosis

- history of antibiotic use (ampicillin, cephalosporins and clindamycin)
- exotoxin B detected in the filtrate of stool samples (bioassay)
- ELISA (Enzyme Linked Immunoabsorbent Assays) to detect toxins
- stool culture for *C. difficile* (by itself not very useful)
- sigmoidoscopy with visualization of pseudomembranes

Treatment

- stop the offending antibiotics
- treat with metronidazole, vancomycin or fidaxomicin

Prophylaxis

- **prudent use of antibiotics**
- contact isolation in case of outbreak
- Is hand washing enough to prevent transmission?

D. *Clostridium perfringens* – cause of Gas Gangrene (histotoxic Clostridia) and the milder food poisoning

- (i) “Invasive and rapidly progressive”- primarily a surgical disease
- (ii) food poisoning – a self limiting watery diarrhea

Bacteriology

- Many species of Clostridia can cause gas gangrene (anaerobic Gram + rod)
- ***Clostridium perfringens*** (the most common), *C. septicum*, *C. bifermentans* and *C. ramosum* (all anaerobes)
- A hallmark of these infections is “**Tissue necrosis**”

Epidemiology

- these are primarily soil organisms
They are also found in the human GI tract and vagina

Pathogenesis

A) Gas Gangrene

- synthesize many toxins and enzymes which lyse host cells and tissues - thus accounting for tissue **necrosis**
- Lecithinase : **alpha toxin** - damage host cell membrane (including capillary and host erythrocytes)
- Collagenase (collagen is the supporting tissue underlying cells)
- Hyaluronidase (hyaluronic acid is a carbohydrate molecule in the host matrix)
- “Can grow **rapidly**” in anaerobic environment (e.g. deep penetrating wound or dead tissues)
- the byproducts of anaerobic growth are **gases** (H₂ and CO₂)

B) Food poisoning

- Ingestion of food contaminated with large number of spores (10^8) with presynthesized enterotoxin
- The enterotoxin is associated with the spore coat
- toxin is released into the intestine

Clinical syndromes

A) Gas Gangrene

- dependent on host anatomy
 - i) cellulitis (superficial)
 - ii) necrotizing cellulitis (invasion into dermis and underlying capillaries)
 - iii) necrotizing fasciitis (invading the fascia around the muscle)
 - iv) myositis or myonecrosis (invasion into the muscle)
- may occur in the absence of open wound (e.g. penetrating but closed wound)
- rarely with uterine infection (septic abortion) or GI lesion in lymphoma or leukemia

B) Food poisoning

- abdominal cramps and watery diarrhea within 8-22 hours, resolved by 24 hours.
- 2nd or 3rd most common cause of food poisoning

Diagnosis (Clinical mostly)

A) Gas Gangrene

- **Crepitus** upon pressing the skin (due to gas in subcutaneous tissue or muscle)
- **discoloration** and edema of the skin, extreme **pain**
- serous dark exudates (this may not be present)
- Gram stain of fluid (What do you see?)
- Culture of wound (remember this is an anaerobe, need special anaerobic media)
- X-ray (What do you see?)

B) Food poisoning

- detecting the enterotoxin by ELISA in feces or implicated food

Treatment for gas gangrene

- **“Surgical”** - wound debridement
- penicillin to kill remaining bacteria
- “Hyperbaric oxygen” in selected medical centers

Outcome

- may be rapidly fatal if left untreated

Normal Microbial Flora and Anaerobic infections:

Learning objectives:

To describe cell shape, morphology, gram stain, how they are identified and role in disease of the following anaerobes: Prevotella, Fusobacterium, *Bacteroides fragilis*, peptostreptococcus and Actinomycetes (also Clostridia from previous lectures)

To describe general principles of antibiotic treatment of anaerobic infections; which anaerobes are resistant to penicillin G and where do they reside in the body?

To describe the names and most common identifying characteristics of the most predominant bacteria comprising the normal flora of the mouth, skin, intestinal and GU tracts.

To describe how the gut flora and their metabolic activities can affect the host (deconjugate bile salts, produce ammonia etc).

Reading assignment: Levinson, Chapter 6, Chapter 14, Chapter 22

Normal Microbial Flora

The Organisms and Their Relationship with Their Hosts

- I. **Normal Flora** (some of these bugs have been mentioned previously in the course, but are briefly reviewed as part of the normal flora – **resident bacteria in the human body – collectively called the human Microbiome**).

Distinguish normal flora (natural flora of body sites) vs. carrier state (carriage of pathogen in body sites)

Importance of the Normal Flora:

1. They constitute a protective host defense mechanism – interfere colonization with pathogens.
2. Normal flora can cause disease, especially in immunocompromised individuals. Suppression of part of the normal flora can allow pathogen to colonize and cause disease (e.g. antibiotic treatment promotes colonization with *Clostridium difficile* in gut, causing antibiotic-mediated diarrhea or pseudomembranous colitis)
3. They may serve a nutritional function – intestinal bacteria produce B vitamins (B12) and vitamin K
4. Bacterial overgrowth in the small bowel (e.g. partial obstruction or decreased motility) can lead to fat malabsorption and vitamin B₁₂ deficiency (**Learn this fact**)
5. Each anatomic location has its own distinct flora; knowledge of the local flora will allow judicious use of antibiotics when the barrier within each normal anatomic location is violated (e.g. penetrating wounds); this knowledge allows judicious use of empiric antimicrobial therapy
6. Recent studies revealed that the intestinal microbiota, the microflora in the intestinal ecosystem, has an impact on many aspects of human physiology and diseases including psoriasis, obesity, asthma, inflammatory bowel disease and cardiovascular disease

I. Normal digestive tract flora

A. Distribution

1. Normal mouth flora (saliva) – Aerobes and Anaerobes

Aerobes or facultative bacteria

- a. Viridans streptococci: **Gram positive cocci**, in chains.

- * These bacteria are present in relatively large numbers (10^6 organisms per ml of saliva or expectorate).
- * Viridans Streptococci (oral Streptococci), the predominant microorganisms in the mouth, are a group of bacteria which are the most common cause of subacute bacterial endocarditis. These bugs are highly adherent, both to dental tissue (e.g. plaques) and to cardiac valves during bacteremia (e.g. causing endocarditis).

The streptococci thrive on sugar, and some species turn sucrose into polysaccharide (dextran), an important ingredient of dental plaque. A byproduct of sugar metabolism is lactic acid which hastens dental caries.

- b. *Neisseria* species (**Gram negative diplococci**, coffee bean-shaped): Next in predominance in most individuals. Some species names are *N. catarrhalis* and *N. sicca* which are **non-pathogens** (Do not memorize these names). You need to differentiate them from related species such as *Neisseria meningitidis*, a cause of meningitis, which can colonize throat or nasopharynx - rarely.

- c. *Diphtheroids*: This is the common name for Corynebacterium species which are not *C. diphtheriae* and thus generally non-pathogenic. *Diphtheroids* are **pleomorphic, Gram positive rods** and encompass many species. These are the **most common contaminants in blood cultures** because they also colonize the skin.

Rare cases of infection due to diphtheroids have been reported, usually with foreign bodies.

- d. *S. epidermidis* is the major species of coagulase negative Staphylococci in the oral flora. **Gram positive cocci**, in grape-like clusters. Not as numerous as viridans streptococci in the mouth.*

S. epidermidis and *diphtheroids* are the most common contaminants of blood cultures (In the presence of foreign bodies - catheters, intravascular artificial devices – they may be pathogens)

- e. **The above four species are most prevalent in the oral cavity** – reported as "normal throat flora" in most microbiology lab without further identification. (Remember that anaerobes are present in large numbers. These are seen on gram stain, but will not grow in routine aerobic cultures.)

Role of normal aerobic flora in disease:

- i) If *the normal throat flora* (NTF) are eliminated by antibiotics, they may be replaced by colonization with more resistant Gram-negative rods or *Staphylococcus aureus*, or yeasts which become the sources for drug-resistant infections seen in patients with malignancy on chemotherapy.

- ii) A small percentage of health individuals (5-40%) have potential pathogens in low numbers in their oral cavity; e.g. *pneumococcus* or *Staphylococcus aureus*. *S. aureus* is a normally inhabitant of the nasal cavity in 25-30% of normal individuals.
 - iii) Upon hospitalization (especially in combination with given antibiotics), **aerobic Gram-negative rods** (e.g. *E. coli* or *Klebsiella*) may colonize the oral cavity.
- f. *Eikenella corrodens*, a facultative Gram-negative rod, is part of the normal oral flora. It causes skin and soft tissue infections associated with human bites and clenched-fist injuries.

Anaerobes in the oral cavity:

- a. **Anaerobes are present in large numbers in the mouth**, on the gums and in the throat. They probably grow in close association with mucous membranes (e.g. gum/teeth interface).

Common oral anaerobes:

- 1) *Fusobacterium* (thin cigar-shaped Gram negative rod), **penicillin sensitive**
- 2) *Prevotella* species (gram negative rods), usually **penicillin sensitive**
- 3) *Anaerobic streptococci* (peptostreptococcus –Gram positive cocci in chains – 15% of bacteria in saliva) – usually **penicillin sensitive**
- 4) *Actinomyces*
- 5) Others such as anaerobic spirochetes

- b. These numerous mouth and throat bacteria **do not extend into the lower trachea**, which is nearly bacteria-free **in healthy individuals**.

Diseases related to anaerobes and/or aerobes in the oral cavity:

i) periodontal infections

ii) *Aspiration pneumonia* is pneumonia cause by aspiration of own oral secretion (e.g. during loss of consciousness or seizures); the offending pathogens are probably due to a mixture of normal mouth flora (mixtures of anaerobes and aerobes - mixed infections)

iii) **Penicillin would be useful** because many of the anaerobes and aerobes in the NTF are sensitive to penicillin

iv) Determination of causative organism(s) might not be fruitful because of the number of organisms involved.

2. **Normal stomach flora:** Because of high acidity, few organisms can be cultured except immediately after meals. **Exception:** In **gastric achlorhydria** (no stomach acid) or **gastric obstruction**, bacteria can proliferate here.

3. **Upper small intestine:** Generally sterile in most normal fasting subjects. When present, bacteria are usually contaminants transported from the mouth and respiratory tract and low in number (less than 10^5 per ml). Some Gram-positive aerobes, few anaerobes.

The relative sterility of the small intestine is largely a result of rapid onward movement of bacteria during peristalsis. Additionally, unconjugated bile acids, as antibacterial agents, may also prevent bacterial growth.

Role of upper small intestine microflora in disease:

- i) Bacterial counts in the upper small bowel may increase (10^5 per ml) if anatomical alterations cause stasis (e.g. after gastric bypass surgery).

4. **Terminal ileum:** In normal subjects, usually 10^6 per ml or less, the species and numbers of bacteria closely resemble those present in feces. This is an area of the intestine where stasis occurs and allows for an increase in bacterial numbers.

Coliforms (*E. coli*, *Enterobacter* and other Gram-negative organism)

Anaerobes (e.g. *Bacteroides fragilis*) predominate - *Bacteroides fragilis* is found primarily in the lower intestine and not part of the mouth flora. This bug is **resistant to penicillin**.

7. **Large intestine and feces:** Marked bacterial proliferation due to stasis and higher pH (10^{10} - 10^{12} bacteria per gram of wet feces). Bacteria probably make up 1/4 to 1/3 weight of feces.

a. *Species of bacteria in normal feces:* predominantly anaerobes (e.g. *B. fragilis* -resistant to penicillin), aerobes accounting for only 0.1 to 1.0% of culturable flora)

- 1) Bacteroides: **Gram-negative** anaerobic rods, non-spore forming, some species look pleiomorphic, 10^{10} - 10^{11} CFU per gram of wet feces.

The metabolic activity of these bacteria generates ammonia, acid, and gas production in the colon. (Ammonia is formed by splitting urea and from proteins.) *
(Learn this fact.)

Species of *Bacteroides* in fecal materials

i) *Bacteroides fragilis*: most common Bacteroides species in the stool.

It is **resistant to penicillin**, and it grows readily under the **routine anaerobic conditions** used in most laboratories.

- It is a **frequent cause of intraabdominal infections (e.g. trauma or bowel rupture) – infections due to mixed flora – anaerobic infection are foul smelling, gas producing and necrotic in nature**

- **Do not use penicillin** for infections originating "below the diaphragm" - **Clindamycin, cefoxitin and metronidazole** more useful for this purpose.

- Aminoglycoside antibiotics, such as gentamicin, useful in combination for treating "aerobic" Gram-negative rods - Intraabdominal infections frequently with mixed flora.

ii) Other Bacteroides species in the stool may be closely related to *B. fragilis*.

2. *Bifidobacterium*: Non-spore forming Gram positive rods, sensitive to penicillin, Found in 2/3 of fecal samples at 10^9 bacteria per gram. Generally non-pathogenic
3. Lactobacilli are Gram-positive rod. They produce lactic acid and help maintain a low intestinal pH

Yogurt "starter" cultures consist mostly of Lactobacilli, which convert milk sugar (lactose) to lactic acid, which lowers growth of harmful bacteria.
4. Clostridial species including *Clostridium perfringens* are found in 1/3 fecal samples At 10^6 per gram of fecal material.
5. Coliforms (Enteric Gram negative rods) and *Enterococcus (faecalis and faecium)* always present at 10^7 CFU per gram. These are the predominant "aerobic" flora in the gut. These two classes of organisms can be found in urinary tract infections in female, probably due to contamination of the vagina and subsequently the urinary tract with fecal flora.
6. Other anaerobes are present sporadically in relatively low numbers; indeed, more than 400 species have been reported present in normal feces.
 - Redox potential, pH, and slow flow are some important factors which prevail in the large intestine.
 - Gases produced by the anaerobic flora include methane and hydrogen.
7. Some less frequent aerobes are *S. aureus*, *Pseudomonas*, *Proteus*, *Klebsiella* (less frequent).

B. Establishment of the normal intestinal flora

- Fetal intestine is sterile.
- At birth, intestine contains at most a few bacteria, but is colonized within several hours.
- Intestinal flora of breast-fed infants consists largely of *Bifidobacterium*. With weaning, or in bottle-fed infants, the flora resembles that of the adult.

C. Maintenance of intestinal flora: The ability of any pathogen or non-pathogen to proliferate and persist will depend on:

1. Redox potential (low oxygen level) and pH
2. Synergistic or antagonistic effect of competing microbes.
 - a. Aerobes provide reducing conditions for anaerobic growth.
 - b. Some bacteria produce growth factors (vitamin K) utilized by other bacteria.
 - c. Acid production drops pH, specific organic acids which prevent bacterial growth and finally bacteriocins which are antibacterial factors secreted by intestinal flora.
 - d. Administration of antibiotics can be harmful to the local flora, and allow growth of pathogens such as *Salmonella* or *Shigella* or *C. difficile*

D. Metabolic activities of the normal flora and their physiological implications on the host:

1. Bile acid deconjugation: Conjugated bile acids are converted by action of normal large bowel flora to free bile acids. Conjugated bile acids are necessary for normal fat absorption. **Abnormal bacterial overgrowth in the upper small intestine will convert more conjugated bile acid to free bile acid, this may cause fat malabsorption.**
2. A major source of ammonia in the blood is the bacterial degradation of nitrogenous substances (e.g. proteins) in the **colon**. (What do you think happens to the blood ammonia level in Germ-free animals?)

- if liver function is impaired, blood ammonia level can rise. In **hepatic coma**, treatment is aimed at reducing the normal intestinal flora with poorly absorbed broad-spectrum antibiotics, cathartics, and enemas.)
3. Conversion of bile pigments: Intestinal bacteria are responsible for converting bilirubin to urobilin and stercobilin
4. Carbohydrates which are not absorbed by host will be fermented by intestinal flora, with resulting drop in pH. The low pH help maintain the local flora.
5. Predominance of Bacteroidetes in obese individuals and thin individuals have more firmicutes in the intestine.

II. Normal Flora of the skin: 10^3 - 10^4 CFU/cm² of skin

1. *Staphylococcus epidermidis* is the predominant organism. This organism is generally nonpathogenic except in the presence of foreign bodies where catheter-related bacteremia (or other intravascular devices) is common.
2. *S. aureus* found in skin and nares; opportunistic pathogen
3. Diphtheroids – nondiphtheriae Corynebacteria, a collection of Gram+ aerobic or facultative, non-spore forming rods.
4. Anaerobes such as *Propionibacterium* and *Peptococcus* reside in deeper follicles of the dermis. *Propionibacterium acnes* (also called *Corynebacterium parvum*) is a diphtheroid that is implicated in the pathogenesis of acne.

III. Upper Respiratory Tract Flora (similar to oral flora)

1. Nose: *S. aureus*, in 25% of normal healthy individuals
2. Throat: viridan streptococci, *Neisseria* species and *S. epidermidis*.
3. Anaerobes: *Bacteroides*, *Fusobacterium*, *Clostridium* and *Peptostreptococcus* (anaerobic oral streptococcus)

IV. Normal Flora of the genitourinary tract

1. Vaginal flora of adult women: *Lactobacillus* species, responsible for acidic pH of the vagina. Essential to the maintenance of normal vaginal flora since their suppression by antibiotics lead to *Candida albicans* overgrowth. *Lactobacillus* species are not dominant before puberty and after menopause.
2. By virtue of location, the vaginal canal can be colonized with fecal flora. *E. coli* and *Enterobacter* can be found in the introitus – pathogens implicated in urinary tract infection and occasionally sepsis.
3. About 15-20% women of childbearing age carry Group B streptococci in the vagina. This organism is an important source of sepsis for the newborn acquired during passage through the birth canal.

ANAEROBIC INFECTIONS AND NORMAL FLORA

ANAEROBIC INFECTIONS

A. Common Features of Anaerobic Infections:

- 2) **Many abscesses** originate from native flora (gut, female genital tract, aspiration, dental, post-surgical) and anaerobes are a major part of the native bacterial flora.
- 2) **anaerobic infections** are characterized by **foul-smelling** discharge, **gas** in the **necrotic** tissue
- 3) Look for **predisposing conditions**: pulmonary aspiration, bowel surgery, poor dental hygiene, abortion, human bites or animal bites, trauma, malignancy
- 4) Most anaerobic infections occur in deep or necrotic tissues where the oxygen is low and access to endogenous local bacteria is available (e.g. anatomic violation of the gut). Thus, healthy tissues, with a normally high oxidation-reduction potential (due to presence of oxygen) will not support growth of anaerobes.

Reduction of oxidation-reduction potential may occur as result of: a) wound; b) impaired blood supply; c) tissue necrosis

- 5) Almost **all anaerobic infections are mixed** (more than one offending organisms –Table 1), **with one or more anaerobes or microaerophilic organisms (facultative aerobes)**. In contrast, most infections caused by aerobic or facultative aerobic organisms involve a single species.

B. Overview:

Table 1: Areas of the Body Susceptible to Infections With Anaerobic Bacteria
 Predominant organisms in these areas of the body
 (Those with italics are common)

	<i>Anaerobes</i>	<i>Aerobes</i>
<i>CNS infections:</i> <i>brain abscesses</i> <i>subdural or extradural empyema</i> <i>meningitis due to ear infections</i>	<i>oral anaerobes</i>	Staphylococcus aureus Streptococcus
Chronic Otitis Media	<i>oral anaerobes</i>	Pseudomonas aeruginosa Staphylococcus
<i>Dental infections:</i> <i>tooth abscesses or dental surgery</i>	<i>Prevotella,</i> <i>Fusobacterium nucleatum</i> <i>Peptostreptococcus</i> <i>Actinomyces</i>	Staphylococcus Streptococcus
<i>Lung infections:</i> <i>aspiration pneumonia</i> pneumonia (obstruction due to cancer) bronchiectasis lung abscesses or empyema	<i>Prevotella</i> <i>Fusobacterium</i> <i>Peptostreptococcus</i>	Staphylococcus Streptococcus Enterobacteriaceae
<i>Peritonitis:</i> <i>appendicitis</i> <i>intraabdominal abscess (rupture, trauma)</i>	<i>Bacteroides fragilis</i> <i>Fusobacterium</i> <i>Peptostreptococcus</i> <i>Clostridium</i>	E. coli, Proteus Klebsiella Pseudomonas aeruginosa Group D Streptococcus
<i>Liver and subphrenic abscesses</i>	<i>Colonic flora</i>	Staphylococcus Enterobacteriaceae Pseudomonas
<i>OB/GYN infections:</i> <i>postabortion sepsis</i> <i>tubo-ovarian abscesses</i> <i>endometritis</i>	<i>colonic flora</i>	group B Streptococcus E.coli Staphylococcus
<i>perirectal abscesses</i>	<i>colonic flora</i>	Staphylococcus
<i>Skin and muscle infections:</i> <i>gas forming cellulites and gangrene</i>	<i>Clostridium</i>	Staphylococcus Streptococcus, <i>E. coli</i>
<i>human bites</i>	<i>oral anaerobes</i>	Eikenella corrodens

- Most anaerobic infections are caused by patient's **endogenous flora**.
- **Species of anaerobic bacteria involved in an infection can usually be predicted by knowledge of normal anaerobic flora located near the site of infection.**
- **Depending upon whether the infection is located above or below the diaphragm, an important distinction can be made: *Bacteroides fragilis, which is penicillin resistant, are found in infections below the diaphragm.*** (Distinguish those in **upper G.I. tract** from those in the lower GI tract, and the genital area.
- **knowledge of the local flora aids in empirical therapy (anaerobic cultures take ≥ 48 hrs):**
 - Oral infections: penicillin sensitive, Gram-positive cocci, Gram- rods, mixed anaerobic infections
 - Intraabdominal infections: penicillin resistant, Gram-negative rods, mixed anaerobic infections

C. SPECIAL ANAEROBIC METHODOLOGY for culture

1. *General Comments on Methodology for Detecting Anaerobes*

If you suspect anaerobic infections (i.e. foul discharge, gas on X-ray or palpation, necrotic tissues in patients with trauma, surgery, dental, GYN or malignancy)

- collect the specimen correctly for anaerobes ----- **very little exposure to air** - Inject samples directly into anaerobic culture tubes or bottles.
- Gram stain is important (+ gram stain without a positive culture is a clue)
- Morphology on Gram stain (*Fusobacterium*) and colonial morphology (*Prevotella*) may be helpful
- Anticipate mixed infections (aerobes and anaerobes)
- Technical difficulties may allow one to detect only one or two species of anaerobes
- Do not submit **oral or fecal cultures for anaerobes**
- Antibiotic sensitivity assays not as reliable as that of aerobic bacteria

2. Common types of anaerobic media

- a. thioglycollate broth. Thioglycollate is a chemical reducing agent that removes oxygen from liquid media

D. Identification and Characterization of Clinically Important Anaerobic Bacteria

- **Gram stain and morphology** (spores), **production of unusual fatty acids** (analyzed by gas chromatograph), and other metabolic characteristics are useful tools in identification.

E. INFECTIONS DUE TO ANAEROBIC GRAM-NEGATIVE RODS

Bacteroides fragilis (pleiomorphic) - **Most common** anaerobic Gram negative rods

- found in **large numbers** in large intestine (10^{11} CFU/gram of feces)
- can resist bacteriocidal action of bile
- does not require very strict anaerobic conditions for survival in clinical specimens. May tolerate traces of oxygen during growth, but is definitely an anaerobe.
- extraintestinal invasion resulting in septicemia (bacteria found in the blood) almost as common as with *Clostridium perfringens*. These two anaerobes cause about 80% of anaerobic septicemias. Only 10% of bacteremias are caused by anaerobes. (8% of all septicemias are caused by *B. fragilis* and *C. perfringens*)
- Common in intraabdominal (bowel rupture) and female genital infections [e.g. pelvic abscesses following OB/GYN procedures including complicated delivery, induced abortion, infected IUD)].
- *B. fragilis* is the most important anaerobe which is **penicillin resistant** (remember this).
- The capsule is the major virulence factor. Only encapsulated strains produce abscesses in animal models. Infections mixed with *E. coli* are more severe than with *B. fragilis* alone in animal models

Prevotella melaninogenica (anaerobic Gram negative rod) – used to be called *Bacteroides melaninogenica*

- more common in oral infections; this bacterium, along with other anaerobes and occasional spirochetes, is a serious etiologic agent of periodontal disease; occasionally found also in genital infections.
- many strains are sensitive to penicillin; they are also more sensitive to traces of oxygen than *B. fragilis* (more difficult to culture and isolate than *B. fragilis*).
- name derives from **black pigment** (Hemin - a precursor for cytochrome synthesis – required for growth) formed in colonies grown on blood agar plates and in infected tissues.

Fusobacterium Species (Gram negative rod)

- microscopically these bacteria have **tapered ends** (needle shape) and are **very thin** in contrast to the usual shape of gram negative rods.
- penicillin and oxygen sensitive (More sensitive to traces of oxygen than *B. fragilis*).
- important in oral infections, lung abscess and other pleuropulmonary infections as well as abdominal infections (note that the species *F. nucleatum* is oral while *F. necrophorum* is intestinal, causing abdominal infections, liver abscess, peritonitis). You do not need to remember species names of *Fusobacterium*.

NONSPORE FORMING ANAEROBIC GRAM POSITIVE RODS

Note: spore-forming Gram + rods are Clostridial species (covered in separate lectures)

Bifidobacterium: present in large numbers in normal large intestine, especially in breast-fed infants.

- Gram + rods with a tendency to be pleomorphic and branching
- almost never involved in infections (do not learn this species for exam)
- Used in probiotics

Corynebacterium (belongs to the genus *Corynebacterium*) – nondiphtheriae *Corynebacteria* are collectively called diphtheroids)
a normal inhabitant of the skin, seen frequently as contaminants in blood cultures.

- formerly termed "anaerobic diphtheroid" (pleomorphic rod)
- some strains "aerotolerant" (will grow in low concentrations of oxygen)
- not usually involved in infections (very rarely, it causes endocarditis in compromised patients)

Lactobacillus: Gram positive rod (not pleomorphic)

- normal flora of intestine and vagina, where it is present in very large numbers
- a non-pathogen, but frequently isolated from GU and GI tract
- important in maintaining low pH (converts sugar to lactic acid) and preventing growth of pathogens (e.g. *Candida*) in vagina and intestine
- some strains are used for making yogurt, sour milk, sauerkraut

Actinomyces: Gram positive branching rods (a common cause of dental abscess, lung abscess - usually mixed infections – and also abdominal abscess)

- some strains aerotolerant
- found normally in oral pharynx and G.I. tract
- involved in cervico-facial infections (oral or dental infections), lung and abdominal infections (GI tract) with **sinus tract**
- **sulfur granules** are yellow granules seen in lesions that are composed of microcolonies of *Actinomyces* plus cellular debris. **This feature is characteristic of *Actinomyces* infections.** The colony grows in "molar tooth appearance"

Clostridia species – covered in separate lectures

ANAEROBIC GRAM POSITIVE COCCI

Peptostreptococcus (resembles streptococci in morphology, gm+ cocci in chains, but obligate anaerobe – anaerobic Streptococcus)

- These bacteria are part of the normal flora of mouth, urogenital and G.I. tract
- Widely involved in infections (often in association with other anaerobes).
- May be found in pure culture in pleuro-pulmonary infections, brain abscesses, and OB-GYN infections.

Peptococcus (resembles staphylococcus in morphology, Gram+ cocci in clusters, obligate anaerobes)

- Part of the oral, upper respiratory tract and gut flora (large intestine)
- Can be involved in oral, lung and abdominal abscesses

ANAEROBIC GRAM NEGATIVE COCCI

Veillonella

- A commensal, not an important pathogen

F. TREATMENT OF ANAEROBIC INFECTION

1. Penicillin, except for *Bacteroides fragilis* (thus penicillin alone is not used for infections below the diaphragm).
2. Clindamycin
3. Newer cephalosporins (e.g. cefoxitin) are more effective against *Bacteroides fragilis*.
4. Chloramphenicol (static drug)
5. Metronidazole (Flagyl is the trade name)
6. If mixed aerobe-anaerobe infections are suspected, as are most anaerobic infections, aminoglycosides are used in conjunction with an antibiotic with anaerobic coverage (e.g. gent + cefoxitin; or gent + clindamycin).
7. Surgical drainage if abscesses are present (No antibiotics can kill bugs inside an abscess)

Staphylococci

Learning objectives:

To describe the laboratory characteristics of this medically important gram positive cocci such as: shape and grouping of individual cells; ability of colonies to cause complete (beta) or greening (alpha) hemolysis on blood agar; relationship to oxygen (aerobic, anaerobic, facultative); catalase positive; coagulase positive and negative reaction.

To distinguish *S. aureus* from *S. epidermidis* and *S. saprophyticus* microbiologically and clinically.

To distinguish Staphylococci from Streptococci

To describe the epidemiology and pathogenesis of *S. aureus* including invasive syndrome and staphylococcal food poisoning.

To describe the mechanism of action and the pathogenic role of the main staphylococcal toxins, including toxic shock toxin and exfoliatin.

To describe the clinical spectrum of *S. aureus* diseases including toxin mediated diseases and invasive syndromes. List the specific diseases.

To explain antibiotic resistance and specific treatment for staphylococcal diseases.

Reading assignment: Levinson, pp. 39-43, 109-116, 495-496 (superantigens on T-cells); 633-634

General comments:

1. Staphylococcal species (*Staphylococcus aureus* and *Staphylococcus epidermidis*) are the major cause of hospital-related bacteremias in the US.
2. In many cases, intravenous catheters (or other implanted prosthetic devices) are the culprit because these devices are frequently colonized with either *S. aureus* or *S. epidermidis*.
3. Some of these organisms have become multiply resistant to antibiotics. Up to 60% of clinical isolates may be resistant to methicillin. Vancomycin may be the drug of last resort. There are recent reports of vancomycin resistance.
4. Despite the deployment of antibiotics, the morbidity and mortality due to these pathogens remain high.
5. The economic costs of these hospital-related infections are very high.

Bacteriology

- Three main species you have to learn; - All Gram + cocci, with **grape like clusters**
 - i) *Staphylococcus aureus* (**coagulase +** staphylococci)
 - ii) *Staphylococcus epidermidis* (**coagulase -**)
 - iii) *Staphylococcus saprophyticus* (**coagulase -**)
- **Coagulase** is an useful distinguishing feature; coagulase is an enzyme that clots human or rabbit plasma by activating fibrinogen
- there are other coagulase negative staphylococci that are of medical importance (e.g. *S. hemolyticus*, *S. hominis*)
- catalase positive (catalase converts $H_2O_2 \rightarrow O_2$ and H_2O)
This contrasts with Streptococcus which produces no catalase
- facultative anaerobes (can grow both aerobically and anaerobically)

	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. saprophyticus</i>
Diagnostic criteria			
microscopic	G+ clusters	G+ clusters	G+ clusters
Colony	yellow	white	white
catalase	+	+	+
coagulase	+	-	-
mannitol fermentation	+	-	-
DNase	+	-	-
hemolysis	+	-	-
resistance to novobiocin	-	-	+
anaerobic growth	+	+	slowly
Properties of organism			
protein A	+	-	-
teichoic acid	+	+	+
phage receptor	+	-	-

(phage receptor useful for typing purposes in an outbreak of *S. aureus* infections)

Staphylococcus aureus (a toxin producer and an invasive and opportunistic pathogen)

Bacteriology

- Gram + clusters, coagulase +, ferments mannitol produces hemolysins, DNase

Epidemiology

- found on skin, mucous membranes and nares of normal individuals
- colonization of nasal passages up to 30-40%, incidence higher in diabetics and drug addicts
- more likely to colonize hospitalized or compromised patients and those with **foreign bodies** (e.g. dialysis catheters or IV catheters)
- *S. aureus* strains can be traced in epidemic outbreaks by several methods:
 - i) serological
 - ii) patterns of sensitivity to different bacterial phages - phage typing
 - iii) DNA fingerprinting (pulse field electrophoresis)
 - iv) ribotyping (specific sequence of ribosomal RNA)
 - v) comparing the DNA sequence of selected genes including protein A and coagulase genes among different strains

Pathogenesis

(A) Toxin-mediated diseases

- i) contamination (e.g. food or tampon) with toxin production, bacteria not required at the site of disease
 - a) enterotoxins - food borne diarrhea
- ii) bacterial colonization with toxin production in the host
 - colonization via specific receptor-ligand interactions (e.g. fibrinogen and fibrinogen receptors on *Staphylococcus aureus*)
 - followed by elaboration of toxins and TOXIN can reproduce the disease in animal model alone
 - a) toxic shock syndrome toxin - of toxic shock syndrome
 - b) exfoliatins - staphylococcal scalded-skin syndrome

(B) Invasive diseases (**Adhesins, toxins, evasive factor**)

- *S. aureus* synthesizes multiple surface **adhesive molecules** that bind to the host's cellular matrix (including fibrinogen, fibronectin, collagen and platelets and possibly others)
- these matrix proteins are found on damaged "skin" (including catheter and wound sites), disrupted airway epithelium, or endothelial cells within the bloodstream
- upon adhesion to tissues, the bacteria will colonize, evade host response, synthesize toxins and enzymes to lyse host tissues and then spread to other target sites (practically any organs accessible by blood)
- the putative **toxins and enzymes** responsible for host cell lysis
 - i) **alpha toxin** (alpha hemolysin) - lyse host cell membranes
 - ii) leukocidins (lysis of leukocytes)
 - iii) proteases (breakdown of host proteins) – at least three different kinds of proteases
 - iv) **coagulase** (forms clot in human plasma)
 - v) staphylokinase (lysis of blood clot or fibrinolysis)
 - vi) hyaluronidase (dissolve hyaluronic acid, a component of the host's cellular matrix)
 - vii) lipase (dissolve lipids and lipoproteins)
- **blunting or evasion of host immune responses**
 - i) **protein A** - a cell wall protein that binds to host IgG via the Fc receptors, leading to "binding on the wrong end", less complement-mediated killing; protein A is a B-cell superantigen, leading to defective production of specific IgM
 - ii) **enterotoxins** A-E, G, H and I are T-cell superantigens, blunting of specific host T-cell response
 - iii) capsular polysaccharides - anti-phagocytic
 - iv) Eap (Map) – a surface protein that impairs neutrophil recruitment

Consequence

- if these inflammatory and enzymatic actions are contained by host immune response (antibody, neutrophils and macrophages), the infection will remain localized (i.e. controlled infections such as localized abscess and not disseminated)
- if the infection is not contained, invasion into the bloodstream would occur and bloodborne infections will ensue; virtually any human organs can be infected in a disseminated infection (liver, spleen, lung, heart, kidney, skin, bone, meninges and brain)

Clinical syndromes

Toxin-mediated diseases (Toxin alone can reproduce the disease in animals)

- I) Food poisoning (a common cause of diarrheal outbreaks)
 - due to enterotoxins A-E, G, H and I (enterotoxin F is TSST-1)
 - enterotoxin is "**heat-stable**"; resist boiling
 - not all strains produce all types of enterotoxins
 - many enterotoxins are **superantigens** (e.g. enterotoxins A and C), stimulating large amount of IL-1, IL-2 and TNF
 - clinical syndromes includes nausea, vomiting and non-bloody diarrhea (all explained by the T- cell superantigen effect)

- II) Toxic shock syndrome (TSS) – require prior bacterial colonization
- produces TSST-1, commonly associated with tampon use in menstruating women or patients with wound infections; occasional patients with nasal packing
 - a **superantigen**, stimulating IL-1, IL-2 and TNF
 - clinical syndrome characterized by fever, rash, followed by desquamation of palms and soles, some may develop hypotension and shock (~5-12% of patients).
- III) Staphylococcal scalded skin syndrome (SSSS)
- caused by two distinct toxins called exfoliatins A and B (EtaA and EtaB), toxins
 - secreted by specific groups of *S. aureus* (phage group II)
 - *eta* genes are encoded within plasmids
 - superantigen
 - site of exfoliation may not be the site of toxin production
 - usually seen in infants, disease characterized by erythematous skin followed by exfoliation (peeling)
 - histology revealed the superficial layer of the epidermis detaching from the underlying dermis in response to the toxin

Many of the toxin-mediated syndromes are immunological, due to the superantigen effect of these toxins. By the time the clinical syndrome develops, the deployment of antibiotic is often not helpful.

Invasive diseases (characterization by **suppuration and abscess formation** according to anatomic locations)

- superficial infections
 - impetigo (red, vesicular lesions of the skin)
 - folliculitis (hair follicle infection - a special case is hidradenitis suppurativa which is folliculitis of the arm pit)
 - furuncles (skin boils)
 - carbuncles (larger boils)
 - cellulitis (spreading superficial infection)
 - lymphangitis
 - wound infections (postsurgical)
 - **catheter site infections** (this is common in hospitals)
 - paronychia (nail bed infections)
 - postpartum breast infections (mastitis)
-
- deep seated infections
 - pneumonia (common in postoperative or hospitalized patients)
 - endocarditis (drug addict or congenital or prosthetic cardiac valve problems)
 - osteomyelitis (traumatic or hematogenous)
 - arthritis (hematogenous, especially in children)
 - abscess (liver, spleen, kidney, brain)
 - meningitis (hematogenous or traumatic)
 - sepsis (originated from localized lesions such as wound, IV catheter site or IV drug abuse)

Treatment

- *S. aureus* abscesses are surgical diseases - abscesses need to be drained
- semisynthetic penicillin (because many *S. aureus* strains synthesize penicillinase which renders them resistant to regular penicillin but not synthetic penicillin)
 - i) dicloxacillin (orally)
 - ii) oxacillin (intravenously)
- if resistant to synthetic penicillin, vancomycin is the drug of last resort. Many methicillin-resistant strains have already developed intermediate level of vancomycin resistance once they are exposed to vancomycin. **“So don’t prescribe vancomycin indiscriminately”**
- *S. aureus* may develop tolerance (i.e. they are inhibited but not killed - MBC/MIC ratio very high) - consider combinations such as adding rifampin and/or gentamicin

Prevention

- hand washing and careful isolation technique by hospital personnel
- judicious use of antibiotic to minimize the development of resistance
- nasal colonization can be prevented by intranasal mupirocin

Staphylococcus epidermidis

Bacteriology

- **coagulase negative Staphylococci**, aware that there are many species of coagulase negative staphylococci, only remember *S. epidermidis* and *S. saprophyticus*
- **Gram +** cocci white colonies (smaller than *Staphylococcus aureus*)
coagulase negative, catalase positive (distinguish staphylococci from group A Streptococcus)
 - very little hemolysis on blood agar plate
 - does not ferment mannitol,
 - sensitive to novobiocin** (different from *S. saprophyticus*)

Epidemiology

- skin colonizer, normal flora of the skin
- very opportunistic pathogen (especially colonizes foreign bodies)
- adheres to **foreign bodies** (IV catheters, dialysis catheters, pacemakers, prosthetic heart valves, prosthetic joints, synthetic vascular grafts)
- susceptible patients includes neonates, patients with renal failure or immunocompromised individuals

Pathogenesis

- *Staphylococcus epidermidis* can **colonize prosthetic surfaces very efficiently**
- attachment can lead to proliferation and invasion into the bloodstream (but much slower than *S. aureus*)
- the surface carbohydrate of the microorganism may mediate attachment to synthetic surfaces
- form biofilms (surface carbohydrate components may mediate intercellular adherence or clustering of bacteria embedded within a carbohydrate matrix – this structure is called a biofilm)
- the exact mechanism or molecules involved in invasion is poorly understood

Clinical syndromes

- low grade fever, pain and discomfort ascribed to infected prosthetic devices (e.g. prosthetic hip), clinical course may be indolent (less toxic than *Staphylococcus aureus* infection)
- bacteremia may ensue as a result of these infections (e.g. prosthetic heart valves, vascular grafts, pacemakers, intravenous catheters)
- if the patient has no signs of infection and blood culture is positive for *S. epidermidis*, it may be a contaminant (remember *Staphylococcus epidermidis* is a skin commensal; always treat the patient not the culture)
- patients do not look as toxic as *S. aureus* bacteremia

Treatment

- remove the prosthetic devices if feasible (IV catheter, dialysis catheters)
- treatment with vancomycin (resistant to semisynthetic penicillin)
If toxic, consider adding rifampin and/or gentamicin in tough cases

Staphylococcus saprophyticus

Bacteriology

- G+ cocci,
coagulase minus
catalase +
does not ferment mannitol
Resistant to novobiocin (contrasting with *S. epidermidis*)

Epidemiology

- skin commensal
- a common cause of urinary tract infection in young women
(Second only to *E. coli* in this age group)

Clinical syndromes

- urinary tract infection, with polyuria and dysuria

Treatment

- trimethoprim sulfamethoxazol (Bactrim)
- norfloxacin (quinolone)
- drug penetration into the urinary bladder is usually very good

STREPTOCOCCI

Learning objectives:

To explain the various patterns of hemolysis.

To describe the basis of streptococcal classification and to distinguish Streptococci Group A,B,C and D (Enterococcus) and non-typable streptococci such as Viridans Streptococci and to describe the medical significance of each of these groups.

To compare sequelae of *Streptococcus pyogenes* infection.

To explain treatment for streptococcal diseases and describe differences in penicillin sensitivity between *S. pneumoniae* and *S. pyogenes* (Group A) and between Enterococcus (Group D) and *S. pyogenes*

Reading assignment: Levinson, pp. 116-125, 134-136 (anthrax), 143-144 (listeria)

General comments:

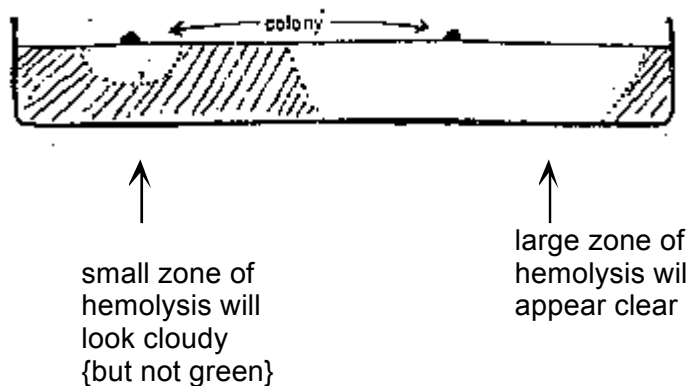
- Gram+ cocci in chains, facultative anaerobes, catalase negative
- Classified by group-specific (Lancefield) carbohydrate antigens (A, B, C, D and nontypable)
- Different patterns of hemolysis help to distinguish Groups A and B (beta-hemolysis) from other groups

STREPTOCOCCUS PYOGENES (Group A)

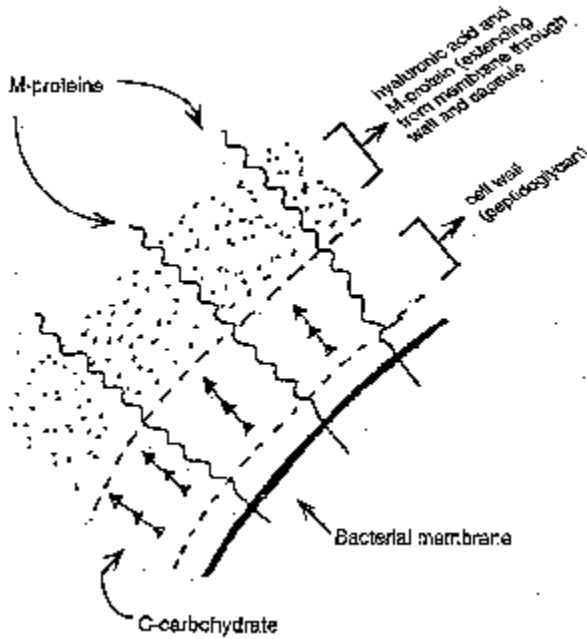
Bacteriology:

- Gram-positive cocci, often found in chains. The streptococci are facultative anaerobes with respect to their metabolism but are aerotolerant (can grow exposed to oxygen in air).
- An important streptococcus classed as group A beta hemolytic Strep. (*Streptococcus pyogenes*).
- Microscopic observation of chains of gram positive cocci.
- Description of zones of hemolysis around colonies grown on a rich medium containing intact red cells:
 - (i) no hemolysis at all [sometimes called gamma hemolysis].
 - (ii) alpha hemolysis: a cloudy green zone (that contains intact red cells with a chemically altered green heme pigment).
 - (iii) beta hemolysis: a clear zone in which all red cells are lysed. Note that a small zone of beta hemolysis may not look completely clear if the zone does not extend through the entire agar layer.

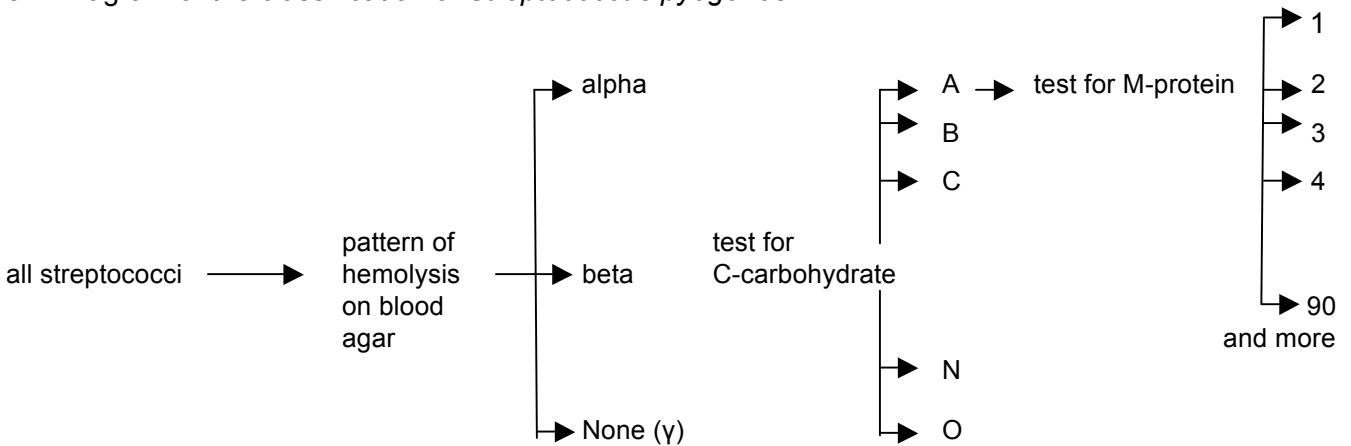
Cross section of a blood agar petri dish with beta hemolysis



- Detection of cell wall carbohydrate antigens (Lancefield classification). Complex carbohydrates extracted from the bacteria are used as antigens in precipitation tests with antisera prepared in rabbits. This test places the beta hemolytic streptococci in groups lettered A through O (exception is Group D which is not beta-hemolytic).
 - The group A streptococci (*Streptococcus pyogenes*) further subdivided by immunological analysis of their M proteins (over 90 types are known) on the surface of the bacteria.
4. Schematic diagram of the *Streptococcus pyogenes* cell wall and hyaluronic acid capsule:



5. Diagram of the classification of *Streptococcus pyogenes*:



Epidemiology:

- found on the skin and in the oropharynx in small numbers
- Respiratory droplets are the usual mode of transmission of streptococcal pharyngitis. A large inoculum (millions of organisms) is required to infect.
- ~5-10% of normal individuals carry group A beta hemolytic streptococci in the pharynx and/or nose. Nasal carriers are more infectious.

Pathogenesis:

1. Invasive diseases including skin infection may involve extracellular proteins (described below) that allow bacterial spread.
 - a. Streptolysins: Hemolytic enzymes that also injure or lyse host cells. They cause the beta hemolysis on blood agar. Two kinds are known:
 - (i) Streptolysin O: oxygen-labile and functions only in a reducing atmosphere, antigenic, reacts with antibody resulting in ASO (Anti Streptolysin O) antibody titers that are important in laboratory diagnosis.
 - (ii) Streptolysin S: poorly antigenic and stable in oxygen.
 - b. Streptokinase: triggers the proteolytic system of the blood that destroys fibrin clots.
 - c. DNAase.
 - d. ~15 other extracellular products.
2. Two factors certainly play a role in all *Streptococcus pyogenes* infections because of their ANTIPHAGOCYTIC activity.
 - a. The hyaluronic acid capsule. Evidence: acapsular bacteria are not pathogenic.
 - b. The M-proteins, which extend from the bacterial membrane through the capsule and into the extracellular medium. Evidence: Strains that make large amounts of M-protein are more resistant to phagocytosis than those that make only a little. Antibody to an M-protein results in immunity to streptococci of that particular M-protein type. The M-protein also plays a major role in adherence to mucosal surfaces.
3. Three factors have been conclusively demonstrated to play roles in specific clinical presentations of *Streptococcus pyogenes* disease.
 - a. SpeA, SpeB, and SpeC are proteases which cause the rash of scarlet fever. Evidence: Antibody against one particular exotoxin prevents scarlet fever caused by that exotoxin (but does not prevent infection by bacterium that produces the toxin).
 - b. SpeA, SpeB, and SpeC cause the streptococcal toxic shock syndrome. Evidence: Antibody against one particular exotoxin will prevent toxic shock in infections with *S. pyogenes* producing that exotoxin.

Clinical syndromes:

Toxin-mediated diseases:

1. Scarlet fever: Some cases of pharyngitis-tonsillitis caused by *Streptococcus pyogenes* are accompanied by the cutaneous rash of scarlet fever which is caused by an exotoxin, the streptococcal pyrogenic exotoxin. The three antigenic types of this exotoxin are designated SpeA, SpeB, and SpeC [The old name for the scarlet fever toxin is erythrogenic toxin]. These

toxins probably cause the rash by acting as superantigens (see item 6, below). This is less common now.

For your interest information: [Immunity to scarlet fever (conferred by antibody to the pyrogenic exotoxins) can be determined by the Dick test: intradermal injection of the toxins. Antibody, if present, prevents a local erythrogenic reaction. Thus a *negative* Dick test (no toxin-caused tissue destruction) means immunity exactly as in the Schick test for immunity to diphtheria. This test is rarely used.]

2. Streptococcal toxic shock syndrome: characterized by severe hypotension (systolic pressure below 90 mm Hg) plus two or more of the following: [impaired renal function; disseminated intravascular coagulation; impaired liver function; respiratory distress; generalized rash; soft tissue necrosis]. The principal cause of these symptoms is streptococcal pyrogenic exotoxins, SpeA, SpeB, and SpeC. These exotoxins act as superantigens that cause massive overproduction of cytokines. Similar in presentation as Staphylococcal Toxic shock syndrome.

Invasive syndromes:

1. Puerperal fever: Infection of the uterus immediately after childbirth. Once common and frequently fatal. Now uncommon in developed countries.
2. Acute pharyngitis and tonsillitis: In its mild form this is a "**strep-throat**." Other symptoms, headache, fever-chills, malaise, elevated white cell count, are common. Accurate diagnosis and adequate treatment (penicillin) are essential to prevent sequela of rheumatic fever (see pages U5 and U6).
3. Impetigo: A minor superficial skin infection, usually in children. The streptococci enter through a pre-existing superficial skin lesion.
4. Erysipelas: a severe cellulitis of the dermis and underlying tissues.
5. Necrotizing fasciitis (or flesh eating bacteria disease): Highly invasive strains of *S. pyogenes* produces rapidly progressing, highly destructive infections that are life-threatening. Surgery may be needed to supplement antibiotic treatment. A poorly defined proteolytic exotoxin may be involved.
6. Other diseases will be considered in SBM: Sinusitis, mastoiditis, otitis media, pneumonia (rare, generally seen only after influenza infection), meningitis.

Important sequelae of *S. pyogenes* infections

(GROUP A BETA HEMOLYTIC STREPTOCOCCUS) INFECTIONS

1. Rheumatic fever: Two to three weeks after recovery from a streptococcal sore throat, some patients may experience heart and skin lesions and joint pains that *do not* result from bacterial growth at these sites. The immunological mechanism is that contain streptococcal M-protein antigens elicit antibodies that cross-react with antigens present in the heart and with antigens in the joints. Not all strains of *S. pyogenes* cause rheumatic fever, and some M-protein types cause more severe rheumatic fever. Rheumatic fever was a major medical problem in the USA 50 years ago. This disease has become rare in the US, still common in developing world. In the absence of good evidence as to which strains are more likely to cause rheumatic fever, treatment of all cases of streptococcal sore throat with penicillin is essential. All of the manifestations of acute rheumatic fever are short term except for the cardiac damage particularly to the mitral and aortic valves. Rheumatic fever patients with prior rheumatic cardiac valvular diseases should receive prophylactic penicillin with invasive procedures.

- Acute glomerulonephritis: One week after infection (more common with skin isolates) with one of a few M-protein types of group A beta hemolytic streptococci (the nephritogenic types), some patients may experience hematuria, edema, and other symptoms of glomerulonephritis. Deposition of immune complexes that contains bacterial antigens is the mechanism.

Comparison of the two major sequelae of <i>Streptococcus pyogenes</i> (Group A beta hemolytic streptococcal) infection		
	<u>glomerulonephritis</u>	<u>rheumatic fever</u>
major signs and symptoms	edema hypertension hematuria	carditis polyarthritis subcutaneous nodules skin lesions [erythema marginatum]
M-protein serotypes	only a limited number	also selected M types, incidence varies markedly from serotype to serotype
site of infection	throat or skin more common with skin strains	only throat
pathogenic mechanism	deposition of antigen-antibody complexes in the kidneys. The antigens are streptococcal	autoimmunity induced by cross-reacting bacterial antigens

Note that viable *Streptococcus pyogenes* bacteria are NOT FOUND in the damaged tissue in rheumatic fever or in glomerulonephritis. Both are immunologically mediated but the mechanisms are different.

Diagnosis

- Elaborate antigenic typing is not routinely done.
- Laboratories look for:
 - Gram positive cocci in chains
 - Beta hemolysis on blood agar plates.
 - Sensitivity to bacitracin, a property that correlates very well with assignment to group A. The other beta-hemolytic streptococci are relatively resistant.
- A physician's-office test uses tiny plastic beads coated with anti-group A streptococcal antibody. When these beads are incubated briefly with a pharyngeal exudate (obtained by a throat swab) the beads agglutinated because they are linked by bacteria. In skilled hands, these tests are reasonably specific but not as sensitive as bacterial culture.
- A titer of 160 or greater of antibody to Streptolysin O (the ASO titer) suggests a recent infection. A four-fold increase in ASO titer is better evidence of infection. Titers of Ab against other extracellular proteins of *Streptococcus pyogenes* are also used.

Treatment

Penicillin resistance has never been a problem with *Streptococcus pyogenes*. ALL *Streptococcus pyogenes* strains ARE SENSITIVE TO PENICILLIN.

GROUP B STREPTOCOCCI

Bacteriology

- Gram+ cocci in chains
- beta hemolysin (but zone of hemolysis less than group A strep)
- bacitracin resistant
- hydrolyze hippurate
- most important species is *Streptococcus agalactiae*

Epidemiology and syndromes

- a. Can be part of the vaginal and colonic flora. It is a major cause (up to 1/3) of neonatal infections. Neonatal group B streptococcal disease includes septicemia, meningitis, pneumonia, and death.
- b. Two epidemiological patterns are seen:
 - (i) Early onset disease (within the first week) is correlated with infection from vaginal bacteria during birth. The incidence of early onset disease has been substantially reduced by a policy of identifying pregnant women who are culture-positive (vaginal and rectal cultures should be done) for group B streptococci late in term. Pregnant women identified by culture or risk factors are treated with intrapartum parenteral penicillin or ampicillin.
 - (ii) Later onset disease is correlated with infant-to-infant spread within the nursery with a less fulminant course and lower mortality.
- c. Neonatal illness is correlated with lack of maternal antibody at the time of delivery. Thus vaginal infections acquired shortly before delivery put the newborn at high risk..
- d. Group B streptococci also cause various infections in children and adults. Currently, the age-specific incidence of infection is highest in the >65 yr. group.

Treatment

Penicillin

ENTEROCOCCI [GROUP D STREPTOCOCCI]

Bacteriology:

- The most important species are *Enterococcus faecalis* and *Enterococcus faecium*
- The pattern of hemolysis differs from strain to strain. Enterococci are common inhabitants of the GI tract.
- Enterococci can grow in high salt (6.5% NaCl) whereas other streptococci cannot
- Enterococci are resistant to many antibiotics. Vancomycin is the antibiotic of last resort. However, Enterococci resistant to vancomycin are an increasing problem, especially with *Enterococcus faecium*

Clinical syndromes:

Enterococci cause urinary tract infections, wound infections and sepsis, especially in elderly patients (to be considered in SBM).

OTHER STREPTOCOCCI

1. Streptococci of Groups C and G (both causing beta-hemolysis, but bacitracin resistant) cause various infections in children and adults. They will not be considered in this course.]

VIRIDANS STREPTOCOCCI (ALPHA-HEMOLYTIC STREPTOCOCCI)

Bacteriology:

- most common species are *Streptococcus mutans* and *Streptococcus sanguis*
- normal inhabitants of the mouth, nose, and pharynx.
- alpha-hemolytic streptococci, a frequent cause of INFECTIVE ENDOCARDITIS. The alpha-hemolytic streptococci of the mouth can produce a transient bacteremia through minor oral trauma, even during chewing tough meat. Alpha-hemolytic streptococci in the blood adhere to pre-existing lesion on the endocardium. usually on a valve. The resulting mass is called a VEGETATION. Vegetations attached to valves can be detected by ultrasound (ECHOCARDIOGRAM). Viridans streptococcal endocarditis generally has a subacute course with intermittent bacteremia. The untreated disease is often fatal.

***STREPTOCOCCUS PNEUMONIAE* (PNEUMOCOCCUS)**

Learning Objectives:

To describe the composition, pathogenic and diagnostic significance of the capsule.

To distinguish *Streptococcus pneumoniae* from *Streptococcus pyogenes*

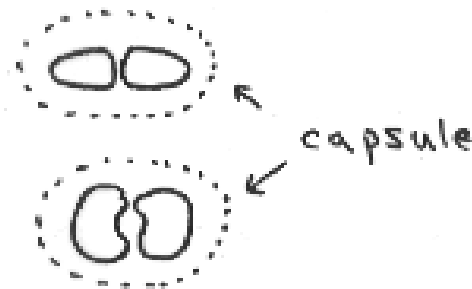
Understand the spectrum of disease caused by *Streptococcus pneumoniae*

To explain the central role of antibody in recovery and in immunity and the role of the vaccine.

Bacteriology

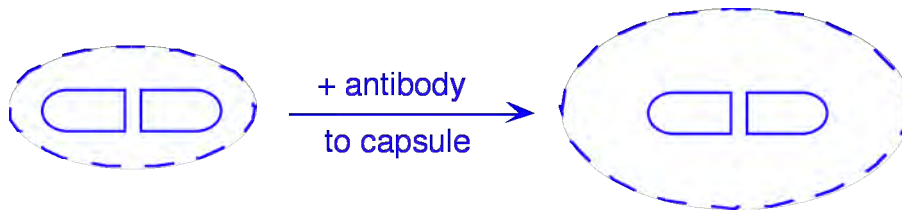
- Gram positive diplococci, lancet-shaped

a. Pneumococci: short dimension apposed:



b. Neisseria: long dimension apposed:

- alpha hemolytic
- optochin sensitive (viridians streptococci are optochin-resistant)
- bile soluble (viridians streptococci are not)
- large polysaccharide capsule (over 90 distinct types), QUELLUNG reaction used to identify the capsule, specific antibody will identify the specific capsular type



Epidemiology

- 10% of adult may be asymptomatic colonizer, incidence higher in children
- no animal reservoir

Pathogenesis

1. These resident pneumococci in oropharynx are the usual source of pulmonary infection. The bacterial population is small and antibody to the capsular polysaccharide is generally not stimulated.
2. Aspiration of mucus is rare; when it happens, the cilia on the respiratory epithelium can push it up to be finally coughed up. Alveolar macrophage can also engulf bacteria. When these capabilities are compromised, pneumonia can develop
3. The pneumococcal capsule hinders phagocytosis and extracellular bacteria can survive. The presence of anticapsular antibody (capsular type-specific antibody) greatly promotes phagocytosis (OPSONIZATION).
4. Pneumococcus produces IgA protease which degrades surface IgA on respiratory epithelium
5. A pneumococcal lytic enzyme called pneumolysin may contribute to the pathogenesis.

Clinical syndromes:

Pneumococcal pneumonia

- (i) The most frequent form of bacterial pneumonia, cough, fever, chills, pleuritic pain, rusty sputum, leukocytosis, 10-20% bacteremic, 5-20% fatality of hospitalized cases even with appropriate antibiotic treatment.
- (ii) One critical factor in natural recovery and in immunity is opsonizing antibody to the type specific polysaccharide capsule. Thus persons with a defect in humoral immunity are at much higher risk. Also at higher risk are splenectomized patients and persons with sickle cell anemia because the spleen plays a major role in combating bacteremia.

Other syndromes include meningitis (children and elderly), otitis media (in children) and sinusitis

Laboratory diagnosis:

- 1) Microscopic examination and culture of sputum sample. Generally the pneumococcal type is not determined
- 2) blood culture
- 3) CSF fluid

Treatment

Multiple antibiotic resistance (including penicillin) is frequent. Sensitivity testing is required. Compare the total absence of penicillin resistance in *Streptococcus pyogenes*.

Prevention:

1. The first pneumococcal vaccine was a mixture of capsular polysaccharide, now reaching 23 serotypes (PneumovaxTM). Pneumovax is a 23-valent polysaccharide vaccine recommended for adults older than 65 years old and for persons 2 years old and at risk for disease (sickle cell, splenectomy, HIV cases, immunocompromised patients). This vaccine is a T-cell-independent antigen that results in poor booster response upon re-immunization.
2. Prevnar 13 is a conjugated vaccine in which the capsular polysaccharides of 13 common pneumococcal types (PrevnarTM) is conjugated to Diphtheria proteins which yields a T-cell-dependent immune response in infants and those with poor immune response. PrevnarTM is part of the recommended pediatric immunization. It prevents pneumococcal meningitis.

LISTERIA MONOCYTOGENES

Learning Objectives:

To describe the epidemiology of infection, why listeriosis acquired from refrigerated foods and the presence of vertical transmission.

To explain the properties of the bacteria that are important for identification and diagnosis.

To explain the mode of spread from cell to cell and how *L. monocytogenes* avoids intracellular and extracellular host defenses.

To explain the diseases caused by *L. monocytogenes* and how listeriosis is prevented and treated.

Bacteriology:

1. a small Gram positive rod distantly related to the diphtheroids, but beta hemolytic, motile (tumbling movement)
2. Grows well at cold temperature (risk of food contamination)

Epidemiology

1. found in soil and water as well as in infected animals and humans.
2. animal → human transmission is usually involved. Dairy products and meat have been implicated in epidemics. Many sporadic cases are food-borne due to cold tolerance.
3. Infection of a pregnant woman can result in bacteremia and a transplacental infection, leading to abortion and stillbirth. Late term infections allow live birth but the newborn may exhibit early onset neonatal septicemia and meningitis. Pregnant women and patients with cell-mediated immunosuppression are susceptible.

Pathogenesis

1. Invade mononuclear phagocytic cells
2. Grow intracellularly
3. Move from host cell to host cells by propelling host actin filament
4. Escape from host phagosome into cytosol by secreting a cytolysin called listeriolysin O

Clinical syndromes

1. Adult infections include meningitis, septicemia and gastroenteritis
2. The fatality rate of hospitalized cases is ~20%.

Prevention

1. High-risk persons (pregnant women and immunosuppressed patients) should avoid raw (unpasteurized) milk and unprocessed soft cheese
2. Drink milk that is pasteurized

For your interest only.

1. Recall that there are only three major obligate intracellular bacteria, *Rickettsia*, *Chlamydia*, and *Mycobacterium leprae*.
2. Among these, *Listeria monocytogenes* is a favorite model for intracellular bacteria in the study of diseases.
3. Concepts to remember include the role of host cell actin in intracellular motility and in the ability to spread directly from cell to cell while evading antibody in the extracellular fluid. These are true of other facultative intracellular bacteria, particularly *Shigella*.

ANTHRAX (*BACILLUS ANTHRACIS*)

Learning Objectives:

To explain the properties of the bacteria that are important for identification and diagnosis.

To explain the three portals of entry for *B. anthracis* in humans and describe the subsequent infections and those at risk for infection.

To describe the roles of spores and toxins in the etiology of anthrax, and the mechanisms of action of the toxins.

To describe how anthrax is prevented and treated.

Bacteriology

1. Aerobic, spore-forming Gram+ rod

Epidemiology

1. A zoonotic pathogen that is commonly transmitted from animal to animal (commonly among rodents), human is the accidental host. Human-to-human transmission is exceedingly rare.

Pathogenesis

1. There are three pathways for human infections, all of which are caused by spores and all of which come from animals.
 - (i) Cutaneous anthrax -
 - (ii) Inhalation anthrax
 - (iii) Intestinal anthrax
2. The pathology is due to two exotoxins and a protein that facilitates cell entry (i) protective antigen – a bacterial protein that facilitates entry of exotoxins into host cells – antibody to this protein protective
 - (i) Edema factor – adenylate cyclase which enters hosts cell and activated by calmodulin
 - (ii) Lethal factor – disrupts signal transduction for cell division

Clinical syndromes

1. CUTANEOUS ANTHRAX results from contamination of existing skin lesions with spores. The lesion progresses to produce a black scab (ESCHAR). If untreated, the cutaneous lesion may lead to proceed to bacteremia. This is the most common and least serious presentation.
2. INHALATION ANTHRAX results from inhalation of spores. An occupation disease in third-world countries with wool or animal hides (wool sorter's disease). The inhaled spores are phagocytosed by pulmonary macrophages where the spores germinate in regional lymph nodes, causing mediastinal lymph node enlargement. Rapidly fatal if left untreated.
3. INTESTINAL ANTHRAX results from ingestion of spores usually in meat. This form of anthrax is rare.

Prevention

A rather crude vaccine consists of *B. anthracis* proteins. It is used for at-risk populations such as veterinarians and troops threatened by biological warfare. It is effective and safe. Efforts at developing a newer vaccine.

Treatment

Ciprofloxacin, tetracycline, or penicillin

INTRODUCTION TO THE GENUS MYCOBACTERIA

Learning Objectives:

- To enumerate the distinguishing characteristics of the mycobacterial genus, including weakly gram positive and acid-fast appearance, aerobic growth, high lipid content in cell wall, fastidious growth, and the tendency to granuloma formation.

I. **Classification** of mycobacteria: Given the awesome public health impact of tuberculosis (TB) and Hansen's Disease (leprosy), the mycobacteria are often categorized as:

1. *M. leprae* (Hansen's Disease, or leprosy)
2. *M. tuberculosis* complex, especially including *M. tuberculosis*, *M. bovis*, *M. bovis*-BCG
3. The nontuberculous mycobacteria (NTM), especially including the important human pathogens, *M. avium* and the so-called rapidly growing mycobacteria (RGM)

II. **Properties** of mycobacteria

1. Weakly gram positive, nonmotile, nonspore-forming, non-encapsulated rods
2. Obligate aerobes (except *M. bovis*)
3. Acid-fastness: mycobacteria stain with the acid-fast (Ziehl-Neelsen or Kinyoun) procedure. A red phenolic dye complexes with the mycolic acids of the mycobacteria and cannot be washed out with acid-alcohol so remain red, whereas all non-acid-fast bacteria lose their red color with the acid-alcohol. In addition to mycobacteria, Nocardia and several parasites may also stain weakly acid fast
4. Mycobacterial cell walls contain mycolic (fatty) acids. Mycolic acids are long chain fatty acids (C78-90) that contribute 40% - 60% of the mycobacterial cell dry weight. Mycolic acids are absent in most other bacteria.
5. Many mycobacteria are very slow growing in culture (but note that *M. leprae* cannot be cultured at all on artificial media). Generation time is 18hrs for TB compared to 1 hour for most common bacterial pathogens.

M. TUBERCULOSIS

Learning Objectives:

- To explain the pathogenesis of *M. tuberculosis* in terms of route of infection, and primary and reactivation disease
- To clearly distinguish between latent and active tuberculosis with regards to presentation, diagnosis and treatment
- To understand principles of the laboratory diagnosis of TB.
- To explain the role and effectiveness of the vaccine *M. bovis*-BCG.



- A. Epidemiology.** Estimated 8 million new active cases with 2 million deaths each year.
- B. Pathophysiology.** Transmission is person to person by inhalation of 1 micron droplet nuclei which are produced by coughing. These droplet nuclei remain suspended in the air for hours and can be inhaled into the terminal respiratory tree. Organisms in the droplet nuclei are ingested by alveolar macrophages, may cause transient bacteremia, and induce granuloma formation in the lung or, less commonly, other parenchymal sites such as brain, liver, kidney. Often, the bacteria remain dormant, but sometimes, the bacteria begin to divide and cause damage. This transition from **latent TB infection** (the asymptomatic stage) to **TB disease** (symptomatic) may occur weeks or even decades following initial inhalation of the droplet nuclei.
- C. Clinical syndromes**
- Ghon lesion, Ghon complex, and Ranke complex: radiographic manifestations of healed primary infection
 - Cavitory tuberculosis: a hole in the lung from TB damage, which may contain a huge number of bacteria, making cavitory TB a more contagious condition than noncavitory TB.
 - Disseminated (miliary) tuberculosis: TB disease occurs throughout the lung and body.
 - Extrapulmonary tuberculosis: miliary, lymphatic (scrofula), skeletal (Pott's disease), genitourinary, central nervous system, or any non-lung site.
- D. Tuberculosis and HIV.** TB is a leading opportunistic infection of HIV-infected patients because the CD4 cellular immunity that usually controls the transition from latent to active TB is compromised in HIV. Extrapulmonary and disseminated forms of TB are more common in HIV positive patients than HIV-negative patients. Whereas patients with LTBI who do not have HIV progress to TB at a lifetime rate of 10%, patients with HIV and LTBI progress to active TB ~10%/year.
- E. Diagnosis of active tuberculosis.**
- AFB (acid fast bacillus) smear. The advantage of AFB smear is it gives same day results, but the disadvantage is limited sensitivity (i.e., patients with TB may be missed by smear alone) and limited specificity (i.e., patients with NTM may be misdiagnosed as having TB).
 - Culture. Culture is more sensitive than smear but takes more time, is more labor intensive and costs more. Culture permits testing sensitivity to antibiotics and performance of DNA fingerprinting for epidemiology. Standard practice has been culture on Lowenstein Jensen egg based medium but growth takes as long as 8 weeks. There are also automated broth culture systems with continuous sampling for CO₂ produced by metabolically active organisms reduces average time to 2-3 weeks.
 - Nucleic acid amplification. Can be used directly on sputum sample on day one to amplify mycobacterial DNA then TB is confirmed by a TB-specific probe

F. Treatment.

Standard treatment

Drug	Mechanism	Dose per day	Side effects
Isoniazid (INH)	-cidal for extracellular organisms	300 mg PO (IM)	Hepatitis, neuropathy
Rifampin	-cidal for intracellular organisms	600 mg PO (IV)	Influenza syndrome, hepatitis, drug interactions
Pyrazinamide	acts on intra-cellular organisms	15-30 mg/kg PO (2 gm)	GI, hepatitis, rash, arthralgias
Ethambutol	static; helps prevent resistance	15-25 mg/kg PO	Optic neuritis, rash

A TB patient receives these 4 "RIPE" drugs for 2 months (what is called the "intensive phase"), then, for INH/rifampin for 4 additional months (the "continuation phase") to complete 6 months total. The international standard is to administer all drugs by directly observed therapy (DOT)

G. Multidrug resistance (MDR). Defined as resistance to at least INH and rifampin. Resistance most commonly occurs in patients who have taken prior treatment. Treatment of MDR is complicated, much more expensive, takes longer (>2 years) and is less effective than treatment of drug sensitive disease. A newer strain called XDR-TB (defined as MDR plus resistance to fluoroquinolone and an injectable drug) is even more difficult to treat. Surgical resection may be necessary.

H. Diagnosis of latent TB infection.

Tuberculin skin testing (TST). Delayed type hypersensitivity to *M. tuberculosis* purified protein derivative (PPD or "tuberculin") occurs 2-10 weeks after initial infection, and is measured by intradermal injection of PPD and measurement of induration (palpable hardness) at 2-3 days. Likelihood of infection depends on magnitude of reaction but also the health status of the patient tested.

TST cutoff

≥5 mm

Health status

HIV infection, any age
Organ transplant or ≥15 mg prednisone/d
TB contact

≥10 mm

Recent immigrant from high TB prevalence area
Worker in high risk congregate setting
Injection drug user

≥15 mm

Persons without risk (should not generally be tested)

Cross reactions with other mycobacteria (such as the TB vaccine strain *M. bovis*-BCG) occur and generally produce smaller reactions.

Interferon gamma release assays (IGRAs), also known as blood assay for *M. tuberculosis* (BAMT). Mononuclear cells from patients with TB infection and normal T cell immunity produce IFN in response to TB specific antigens.

I. Treatment of latent TB infection.

INH (isoniazid) x 9 mos (best regimen, preferred for HIV)

INH x 6 mos

Rifampin for 4 mos

Rifapentine and INH once weekly for 12 weeks

- J. Infection control.** Key element is diagnostic suspicion and prompt patient isolation (negative air pressure, approved respirator for all entering room). Patients should be instructed to cover their mouths when they cough. Health care workers in some settings of high TB risk have annual TST or IGRA, and those with exposure to patient should have additional testing 8-12 weeks after exposure. At most hospitals in the US, pos AFB smears are more likely to grow *M. avium* than *M. tuberculosis*, but initial assumption should always be TB. When working in a developing country where TB is endemic, examine patients in rooms with adequate ventilation, wear a mask if possible (alternative: patient wears a mask).

***Mycobacterium bovis*, BCG Strain**

- A. General:** *M. bovis* is a pathogen closely related to *M. tuberculosis* and causes TB in cows and other ungulates; *M. bovis* in milk can produce gastrointestinal or other forms of "tuberculosis" in humans. An attenuated strain known as *M. bovis*, Bacille Calmette Guerin (BCG) strain is used as a live vaccine against tuberculosis in humans.
- B. Efficacy:** Reduces risk of childhood tuberculosis by ~80 % and all tuberculosis by ~50%. Also prevents infections due to other mycobacteria. Greatest efficacy against tuberculosis against bacteremic complications (meningitis) and childhood forms of progressive tuberculosis.
- C. Use:** administered as intradermal childhood vaccine in most parts of the world where TB is endemic. Also used as a topical treatment of bladder carcinoma.
- D. Effect on PPD skin test:** childhood BCG immunization usually converts PPD skin test to positive for several years, but reactions ≥ 10 mm unlikely from BCG alone. Prior BCG or prior infection with NTM can lead to gradual increase in PPD with repeated testing. IGRAs (BAMTs) for LTBI are not affected by previous BCG vaccination.

MYCOBACTERIUM LEPRAE AND LEPROSY (Hansen's Disease)

Learning Objectives:

- To characterize *M. leprae* as an unusual member of the mycobacterial genus by its historic impact, inability to be cultivated in vitro, and as an obligate intracellular pathogen.

A. Prevalence

1. Falling globally: Widespread in southern hemisphere especially Brazil, Uganda and India
2. United States. 300-400 patients in National Leprosarium, Carville, LA, many due to immigration from endemic settings, but some patients are without travel or known source

B. Pathophysiology. Temperature preference is 27-33 C, hence predilection for cooler areas of the body (skin, nose, mucous membranes of upper respiratory tract). Humans and armadillos (core body temp = 34C) are the only known natural hosts. Can be adapted to growth locally in the foot pad of mice for experimental but not diagnostic studies. Also can be grown in the nine-banded armadillo. *M. leprae* has never been cultivated in artificial media.

1. Transmission – probably respiratory, perhaps sometimes thru breaks in skin
2. Genetic predisposition?
3. Incubation period usually 2-5 yrs (range few months to 30 years)

Feature	Tuberculoid	Lepromatous
Pathology	Well-formed noncaseating granulomas	Lepa cells of macrophages stuffed with <i>M. leprae</i> ; few granulomas
Bacillary count	Paucibacillary	Multibacillary
AFB smear of lesions	Often negative	Positive
Gross	Flat, red or hypopigmented anesthetic lesions	Nodules, diffuse thickening, may ulcerate
Predominant immune reaction	Cellular	Antibody
Lepromin skin test	Positive	Negative
Prognosis	Good	Poor

C. Diagnosis

Based on gross appearance of lesions, histological examination, acid-fast stains of material from nodules, margins of flat lesions or nasal scrapings

D. Chemotherapy. Major improvement in outcome with treatment, typically for 1 year

1. Dapsone
2. Rifampin
3. Clofazimine (only if multibacillary)

Nontuberculous Mycobacteria (NTM)

Learning Objectives:

- To provide an introduction to the epidemiology and disease spectra of nontuberculous mycobacteria, especially *M. avium* and the rapidly growing mycobacteria (RGMs)
 - To understand the nontuberculous mycobacteria in terms of general laboratory characteristics.
1. General: numerous hardy species present in soil, water and various animals. Not transmitted human to human.
 2. Runyon classification still in use: I-IV according to how fast they grow and whether they express color in light or dark.
 3. Clinical significance: lab contaminant (i.e., no clinical significance); colonizing (i.e., no clinical significance); pathogenic in immunosuppressed or normal hosts. ATS guidelines suggest an approach to distinguishing between disease or other states. Often when the AFB smear shows organisms, clinical challenge to distinguish between NTM and TB, which usually depends on laboratory results such as probe or amplification tests.

Mycobacterium avium complex (MAC)

1. General: Most common NTM causing human disease. Widespread distribution in water, soil, and birds. INCIDENCE INCREASING.
2. Clinical syndromes:
 - Cervical adenitis in children (scrofula - also caused by MTBC and bovis)
 - Pulmonary disease in patients with chronic lung disease
 - Pulmonary disease in normal hosts, especially older women.
 - Disseminated infection in advanced AIDS.
3. Treatment multidrug on average for 18 months.

Rapidly Growing Mycobacteria (RGM)

1. Includes *M. chelonae*, *fortuitum* and *abscessus*. Like MAC, widespread distribution in water, soil, and birds. INCIDENCE ALSO INCREASING.
2. Lab features: have to grow on solid media within 7 days.
3. Clinical syndromes:
 - Skin disease, especially in immunocompromised, surgical wound infections, or direct inoculation
 - Prosthetic device infections
 - Pulmonary disease in patients with chronic lung disease (e.g., cystic fibrosis)
 - Disseminated infection in advanced immunocompromise.
4. Treatment multidrug and prolonged, but dependent on species and susceptibility testing results.

Plague, Tularemia, Brucellosis,

Learning Objectives:

To explain the characteristics of microbes that cause plague

To explain epidemiology of plague, the animal reservoirs and arthropod vectors that cause plague and the geographic distribution of plague in the US.

To describe the virulence factors important for pathogenesis and the role of toxins in plague.

Explain how plague is diagnosed.

To recognize the difference between bubonic and pneumonic plague, and explain the clinical syndromes associated with plague and how plague is prevented and treated.

Reading assignment: Levinson, Chapter 20 (plague, tularemia, brucellosis), pp. 643

Yersinia pestis and Plague

Yersinia pestis is the cause of plague, including bubonic and pneumonic plague.

Bacteriology General properties of *Y. pestis*

- Large rod-shaped or coccobacillary gram negative
- Aerobic or facultative aerobic, non-lactose fermenter
- Family Enterobacteriaceae, genus includes 2 other zoonotic bacteria (*Y. pseudotuberculosis*, *Y. enterocolitica*)

Epidemiology

A. *General.* Cycle of transmission involving mammals (usually rodents) and their associated ectoparasites (usually fleas) with incidental involvement of humans

1. Transmission from flea to mammal (bubonic)

- Flea acquires *Y. pestis* after blood meal
- *Y. pestis* multiplies, obstructs foregut
- Obstructed flea attempts to feed, regurgitates 24,000 organisms on bite site
- Organisms enter lymphatics, cause regional adenitis ("bubo") in the mammal

2. Transmission from mammal to mammal (1° pneumonic)

- Bubonic plague leads to 2° pneumonia in index case
- Spread via respiratory droplets to cause 1° pneumonia in a contact

B. *Urban plague* (domestic, murine)

- 3 major human epidemics (A.D. 546, 1346, 1894)
- Epizootics (animal epidemics) among urban black rats and their fleas (*Xenopsylla cheopis*)
- Humans involved as rats die and their fleas seek new hosts
- Initial cases bubonic, then pneumonic

C. *Rural plague*

- Sporadic human cases related to travel or residence in rural areas
- Enzootic and epizootic pattern among wild rodents and their fleas
- Distribution: India, South America, South Africa, southern Russia
- US cases: 10 cases/year in southwestern US
- Mode of acquisition in US
 - a. flea bite
 - b. hand contact with infected mammal
 - c. contact with domestic pets

Pathogenesis

A. Extracellular pathogen

- Anti-phagocytic **capsule** required for virulence (F1 antigen)
- V and W antigens needed for survival within macrophages
- Anti-phagocytic properties present at 37° (mammalian temperature) but not at 28° (flea temperature)

B. Intracellular pathogen

- Persistence within mammalian monocytes

C. Toxins

- Classical LPS endotoxin
- Exotoxin

Clinical features

A. Bubonic plague (flea bite): fever, malaise and painful lymphadenopathy

B. Pneumonic plague (1° and 2°): fever, cough, shortness of breath

C. Septicemic plague (flea bite): no bubo, widespread dissemination via blood

D. Complications:

- Disseminated intravascular coagulation (DIC): skin hemorrhages ("black death")
- Plague meningitis

Immunity

- Antibody develops and is protective
- Inactivated vaccine protects against bubonic plague; used by US troops in Vietnam

Diagnosis

- Bubo aspirate: Usually gram stain and culture positive, confirmed by fluorescent antibody (FA microscopy)
- Blood culture: Usually positive; may have very high numbers of organisms ($>10^6$ and visible on peripheral smear).
- Serology: 4 x rise in antibody (passive hemagglutination) to F1 capsule is diagnostic

Immunity

- Antibody develops and is protective
- Inactivated vaccine protects against bubonic plague; used by US troops in Vietnam

Treatment/prognosis

- 10 days of tetracycline or streptomycin or chloramphenicol
- Mortality 60-90% untreated, 5% with early antibiotics
- US mortality: 15% overall
- Adverse prognostic features: axillary adenopathy, 2° plague pneumonia, positive blood cultures, organisms on peripheral smear

Prevention

- Flea control programs in enzootic areas frequented by humans
- Avoid ill rodents
- Inactivated vaccine

***Franciscella tularensis* and Tularemia**

Learning Objectives:

To explain epidemiology of tularemia, the animal reservoirs, arthropod vectors and individuals at risk.

To explain pathogenesis related to intracellular infection of monocytes (diffuse RE infection).

To recognize that the clinical presentation is similar to plague (less severe) and explain how tularemia is diagnosed, prevented and treated.

Bacteriology

- Small unencapsulated pleomorphic Gram negative
- Fastidious aerobic slow grower, requires cysteine, glucose on blood agar for growth (or thioglycollate)
- Cold tolerant (survives in water for up to 90 days)
- Infectious dose: 5-10 organisms
- Category A priority agent (NIH)

Epidemiology Zoonotic disease transmitted from infected animals or arthropods to human; widespread distribution in northern hemisphere in 100 wild mammals, 9 domestic animals, birds, insects and water

- Routes of human infection:
 1. rabbit: hand contact or ingestion partly cooked meat; winter disease in eastern US
 2. arthropod-borne: ticks (*Dermacentor variabilis*), deer flies (*Chrysops discalis*) and others: summer disease in western US
 3. Other: handling infected tissues (muskrat trappers in Vermont), animal bite (cat), laboratory aerosol (dangerous to handle in a regular lab), shaking dog aerosol (infected wet dog aerosolized Tularemia by shaking itself dry inside a cottage in Martha's Vineyard in 1978)

Pathogenesis

- Tick bite: organisms injected directly while feeding or bite wound contaminated by feces
- Organisms cause skin lesion, enter lymphatics, produce local lymphadenopathy then bacteremia with granuloma formation in reticuloendothelial system (spleen, liver)
- Intracellular survival in monocytes
- Endotoxin plays role in initial systemic symptoms

Clinical Features

- Abrupt onset fever, chills, malaise
- Specific syndromes
 1. ulceroglandular: most common, skin ulcer and painful adenopathy (inguinal, axillary)
 2. others: typhoidal (bacteremia), pneumonia

- Complications: pneumonia in 10-15%
- Mortality < 1% with treatment

Diagnosis

- Difficult and dangerous to culture; laboratory accidents
- Fluorescent antibody staining of node biopsy
- Serologic: [4x titer rose or single titer \geq 1:160 in 50-70% at 2 weeks (but cross-reacts with brucella)]

Treatment

- Streptomycin bactericidal, Rx for 7-10 days (alternative: tetracycline 14 days)
- Relapses occur from intracellular persistence

VII. Prevention

- Ticks: check tick frequently in endemic areas, remove by mouth parts
- Rabbits, muskrats: protective gloves when dressing animals
- Vaccine (live attenuated): for lab workers, trappers at risk

Brucella species and Brucellosis

Learning Objectives:

To explain the importance of unpasteurized milk products and animal vectors in transmission of Brucella and the mode of transmission in abattoirs.

To explain that the clinical manifestations of a diffuse RE system disease can be nonspecific, characteristics of the microbe and how infections are diagnosed.

To describe the basis for control of Brucella in the United States.

Bacteriology

- Pleomorphic fastidious gram negative
- Grows slowly, requires 10% CO₂ for optimal growth

Epidemiology Zoonotic disease common in developing countries due to transmission from unpasteurized cheese and milk. Uncommon in US (200 cases/year), most cases from Midwest (Illinois, Iowa)

- *B. abortus* (cattle): contaminated milk or direct tissue contact (abattoirs)
- *B. suis* (swine): tissue contact, airborne, abattoir workers (slaughterhouse) at high risk
- *B. melitensis* (goats, sheep): unpasteurized milk, goat cheese
- [*B. canis* (dogs): urine from kenneled dogs]

Pathogenesis

- Causes infectious abortion in cows, sheep, pigs and goats (localization in placenta)
- Disease of reticuloendothelial (RE) system in humans
- Organisms ingested by PMN, multiply in monocytes, then form granulomas in liver, kidney, spleen and bone marrow

Clinical Features [also called Malta fever, undulant fever]

- Systemic and non-focal, including FUO (fever of undetermined origin – diagnosis difficult unless you think of it)
- Fever, chills, myalgias (muscle pain), headache, arthralgias (joint pain)
- Intracellular persistence causes prolonged initial symptoms and high risk of relapse
- Complications: osteomyelitis (vertebral) and endocarditis (culture negative)]

Diagnosis

- Occupational history
- Blood culture: 21 day incubation, prolonged bacteremia common
- Serologic: 4x titer increase [cross reactions from tularemia, typhoid, cholera vaccine, brucella skin test]
- Bone marrow biopsy in difficult FUO cases

Treatment

- Doxycycline plus rifampin for 6 weeks.
- 25% relapse, 1-2% mortality

Prevention

- Pasteurization of milk, cheese
- Control of animal reservoir
 1. vaccination of young female calves with *B. abortus* vaccine
 2. herd testing: antibody test on pooled milk samples from herd, individual animal testing and destruction of positive animals
- Occupational protection: mesh gloves, eye protection

SPIROCHETES

Learning Objectives:

- To compare and contrast the diseases caused by the 3 medically important spirochetes
- To compare and contrast the reservoirs and modes of transmission of these spirochetes
- To describe the geographic distribution of the illnesses associated with spirochetes
- To describe the treatment procedures for disease caused by these spirochetes
- To be aware that there are no vaccines for any of them
- To explain how size and shape of *Treponema pallidum* affects the capacity to see these bacteria by light microscopy
- To describe the different stages of syphilis and Lyme disease and how they are diagnosed
- To describe the treatments for each spirochete infection covered in this session
- To explain the life cycle of deer ticks and its relationship to the spread of Lyme disease
- To discuss the Tuskegee experiment as an unethical example of neglecting ethics related to medical human experimentation

Reading assignment: Levinson, Chapter 24; pp. 645-646

DESCRIPTION:

Organisms are elongated, flexible, and motile; twisted spirally on their long axis (helicoid). They possess a flagellar structure (axial filament, or internal flagellum) spirally wound around the cell and anchored through hook-like bases at the two poles of the cells.

Spirochetes multiply by binary fission; have a gram-negative type of structure.

There are three major groups of spirochetes that cause human disease:

1. Genus *Treponema* (causes syphilis, yaws, and pinta; also non-pathogenic organisms in this genus are found in oral cavity, intestine, and genitalia of both humans and other mammals).
2. Genus *Borrelia* (cause of relapsing fever and Lyme Disease) named after the French microbiologist, Borrel.
3. Genus *Leptospira* (cause of leptospirosis).

I. *Treponema pallidum*:

- A. General Characteristics: Agent of human syphilis. Although 5-15 microns (μm) long, width is only 0.2 μm , which is less than the resolving power of the light microscope and hence cannot be seen by gram staining. Can be made visible, however, by "darkfield" microscopy; by immunofluorescence; by deposition of silver salts on bacterial surface; or by electron microscopy. The structure is like that of a gram-negative bacteria, but it is too thin for a Gram stain to be useful.

Live organisms cannot be grown in culture, but can be kept motile for several days in very rich media (albumin, serum), under anaerobic conditions. Will survive in whole blood for only about 24 hours, or in tissue specimens for several days (hence if blood is stored for at least several days, as it is in a blood bank, the organisms do not survive).

Easily killed by heat, drying, or soap and water, but live bacteria can be stored at -80°C for many years, and then revived. Can be perpetuated in the laboratory through injection into the testicular tissue of rabbits (but rabbits do *not* carry this disease naturally).

B. Mode of transmission:

1. Usually passed by direct contact of genitalia or mucous membranes; Generally not transmissible during late stages of disease (after approximately 4 years post-infection).
2. Also transmitted from mother to fetus--causing "congenital syphilis"--with serious pathological consequences to the child; still birth, abortion, or general signs at birth generally similar to secondary syphilis. Preventable by treatment of mother during early months of pregnancy;
3. May be transmitted--by transfusion--to a recipient of *freshly* obtained blood (transfusion syphilis) or, very rarely, to nurses and physicians, through cuts.

C. Diagnosis:

1. Pathogenesis:

- a. Incubation: long incubation period of 2-6 weeks. Clinical evidence of disease is absent, but bacterial replication at the site of entry as well as secondary sites is very active.
- b. Primary lesion (*chancre*): usually appears 1-4 weeks after infection, typically on genitalia, accompanied by focal lymphadenopathy. Chancre heals spontaneously in 1-5 weeks after appearance.
- c. Secondary lesions: organism has disseminated. A generalized skin rash or mucosal lesions erupt as early as 2 weeks or as late as 20 weeks after the primary lesion (*chancre*) first appeared. This phase (secondary syphilis) may result in arthritis, renal dysfunction, or other abnormalities; hence the term applied to syphilis: Mucosal lesions are highly infectious.
- d. Tertiary lesions occur late in disease--many years after initial infection, and may occur in the central nervous system, aortic valves of the heart, and other locations (the lesion has a rubbery consistency and is called a "gumma"). Organisms generally not seen, much damage due to hypersensitivity.

2. History of exposure. An important aid in diagnosis.

3. Demonstration of organisms in lesions. Involves darkfield examination of exudate from open genital lesion, or from "secondary lesions." (Note: *T. pallidum* is indistinguishable from saprophytic *T. microdentium*, a human mouth dweller.)

4. Serological tests (positive in late primary)

a. Early tests: Wassermann, *et al.*:

"Wassermann antibody" reacts with a specific lipid: diphosphatidylglycerol ("cardiolipin", originally extracted from beef heart) to which is added lecithin and cholesterol. Complement fixed in reaction, and degree of fixation measured.

Origin of antigen that infected individual actually makes antibodies against is unknown. May be in *T. pallidum* or released from host tissue.

b. Modern tests:

1. Nonspecific tests: These are very sensitive and generally done first

a) Screening tests widely used: VDRL (Venereal Disease Research Laboratory) test; a flocculation test (clumping on a slide) with cardiolipin.

2. Specific tests: These are generally used to confirm results of the nonspecific tests

FTA-ABS (fluorescent treponemal antibody - absorption) test: patient's serum is first *absorbed* with non-pathogenic treponemes to remove non-specific antibodies. This serum is then added to a slide with T-pallidum (obtained commercially), washed, and made visible by the addition of a second (fluorescent) antibody.

Micro-hemagglutination test: involving red cells coated with *T. pallidum* antigen, and serum from patient.

ELISA: utilizes several *T. pallidum* antigens to coat the microtiter plate. The major test performed at DHMC.

TPI (*Trepomena pallidum* immobilization) test: antibody from patient reacts directly in presence of complement with living *T. pallidum*, causing organism to lose motility. A difficult test, but important if a false positive is suspected. Test carried out only in special laboratories.

c. False positives and negatives:

The test for syphilis may read *positive* in the presence of infectious mononucleosis, malaria, and even in the absence of any disease; such reactions are called *false positives*. *False negatives* may occur in a small number of patients who *do* have syphilis. The incidence of false positives and negatives is higher in the VDRL test than in FTA-ABS.

d. Despite what seems to be a somewhat cumbersome set of diagnostic procedures, PCR is NOT used for diagnosis. Specific primers have not been tested to the point of diagnostic use. In addition, the serologic tests may be more sensitive depending on the stage of infection.

D. Epidemiology and Patient Populations of Special Interest

In the US there is a wide geographic distribution with some concentration on the coasts and the southeast and southwest regions. The highest incidence is among sexually active individuals, especially in males who have sex with males.

E. Antibiotic treatment:

T. pallidum is very sensitive to *penicillin*. Resistant organisms not yet found! Duration of therapy depends on stage of disease; therapy must be adequate to completely eliminate infection. Alternative antibiotics: tetracycline, erythromycin.

F. Vaccine:

No effective vaccines have been developed. This is an example where genomics might provide a huge influence. Since *T. pallidum* cannot be cultured in vitro, it has been difficult to study. It is likely that the genomic sequence will provide clues about surface molecules that can be expressed by recombinant DNA methods and used in vaccine formulations.

II. OTHER TREPONEMAL DISEASES

A. Yaws

A tropical disease caused by *T. pertenue*. Organism closely resembles *T. pallidum*, and has closely related antigens. Non-venereal transmission, through open sores of skin. Often occurs in children. Patients show a positive syphilis test. No transplacental transmission. Primary lesion ("mother yaw") resembles a raspberry; yaws is an African word for raspberry.

B. Bejel

A disease common among children in Syria. Similar to yaws. Non-venereal transmission. Organism is called *T. pallidum*, subspecies *endemicum*. Treated with penicillin.

C. Pinta

A disease of Central and South America, caused by *T. carateum*. Also called carate, mal de los pintos, azul. Rarely serious; gives rise to flat, non-ulcerating skin lesions of hands, feet, and scalp, that heal spontaneously, leaving depigmented areas. This organism is also sensitive to penicillin.

III. Genus *Borrelia*

A. *Relapsing fever* is caused by any of a dozen different species of *Borrelia* (most notably *B. recurrentis*, *B. hermsii*). Stained organisms *visible by ordinary light microscopy*. They have not been grown on artificial media, but grow reasonably well in the chick embryo. *B. hermsii* is endemic in the western U.S. The disease is transmitted by ticks to rodents, and then to humans. The natural reservoir is wild rodents. *B. recurrentis* is epidemic, not found in U.S. and involves transmission from human to human, via body louse.

1 Course of disease: Fever of 4-5 days; patient becomes afebrile for 7-10 days. Then fever recurs and disappears again. 3-10 relapses before complete recovery. Relapse appears to result from antigenic variation of surface proteins. The variable membrane protein is encoded on a *linear plasmid*.

2 Diagnosis: based on clinical symptoms plus observation of *Borrelia* organisms in stained blood smears (Wright's stain) or dark field microscopy. Blood can be injected into a mouse and the mouse blood examined for *Borrelia*. Tetracycline derivatives are the drugs of choice. Penicillin or erythromycin for young children or pregnant women.

3 Epidemiology and Patient Populations of Special Interest: Tick-borne relapsing fever (TBRF) is linked to sleeping in rustic, rodent-infested cabins in mountainous areas in the western U.S. Louse-borne relapsing fever (LBRF) is currently associated with regions affected by war and refugee camps in eastern Africa.

B. Lyme disease: caused by *Borrelia burgdorferi*. Now the most common tick-borne disease in the U.S. (8,000 cases/yr). Endemic on east coast, northern midwest, and Pacific northwest. The white-footed mouse is the major reservoir. The organism is transmitted from the mouse to humans via the deer tick. Virulent *B. burgdorferi* carries 7 linear and 2 circular plasmids.

1. Pathogenesis - 3 stages

a. First: papule with expanding erythema 3-14 days after bite--fever, headache, stiff neck, malaise.

b. Second: various neurologic and cardiac involvements.

c. Third: migrating episodes of arthritis. These occur weeks to months after the tick bite and may last several years.

d. Diagnosis: based on these symptoms and expanding bright red rash. It is difficult to isolate the organism. A diagnostic ELISA test to detect circulating antibodies against *B. burgdorferi* is available.

e. Late manifestations: without treatment

chronic arthritis: 3-5 years after first symptoms. Also in those with HLA-DR4 that don't respond well to tetracycline treatment.

2. Epidemiology and Patient Populations of Special Interest – Regional distribution in the northeast and northern Midwest. Although *B. burgdorferi* is spread to humans by the deer tick, the source of infection for the tick is the white-footed mouse. The disease is associated with smaller patches of forest (less than 5 acres) rather than large contiguous regions of forest, where predators of the mouse are prevalent. Individuals who frequent forests fields are at greater risk than the general population.
3. Treatment – Tetracycline derivatives

Ampicillin is a safe alternative for young children.
4. Vaccine - A vaccine was developed for people with a high risk of being exposed to Lyme Disease, such as forest workers. The vaccine is no longer available. The vaccine contained outer surface protein A (OspA).
5. Unique iron Non-requirement - Both biochemical and now genomic analyses suggest that *B. burgdorferi* does not have a requirement for iron. There is no evidence of iron uptake systems and no iron requiring enzymes. Instead, the organism seems to have a higher than average number of manganese requiring metalloenzymes. This is unique among pathogenic organisms where the paradigm is to rely on iron for biological processes as well as a signaling mechanism with respect to virulence gene regulation.

IV. Genus *Leptospira*

Leptospirosis is an uncommon human disease transmitted by rats, dogs, and other animals, usually from infected animal urine. Transmitted through skin or upper alimentary mucosa, *not arthropod-borne*. Manifestations of leptospirosis include nephritis, jaundice, meningitis. Multiple serotypes and animal reservoirs of *Leptospira* can cause disease.

1. Pathogenesis – Enters blood and invades various tissues and organs; particularly kidney, liver, meninges, conjunctiva.
2. Symptoms – Muscular pain, headache, photophobia, fever, chills. Generally lasting for a few weeks.

Weil's Disease – infectious jaundice caused by one serovar. Leads to renal failure, hepatic injury with a fatality rate as high as 25%.

3. Epidemiology and Patient Populations of Special Interest - Generally an occupational hazard of sewage workers, slaughter-house workers, or people in rat infested areas.
4. Diagnosis – Can be grown and identified by serological methods.
5. Treatment – penicillin, erythromycin, tetracyclines.
6. Vaccine – no vaccine for humans.

THE MYCOSES: INTRODUCTION & CUTANEOUS/MUCOSAL INFECTIONS

LEARNING OBJECTIVES:

I. General Concepts of Fungal Biology

- To describe the terminology used to characterize fungi.
- To explain the relatedness of fungi to animals, and bacteria on a phylogenetic tree based on 16S and 18S ribosomal RNA sequences.
- To explain the major differences in cell structure between fungi, bacteria and mammalian cells.
- To name the Phyla that includes fungal pathogens.
- To describe the two basic morphologies of fungal cell structure and growth and describe their respective advantages.
- To describe fungal structures including spore, conidia, arthrospore, yeast, germ tube, hypha and mold.
- To define the term "dimorphism".
- To recognize that each pathogenic fungus is classified as a mold (filamentous), a yeast or is dimorphic.

II. Important molecules

- To name a cholesterol-like molecule located in the fungal cell membrane.
- To explain the biochemical structure and basis of the rigidity of fungal cell walls.
- To describe the names, usages, and mechanisms of action of the major antifungal drugs.

III. Fungal Pathogens and Medicine

- Compare sites of cutaneous, mucosal and deep mycoses.
- To describe portals of entry into mammalian hosts and to define the terms "endogenous" and "exogenous" in reference to fungi.

IV. Cutaneous Mycoses

- To define the terms dermatophyte, Tinea, endothrix and ectothrix.
- To list the three genera of fungi termed dermatophytes and describe their morphologies, sources, modes of spread and role in causing outbreaks.
- To define the terms geophilic, zoophilic or anthrophilic and their utility in controlling outbreaks of superficial fungal disease.
- To describe the features of Tinea Versicolor and Tinea Nigra and the organisms that cause these diseases.
- To explain how cutaneous mycoses are diagnosed, treated and prevented.

V. Subcutaneous Mycoses

- To name and describe fungi that cause subcutaneous infections and describe the route of transmission.
- To describe how subcutaneous fungal infections are diagnosed and treated.

VI. *Candida* sp.

- To describe the morphological forms exhibited by *Candida albicans*, explain their role in virulence and contrast *C. albicans* growth forms with those of other pathogenic fungi.
- To describe individuals at risk for candidiasis and important mechanisms of immunity.
- To describe how *C. albicans* gains entry into the blood stream.
- To describe a rapid test for presumptive identification of *Candida albicans*
- To describe the role of *Candida glabrata* and other *Candida* sp. in fungal disease.

READING ASSIGNMENTS

Reading and assignments: See Assignments in the Reading and Practice Question Assignments for BSMD located at the beginning of the syllabus. These include Levinson, Chapter 47, 48,10 (see sections on antifungal drugs) and Chapter 50 pages 400 to 402.

General Concepts

Fungi are very important components of the ecosystem. Their capacity to break down complex polymers into smaller molecules that can be absorbed facilitates recycling of organic matter. Fungi are intimately associated with plants both beneficially through mycorrhizal associations and detrimentally as pathogens. Fungi are ubiquitous in the environment via their abundant airborne spores, as well as growth in the soil and on plant surfaces. In nature, fungi are primarily pathogens of plants, whereas fungal infections of animals are less common. Yet, when fungal diseases occur, they can be associated with extinction of their host species. Recent examples of fungal outbreaks include White Nose Syndrome in bats, Chytridiomycoses in frogs, and even here in specifically in New England an outbreak of fungal disease in a rare threatened population of rattlesnakes.

Fortunately, mammals are highly resistant to fungi. One reason for this resistance is that the core body temperature of 37°C prevents growth of many fungi, which are adapted to environmental temperatures between 25 and 35°C. Most environmental fungi cannot grow in human hosts. Secondly, mammalian hosts have evolved innate and adaptive immune mechanisms that prevent the germination and growth of fungal spores.

Nonetheless, some fungi have co-evolved with mammalian hosts living either commensally or pathogenically on mucosal surfaces. Other fungi have emerged as pathogens in the context of advances in medicine that prolong life while reducing host immune defenses. Clinicians must have knowledge of fungi to incorporate fungal pathogens into their differential diagnoses when managing patients that present with unknown infectious etiologies. Therefore, fungi are an especially critical part of any differential diagnosis in patients with known immune suppression.

Fungus (pl. fungi):

A eukaryotic, unicellular (yeast) to filamentous (multicellular), achlorophyllous organism having an absorptive nutrition. Reproduction can be sexual, asexual or both.

Fungi are in the Eukarya domain having a true nucleus bounded by a nuclear membrane.

Terminology

MYKOS: FUNGUS

MYCOLOGY: STUDY OF FUNGI

MYCOSIS: DISEASE CAUSED BY FUNGI

MYCOTOXICOSIS: DISEASE CAUSED BY A FUNGAL TOXIN

The spectrum of antifungal drugs is limited. Since fungi are eukaryotic, therapeutic agents that will selectively inhibit growth of fungi without harming mammalian hosts are of limited availability. Discovery of new antifungal drugs is an important goal of medical research.

Taxonomy - Fungi have their own Kingdom consisting of several Phyla historically based on the characteristics of structures harboring sexual spores.

Ascomycota - the largest and most diverse group of fungi. Their sexual reproductive structures are characterized by asci, which are sacs containing ascospores. They include medical pathogens such as *Aspergillus* and *Candida*, and other famous, intensely studied, fungi that are used as models of mammalian cells such as *Saccharomyces*, and *Neurospora*. Many advances in modern medicine have come from the study of these model eukaryotic organisms.

Basidiomycota - a large and diverse group named for the basidium where sexual spores are produced. Includes the fungal pathogen *Cryptococcus neoformans*. The common mushroom found in/on many food items, *Agaricus bisporus*, is in this phylum.

Glomeromycota – (formerly named Zygomycota) named for the zygosporangium where sexual spores develop. Includes the medical pathogens *Mucor* sp, *Rhizopus* sp. and others.

Motility - The medically important fungi are non-motile

Cytoplasmic membrane - The main distinguishing feature of fungal cell membranes is that **ergosterol** is used instead of cholesterol in most fungi (except *Pneumocystis*) (note –it is not necessary to memorize the structures)

General properties of the cell wall

- The cell wall constitutes a large proportion of the cell mass.
- All medically important fungi have a cell wall.
- The fungal cell wall is biochemically unrelated to bacterial cell walls.
- The fungal cell wall contains important antigens used in diagnostic tests.
- The fungal cell wall is the target of the Echinocandin class of antifungal drugs.

Cell wall composition and function

Inner layers:

- β (1,3) and β (1,6) glucan and chitin comprise 60-70% of the cell wall mass
- β (1,3) and β (1,6) glucans provide osmotic stability, strength and rigidity.
- β (1,3) glucan is a target of innate antifungal immunity. Many pathogens mask this polymer for protection from host defense mechanisms.
- Glucan polymers allow the wall to expand and contract and prevent lysis.

Outer layers: heavily modified with polysaccharides such as polymannose (70% or more of the glycoprotein mass is carbohydrate)

Glycoprotein function:

- Protect and mask inner beta 1,3 glucan from glucanases that would interfere with function of glucan.
- Adhere to host, other microbes, catheter material, etc.
- Elicit antibody production.
- Maturation is typical of proteins secreted through the classical secretory pathway.
- Both N- and O- carbohydrate linkages are used.
- Outer glycoproteins are attached to the glucan layer through a glycosylphosphatidyl inositol anchor remnant.

ANTIFUNGAL DRUGS

Learn the contents of Table 47-3 and the accompanying text in Levinson. Know the major drugs and their targets. The three most commonly used classes are listed below:

1. Polyenes – target ergosterol directly (i.e. amphotericin B, natamycin A)
2. Azoles – target the fungal P450 Cytochrome Erg11A involved in Ergosterol Biosynthesis (i.e. fluconazole, voriconazole)
3. Echinocandins – target fungal beta glucan biosynthesis (i.e. Caspofungin, Micafungin)

I. DRUGS THAT AFFECT MEMBRANE PERMEABILITY

A. Polyene compounds (amphotericin B and Nystatin). These are very important antifungals.

1. Polyenes bind to sterols in cell membranes – damaging the membranes

2. Selective toxicity: binding is much better to ergosterol, the principal sterol in fungal (and plant) membranes, than to cholesterol, the principal sterol in animal cell membranes.
3. These agents are poorly absorbed from the GI tract; amphotericin B is used systemically but is toxic; Nystatin is too toxic for systemic use.
4. Nystatin is used topically and for oral fungal infections using the "swish and swallow" technique. This is possible because nystatin is only toxic to humans if it were to gain systemic access. Since it is not absorbed from the GI tract this method does not lead to toxicity.
5. As you might expect, amphotericin B is fungicidal as it destroys membrane integrity. *However* nystatin is fungistatic, except at high doses that are not physiological achievable.

II. INHIBITORS OF CELL MEMBRANE SYNTHESIS

- A. Azoles – Systemic antifungal agents useful for the treatment of oral candidiasis (thrush) and other systemic mycoses; important in patients (e.g. AIDS) who may require chronic suppression of recurrent fungal infections.
 1. Fluconazole (trade name Diflucan), itraconazole, ketoconazole, miconazole (topical), voriconazole (trade name Vfend), posaconazole
 2. Can be given orally, but hepatotoxicity occurs in approx. 0.01% of patients. Visual disturbances are common with voriconazole treatment.
 3. Major activity is the inhibition of ergosterol synthesis, principally by inhibition of cytochrome P450 14a-demethylase—membrane and cell properties are disturbed; because hyphae synthesis is inhibited the fungi are more easily phagocytosed by PMNs and macrophages. The effect is fungistatic.

III. INHIBITORS OF CELL WALL SYNTHESIS

Echinocandins – The newest class of antifungal drugs. They are inhibitors of glucan synthesis. They function by inhibiting the activity of 1,3-b-D-glucan synthase.

1. Caspofungin - The first of these new drugs to be approved. It goes by the trade name Cancidas
2. Anidulafungin - This drug was just approved by the FDA in February of 2006. It goes by the trade name Eraxis.
3. Micafungin

B. Echinocandins are either fungicidal or fungistatic depending on the fungus.

IV. ANTIMETABOLITES

- A. Flucytosine - Flucytosine is the only available antimetabolite drug having antifungal activity. It inhibits fungal protein synthesis by replacing uracil with 5-fluorouracil in fungal RNA. Flucytosine also inhibits thymidylate synthetase via 5-fluorodeoxy-uridine monophosphate and thus interferes with fungal DNA synthesis.

- B. Interestingly this drug can be either fungicidal or static, depending on the fungal isolate. The basis for this is not known.

V. MECHANISMS OF RESISTANCE TO ANTIFUNGAL COMPOUNDS

- A. While mechanisms are becoming more defined, generally less is known about these resistance mechanisms than for antibiotic resistance.
- B. MAJOR THEME of resistance mechanisms as they become better understood is that so far, the resistance is conferred by changes within the fungal genome itself. In other words NO acquired resistance mechanisms that inactivate the drug have thus far been discovered. Normal cellular efflux pumps that have increased levels of expression represent a common mechanism, as does alteration in the targets of the drugs.
- C. The mutation rates to resistance are quite high for some of the agents noted above. As a result some of them are often used in combination.
- D. The CDC recently noted that drug resistance in *Candida* species is at a critical level.

Physiology and growth

Fungi are **absorptive heterotrophs** and require that carbon be supplied in organic form. Fungi grow in their food, secreting digestive enzymes that break down complex molecules into simpler ones that they can absorb.

Fungi are acid tolerant. **Sabouraud's agar**, which has an acidic pH, is often used to enrich for fungi in cultures of patient specimens.

Secondary metabolites.

Secondary metabolites are synthesized by non-ribosomal peptide synthases (nRPSs) and polyketide synthases (PKS) and include several medically useful compounds such as the cholesterol-lowering drug lovastatin, produced by *Aspergillus terreus*, and the anti-viral drug equisetin, produced by *Fusarium heterosporum*. Penicillin, one of the most revolutionary discoveries in modern medicine, is produced by fungi in the genus *Penicillium* (some are human pathogens!).

Other secondary metabolites of fungi are toxic such as the aflatoxins. These compounds produced by *Aspergillus* and *Fusarium* are toxic at very low concentrations and are potent carcinogens. They are of primary concern in the grain industry. Grain may become contaminated by fungi, leaving behind toxins that are not destroyed during processing into flour. Peanut crops that are left to dry in the sun are of particular concern as they are easily contaminated with fungi. Diseases caused by toxic metabolites of fungi are termed **mycotoxicoses**.

Fungal Structures

Fungi grow in two basic forms termed **yeast** and **hyphae** that are described below. The term **mycelium** refers to a mass of hyphae and is a characteristic of the commonly used term "mold". Molds are fungi that grow almost exclusively as filamentous hyphal organisms. Molds are multicellular organisms. The principles described previously in the notes for bacterial growth, measurement of cell number, the growth curve, stages of growth are also applicable to unicellular yeast cells. Hyphae are more difficult to enumerate.

Size of fungi

Fungi are larger than bacteria. Yeast forms generally range between 2-6 microns, slightly smaller than red blood cells. Hyphae are very long. The size and morphology of fungi often makes them easy to visualize and identify microscopically. The size of the infectious propagule of human pathogenic molds, the conidia (spore in jargon), can be as small as 1 μm in size allowing deep penetration into the lower respiratory tract.

Yeast - oval cell that reproduces by budding or fission. Growth is polarized at the tip when a new bud emerges from a mother yeast cell. Growth becomes isotropic, with even expansion around the spherical cell during yeast maturation.

Yeast cells form smooth pasty colonies.

Hypha (*pl.* hyphae) - thread-like filament that may branch and may contain septa that divide the cytoplasm into segments, each segment having a nucleus. Alternatively, hyphae may not have septa. A new hypha emerging from a yeast or conidia is termed a **germ tube**. Hyphal growth occurs by apical extension from the hyphal tip and is exclusively polarized.

The term for the lack of hyphae is **aseptate or coenocytic**. A multinucleate mass of protoplasm results from repeated nuclear division unaccompanied by cell fission.

Hyphae exhibit apical growth **exclusively** from the tip.

Spitzenkorper - a collection of vesicles near the tip that is specific to hyphal growth.

Hyphae may be **aerial** or **vegetative**, which are submerged within growth media. Aerial hyphae produce spores that are easily air born facilitating spread.

Spore - A reproductive propagule that forms sexually. However, this term is often used interchangeably (albeit incorrectly so) for any fungal propagule such as conidia.

Conidia - asexual spores classified as microconidia (small, usually easily airborne and are often the infectious form of fungal pathogens) or macroconidia (large, and are useful for identification).

Arthrospore – a spore resulting from fragmentation of a hyphal filament.

Advantages of hyphal growth

Apical growth enables fungus to extend into fresh zones of substrate.

Tip extension can be rapid (40 micrometers/sec).

Hyphal tips have penetrating power resulting from turgor pressure.

Polymer degrading enzymes that are secreted by hyphae allow fungi to breakdown complex organic molecules.

Hyphae produce spores of variable shapes. A network of numerous hyphae and spores is described as a **mycelium**.

Intermediate growth form

Yeasts (primarily candida species) can become elongated under certain conditions. Elongated yeasts are termed **pseudohyphae**.

Dimorphism – growth in two different forms. Thermal dimorphism means that the two different forms occur at different temperatures.

Some fungi grow in hyphal (mold) form in the soil at environmental temperature but as yeast forms in animal tissue at physiological temperatures. This property is termed “classical thermal dimorphism”.

Cutaneous Mycoses

General Concepts

Historically, mycoses have been classified according to the depth of invasion by fungi.

Superficial infections of the skin and nails are the most common fungal diseases in humans and affect ~25% (or ~1.7 billion) of the general population worldwide. The dermatophytes are the primary cause of these infections, which are termed dermatophytoses. Three fungal genera cause the dermatophytoses; *Epidermophyton*, *Trichophyton*, and *Microsporum*.

I. General Properties

The dermatophytes are filamentous fungi with septate hyphae that invade superficial keratinized structures (skin, hair and nails). Hyphae produce microconidia and macroconidia in laboratory cultures.

II and III. Pathogenesis and Immunity

The consequences of these diseases are primarily cosmetic and mild to moderate irritation and little is known about their pathogenesis and immunity. Fragments of infected, desquamated human or animal skin, fallen hairs or other superficial tissue, containing spores or hyphal fragments come into contact with uninfected hosts. Fungal elements may also spread through fomites associated with combs, hairbrushes, hats and other materials that come into contact with infected hosts. Hyphae invade and spread through uninfected tissue. One of the most important factors influencing the presence of dermatophytes on the skin is moisture. A warm, moist, humid environment predisposes to dermatophyte infections.

IV. Clinical Features

The diseases are named tinea followed by the Latin term of the affected body part. The clinical manifestations are different for different parts of the body. (see Levinson Image Gallery).

<http://www.langetextbooks.com/0071774343/gallery.php?id=t>

Infections of the skin may be red and inflamed. Crumbling of nails and skin around nails may occur accompanied by itching. In some cases vesicles, indicative of an allergic hypersensitivity reaction may form. Hair may break and fall off.

V. Epidemiology and Patient Populations of Special Interest.

The natural habits of the dermatophytes are primarily the surfaces of humans (anthropophilic species) or animals (zoophilic species). One species inhabits soil (geophilic). The dermatophytes are distributed worldwide, however some species are restricted to certain geographic locations. Population groups affected may include children in daycare centers or groups sharing locker room spaces. Infections may quickly spread through families upon infection of one family member. Host factors including male gender, increased age, nail trauma and poor peripheral circulation due to vascular disease or diabetes may be risk factors for infection. Other risk factors include the socioeconomic status and personal hygiene of the patient.

Prevention of the spread of infections depends upon identification of the source, diagnosis of the infecting pathogen and frequent disinfection of shared items in the home and of public floors where persons are likely to go barefoot. Persons and pets living in the same household of an infected person should all be treated.

VI. Diagnosis and Treatment.

Direct Examination

KOH mounts of clinical specimens, collected with instruments such as a sterile blade or brush, are performed to clarify the tissue allowing detection of fungal elements upon microscopic examination. Fungi may be seen infecting hair from the outside "ectothrix" or inside "endothrix". A Wood's ultraviolet

light is useful for detecting ectothrix infections because *Microsporum fluoresces* when exposed to UV light.

Culture

Clinical samples should be plated onto selective and non-selective media. This allows for inhibition of saprophytic non-pathogenic fungi and for recovery of pathogens that may be inhibited by selective media. Positive cultures will show growth after 1-2 weeks, however cultures should be held for up to 4 weeks before discarding because the false negative rate is high. Species can be identified by colonial appearance, by microscopic examinations by Scotch tape preparations and biochemical tests.

Treatment

The choice of treatment should be geared to the specific tinea involved. Some agents may be effectively cured by the use of several topical agents. Oral treatments may include griseofulvin or triazoles. Despite the fact that the dermatophytes are susceptible to antifungal drugs, long courses of antifungal drugs may be required to elicit cure especially for nail infections, but may also cause hepatotoxicity.

Other cutaneous fungal pathogens.

See Levinson for information about fungal pathogens that cause Tinea Versicolor and Tinea nigra.

Subcutaneous mycoses

Subcutaneous mycoses

General concepts

In contrast to the superficial mycoses, the subcutaneous mycoses involve the tissue beyond the skin. These mycoses are usually the result of inoculation of fungi that grow in soil or on vegetation directly into the deeper layers of the skin and subcutaneous tissues by puncture or abrasion.

Sporotrichosis

I. General Properties

Sporothrix shenkii displays classical dimorphism, growing as a budding yeast at 37 °C in host tissue and hyphae at 25 °C in the environment. Yeasts in tissues are cigar shaped.

II Pathogenesis

Organisms are generally introduced traumatically by puncture. After inoculation into the skin, *S. shenkii* grows locally and can be limited to the site of the inoculation or may cause a chronic, slow invasion of skin and underlying lymph nodes and subcutaneous tissue.

III Immunity (see end of section on subcutaneous infections)

IV. Clinical Features

Lesions initiate as surface plaques with irregular borders or *S. shenkii* may gain entry to, and spread along, the proximal lymphatics. After an incubation period of about three weeks, nodular lesions emanate from site of inoculation. Dissemination may occur in immunocompromised hosts.

V. Epidemiology and Patient Populations of Special Interest

Sporotrichosis occurs globally with the majority of cases being reported in the Americas, Australia, Asia or Africa. *S. shenkii* grows in the mold form on vegetable matter in humid climates. Agricultural workers, farmers gold miners and florists are at increased risk due to occupational exposure. Males are more commonly affected than females.

VI. Diagnosis and Treatment

The diagnosis is made by clinical suspicion and the isolation of *S. shenkii* in cultures of infected tissue. The yeast form may be visible histological sections of infected tissue. Treatment involves long course of antifungal therapy with azoles and other antifungal drugs.

Chromomycosis

I. General properties

Several soil fungi termed dematiaceous fungi (fungi with brown to black melanin) cause chromomycosis.

II. Pathogenesis

Slow growing wart-like lesions containing brown fungal cells are seen following abrasion with plant material containing fungi. The local host reaction involves a hyperplastic inflammatory response that contributes to lesion formation.

III Immunity (see end of section on subcutaneous infections)

IV. Clinical Features

The initial lesion is a single nodule with scaling at the site of inoculation. After months or years, new nodules and verrucous plaques appear. Over time, lesions become large and cauliflower-like that bleed easily.

V. Epidemiology and Patient Populations of Special Interest.

Chromomycosis is most common in tropical and subtropical America ranging from Mexico to Brazil and in Africa, affecting workers in rural areas. Males are more commonly affected than females.

VI. Diagnosis and Treatment

Diagnosis is performed by biopsy and histological analysis of infected tissue. The characteristic feature are dark-walled fungal cells. Cultures may be useful for identifying the specific pathogen, but the yield is poor. Surgical removal of infected tissues is the best choice for management of the disease however long periods of antifungal drug treatment may be partially effective.

Mycetoma

General Concepts

Mycetoma or eumycetoma is a chronic infection of the skin and subcutaneous tissue that is caused by fungi. A similar condition termed actinomycetoma is caused by several species of bacteria. Actinomycetomas grow faster and are effectively treated with antibacterial drugs.

II. and IV. Pathogenesis and Clinical Features

The organisms are implanted into host tissue after a penetrating skin injury caused by trauma. The organisms may persist in the tissue for many years without causing clinical signs of disease. Eventually a mass of inflammatory cells accumulates. Inside the mass of inflammatory cells, fungal grains up to 5 mm in diameter form.

V. Epidemiology and Patient Populations of Special Interest.

Adults are usually affected and are exposed during outdoor activities. Fungi such as *Madurella grisea* are associated with plants and are broadly distributed with concentrations in Africa and India.

VI. Diagnosis and Treatment

The grains found in tissue exhibit distinctive shape and color specific to the pathogen involve. Microscopic examination of the grains may reveal the presence of hyphae or other microbial elements.

Cultures should be performed to isolate and identify the etiologic microbe. Fungi may respond to azoles however surgery may be required in cases that are refractory to medical treatment.

Immunity to fungi that cause subcutaneous mycoses.

Cell mediated immune responses are important for inhibiting spread. Patients with defects in the cellular immune responses are predisposed to disseminated and life-threatening cases in which the skin is the portal of entry.

Candida albicans and candidiasis

General Concepts

Many fungal pathogens are free-living in nature and are termed “**exogenous**” in that their ecological niche is outside of the host.

In contrast, “**endogenous**” fungi are associated with human or animal hosts rather than being free in nature. The most important fungal pathogen in this group is *Candida albicans*, which is part of the normal flora of the gastrointestinal tract of warm blooded animals. Other *Candida* spp. are also associated with the gastrointestinal tract. For these organisms, when isolated from cultures of clinical specimens, it can be difficult to ascertain whether *Candida* sp. are responsible for the disease etiology or are simply present as normal flora.

Because of its association with human hosts and its capacity for tissue invasion in the presence of numerous risk factors, *C. albicans* causes a wide spectrum of disease that range in anatomic location from orogastric mucosal infections to deep seated internal infections and hematogenous infections.

I. General Properties

Candida species are the most common etiological agent of life-threatening invasive fungal infections in patients who (i) are severely immunocompromised, (ii) have endured invasive clinical procedures, or (iii) have experienced major trauma, and treatment requires extended stays in intensive care units. *Candida* species are the fourth most common cause of nosocomial (hospital-acquired) bloodstream infections. Advanced medical procedures—such as the use of catheters, neonatal intensive care, major gut surgery, or liver transplantation—are predisposing factors to disseminated *Candida* infections¹.

Although more than a dozen *Candida* species can cause disease, in almost all patient groups *Candida albicans* dominates in terms of incidence and disease manifestations.

Candida albicans is a budding yeast at room temperature. At 37°C under specific culture conditions it produces germ tubes, which extend into hyphae. The hyphae are vegetative, forming within culture media or beneath the surface of the agar. Aerial hyphae are not formed. Intermediate forms termed pseudohyphae appear as short or highly elongated yeasts. In contrast, *C. albicans* undergoes a special kind of dimorphism, growing in yeast form in vitro at environmental temperatures and hyphal form at physiological temperatures. An important difference from classical dimorphism is that both growth forms as well as pseudohyphal forms of *C. albicans* are found in infected tissue. Polymorphic is another term used to describe multiple growth forms. All of these forms may be found in diseased host tissue. (See Figure 50-1 in Levinson)

C. albicans is a common endogenous inhabitant of the mucous membranes and digestive tracts of humans and is not found free-living in nature. Normally, *C. albicans* resides in the host without disease. *C. albicans* may be cultured from the oral cavity and vagina of normal hosts.

C. dubliniensis is closely related to *C. albicans* and also forms germ tubes although not as readily as *C. albicans*.

Candida glabrata is the second most prevalent yeast pathogen in humans after *Candida albicans*. *C. glabrata* is monomorphic, growing in the yeast form only. Germ tubes and hyphae are not produced. Both species are normally commensals, and can be isolated from the mucosa of healthy asymptomatic individuals. In the environment, both species are found exclusively in association with mammals. Both species cause a similar constellation of mucosal and blood stream infections, with *C. glabrata* accounting for approximately 15% of infections worldwide. *C. glabrata* is more resistant to azole antifungal drugs than *C. albicans*.

C. tropicalis, *C. parapsilosis* and others may also cause infections similar to *C. albicans*.

II. Pathogenesis

The source of infections is usually the patient's own endogenous organism although person to person spread has been documented in intensive care units. In the presence of antibacterial therapy or immunosuppression causing a deficiency in cell mediated immunity, an increase in the number of organisms above that found in commensalism occurs. Attachment of *C. albicans* hyphae to surface epithelial cells follows. Invasion of the keratinocyte layers lining the mucosal surfaces occurs leading to the formation of white punctate lesions, which spread horizontally over the mucosal surfaces leading to thrush or pseudomembranous candidiasis. *C. albicans* has numerous adhesins that are important for attachment to mature keratinocytes. The hyphal form is more adherent and invasive than yeast forms and hyphal forms are found in deeper keratinocyte layers than yeast. Overall, invasion is limited to the upper most layers of the epithelium unless immunosuppression is very severe. Yeast forms are thought to promote escape from biofilms, facilitating spread to new locations in the host. Yeast forms also are more resistant to stresses. The ability of *C. albicans* to interconvert between growth morphologies in the host is considered to be a virulence property of the organism.

Entry of *C. albicans* into the bloodstream and potential invasion of internal organs may occur following heavy colonization of skin and mucosal surfaces in neonates or in individuals treated with antibiotics, or through prolonged hospital stays that increase the chance of colonization from health care workers. Damage to the integrity of the gut mucosa through surgery following total parenteral nutrition or therapy induced mucositis can also increase the risk of hematogenous candidiasis. Formation of biofilms on catheter material may provide a protected site that seeds the bloodstream with organisms that can spread to various regions of the body. *C. albicans* may proliferate in any body site.

III. Immunity

Both innate and adaptive immunity are important for protection against candidiasis. Innate immune defenses consist of neutrophils, macrophages and monocytes as well as antimicrobial factors in mucosal secretions and epithelial cells. Because of exposure to *C. albicans* at birth, adaptive immunity develops during childhood and healthy immunocompetent adults are protected by adaptive immunity to *C. albicans*. Protective adaptive immunity consists of cell mediated immune responses, which involve cooperation between T cells that are activated by fungal antigens and phagocytic cells. The activated T cells recruit and activate phagocytic cells. Recently, IL-17, a cytokine produced by Th17 cells has been found to be critically important for protection. IL-17 recruits neutrophils to sites of infection and increases the expression of antimicrobial peptides by epithelial cells. Macrophages, dendritic cells, epithelial cells and stromal cells produce cytokines that are responsible for maturation and recruitment of IL-17 producing cells.

DTH skin testing using candidal antigens is uniformly positive in immunocompetent adults. The test is used as an indicator of the presence of cell mediated immunity when assessing the host immune status.

IV. Clinical Features

Cutaneous diseases include diaper rash and other moist areas of the epidermis.

Clinical features of candidiasis in the oral cavity depend on the immune status of the host. *C. albicans* may cause erythema of oral surfaces even in normal hosts. Thrush may occur in infants prior to the development of mature cell mediated immunity.

Oral candidiasis is one of the most common opportunistic infections in the presence of HIV and occurs very early after infection with HIV.

Oral thrush also occurs in patients receiving chemotherapy. It causes pain, inability to eat and increased risk of additional infections.

Esophageal candidiasis is a severe form of candidiasis that occurs in hosts with leukemia and lymphoma or other forms of immunosuppression.

Vaginitis commonly occurs in immunocompetent hosts.

Individuals with genetic deficiencies in IL-17 mediated T-cell immunity suffer from chronic mucocutaneous candidiasis (CMC). CMC is characterized by extensive invasion of skin and mucosal surfaces over prolonged periods of time.

For disseminated candidiasis, the symptoms may include fever and sepsis and cutaneous lesions.

V. Epidemiology and Population Groups of Special Interest

- Most infections occur in the economically developed regions of the world. It is estimated *C. albicans* causes over 400,000 life-threatening infections/yr worldwide².
- Newborns and premature infants acquire *C. albicans* from the maternal gastrointestinal flora and are susceptible to cutaneous and mucosal infections resulting from immature cell mediated immune responses.
- Several primary immunodeficiency disorders conferring susceptibility to chronic mucocutaneous candidiasis (CMC) are known. Deficiencies in Th17 mediated immunity resulting from auto-antibodies to IL-17 or mutations in signal transduction pathways upstream of IL-17 expression are responsible for CMC. The immunodeficiencies include Autosomal-dominant hyper-IgE syndrome (AD-HIES), Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED) and others.
- Individuals with HIV are at high risk of infection as are those that are iatrogenically immunosuppressed for treatment of malignancies or organ transplantation.
- Candidiasis is the most common form of fungal infection affecting the transplant recipient.
- Those with central venous catheters may experience *C. albicans* biofilm formation on the catheter and seeding of the bloodstream.
- Numerous underlying diseases including diabetes can increase susceptibility to candidiasis.
- Those treated with antibacterial drugs exhibit increased susceptibility to fungal infections.
- Women in the third trimester of pregnancy are especially susceptible to vaginal candidiasis.

VI. Diagnosis and treatment

Direct Examination

The KOH mount may be useful for detecting fungal growth on mucosal surfaces in vaginitis or oral infections. *C. albicans* may be detected with the Gram stain as it retains the crystal violet iodine complex.

Histologic stains such as the PAS stain or the GMS stain will allow detection of *Candida* species in tissue sections.

Culture

Specimens from mucosal and skin infections caused by *Candida* species are easily cultured on blood agar or on Sabouraud's agar to deter bacterial flora and enrich for fungi. Smooth white colonies form after two days. CHROMagar is useful for differentiating species of *Candida*.

C. albicans is differentiated from other yeasts by the rapid germ tube test in which germ tubes form within an hour of inoculating serum at 37°C. Chlamydospores form on specialized media, but this is not usually performed for laboratory diagnosis.

Definitive identification of *C. albicans* and other yeasts is based on patterns of fermentation and assimilation in various carbon sources.

Non-culture methods

Diagnosis of deep seated infections may be difficult due to the lack of available tests that can be performed on serum. Blood cultures are insensitive and may be negative in the presence of bloodstream infection. Molecular methods are increasing the sensitivity of detection of *C. albicans* in blood cultures. A method for rapid identification of *C. albicans* termed PNA FISH, which identifies *C. albicans* within 2-3 hours in blood cultures, consists of a fluorescent probe that detects *C. albicans* 26S rRNA. Applying this method results in identification 24-48 hours earlier than with conventional methods.

Treatment

Various azoles and echinocandins are useful for mucosal infections, which may also be treated with oral troches. Amphotericin B and fluconazole and other azoles are used for disseminated infections. Treatment regimens are complex and are tailored to the specific clinical settings according to the degree of immunosuppression and other factors. Decreasing risk factors is also important in preventing candidiasis.

Sources for notes.

Enaissie, EJ, McGinnis, MR and Pfaller, MA, Clinical Mycology, Second Edition. Churchill, Livingstone, Elsevier. 2009. Chapters 8, 16 and 24.

Vinh, DC. 2011. Insights into human antifungal immunity from primary immunodeficiencies. The Lancet vol. 11. 780-792

SYSTEMIC MYCOSES (Endemic Dimorphic Mycoses) Primary Fungal Pathogens

Learning Objectives

To explain the common features of *Coccidioides immitis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Paracoccidioides brasiliensis* and the diseases they cause.

To compare and contrast the morphologies, ecological niches, diseases and routes of transmission of *C. immitis*, *H. capsulatum*, *B. dermatitidis*, and *P. brasiliensis*.

To explain how systemic mycoses are diagnosed and provide an example of how fungal infections can be misdiagnosed.

To define an endemic mycosis.

To recognize that global travel is a risk factor for these endemic mycoses.

To recognize that the incidence of *C. immitis* infections is on the rise in the SouthWest USA.

To recognize that laboratory culture of these organisms requires great care and any suspected cases should be dealt with under BSL3 laboratory conditions.

READING ASSIGNMENTS

Levinson Chapter 49.

General Concepts

Primary versus opportunistic fungal pathogens

The organisms that cause systemic infections are responsible for the increasing numbers of fungal infections. Organisms that cause systemic infections, or infections that affect the entire body, have traditionally been divided into two categories termed **primary** fungal pathogens and **opportunistic** fungal pathogens. Primary fungal pathogens are those that are capable of causing infections in hosts that are not immunocompromised, or otherwise do not have host defense defects. Opportunistic fungal pathogens on the other hand, cause diseases that are manifested almost exclusively in patients debilitated by some other underlying non-fungal cause and whose normal defense mechanisms are impaired. Both the primary pathogens, as well as opportunists, cause more frequent and severe infections in compromised hosts.

Classic Thermal Dimorphism

The primary fungal pathogens grow in nature as soil saprophytes, usually in filamentous growth forms that produce conidial spores, and usually in defined geographic areas. When their conidia are inhaled, the organisms adapt to and grow in an environment that is unnatural from their perspective, and major changes in cell shape and metabolism occur. Growth in the host is as “yeasts” rather than hyphal filaments. Infection for the fungus is a “blind alley” in that spores are not produced in infected hosts. Person to person transmission does not occur for this type of pathogen. Thermal dimorphism is demonstrated in the laboratory by growth as yeasts (or spherules in the case of *Coccidioides* sp) at 37°C and growth in hyphal form with characteristic conidia at 25°C.

Epidemiology

These organisms occur in many places throughout the world, however they are **endemic** in specific regions of the US. Official data on disease incidence is unavailable. Estimates for endemic regions range from 3,000 to 24,000 cases per year. Mortality rates range from <1 to 75% depending on immune status of the host and therapy. A travel history for all patients exhibiting illness is now a necessity in our global interactive world and fungal infections play an important role in differential diagnosis.

Common features of pathogens, host response and disease

- Disease results from inhalation of spores.
- Geographic distribution is specific to the fungus.
- Fungi exhibit “Classic” Thermal Dimorphism;
Environmental form (mold):
Tissue form (yeast or other specialized form)
- The host immune response that is protective consists of cell mediated immunity. Tissue macrophages become activated and fuse to “wall off” fungi from surrounding host tissue by forming a granuloma. This prevents the fungus from causing disease. However, if the host becomes immunocompromised, the granuloma may dissociate, releasing live fungi into the tissues.
- May become latent (like TB) and form granulomas as stated above.
- Diseases are much more severe in the presence of HIV infection.
- Exposure is determined by skin test (DTH).
- Fungi are rarely transmitted from person to person.
- Laboratory cultures of the mold forms are hazardous.

***Coccidioides* species and coccidiomycosis (Valley Fever)**

I. General Properties

The disease is caused by two highly related species, ***Coccidioides immitis* and *C. posadasii*** The environmental mycelial phase of *Coccidioides* species contains barrel- shaped arthroconidia (2 - 6 micron), which are easily fragmented and airborne. Spherules consisting of large (12 - 100 micron) thick-walled structures containing numerous endospores, are formed in the host.

Coccidioidomycosis is endemic in certain areas of North, Central and South America. Central Texas is situated at the Eastern edge of the endemic area in the US. 100,000 people are infected annually in the US, almost all of them in the southwestern states. The fungus grows in the “Lower Sonoran Life Zone”, i.e. arid climates, hot summers, low altitude, alkaline soil and sparse flora. Positive cultures have been obtained from soil. Arizona and southern California (where the disease gets its name) are endemic areas.

II. Pathogenesis

Inhaled arthroconidia lodge in the alveoli and develop into spherules in the tissues. Rupture of spherules releases large numbers of endospores into the tissues. Each endospore develops into a new spherule.

III. Immunity

The initial host response consists of macrophages and neutrophils. Spherules are resistant to killing by neutrophils. The onset of cell mediated immunity, which is detected by a positive DTH skin test within 2-4 weeks of the onset of infection, indicates the generation of a protective host response. If a protective response is not generated, endospores may gain entry into the blood, lodge in host tissue and produce spherules.

IV. Clinical Features

Infections are often asymptomatic. 40% of infected individuals have symptoms consistent with a lower respiratory infection and/or systemic illness with some of the following symptoms: cough, sputum production, chest pain, malaise, fever, chills, night sweats, anorexia, weakness and arthralgia. The disease lasts 2 - 6 weeks and is called Valley Fever. A small number of cases progress to a chronic pulmonary form characterized by cavity formation.

Disseminated disease occurs more commonly in those with immunosuppression, but can occur in immunocompetent adults as well.

Erythema nodosum (EN) occurs in 10% of infected individuals. Nodules do not contain organisms and are not indicative of disseminated disease. EN is indicative of protective immunity.

V. Epidemiology and Patient Populations of Special Interest

Coccidioides only occurs naturally in the Western hemisphere. Hyperendemic areas include Kern, Tulare, and Fresno counties in the San Joaquin Valley of California, and Pima, Pinal, and Maricopa counties in Arizona. Endemic areas also include parts of Central and South America. Cycles of rain and drought enhance dispersion of the organism. Natural events such as earthquakes and dust storms often stir up fungal particles and increase the number of infections. Person with occupations that expose them to soil are also at increased risk in endemic areas. Immunocompromised individuals are at high risk in endemic areas. Symptoms are more common in men of dark-skinned races, particularly Filipinos. The risk of disseminated disease in females increases during the third trimester of pregnancy. The increase in travel to these warm climates has extended the need for clinicians to be aware of their patients travel history and the signs and symptoms of these endemic mycoses. It is not uncommon for initial diagnoses of lung cancer to be made in patients with *Coccidioides* or *Histoplasma* infections.

VI. Laboratory Diagnosis

Spherules may be detected with KOH mounts or fungal stains in infected tissue.

Organisms can be cultured on Sabouraud's agar; demonstration of dimorphism and the tissue form is necessary for identification. The environmental form produces arthrospores that are hazardous to laboratory workers.

Serologic tests are useful for monitoring the progress of the disease. IgM precipitating antibody becomes positive in the first 2-4 weeks of illness. Complement fixing IgG antibodies develop later; titers increase with disseminated disease. Antibodies disappear with resolution of disease and persist with continuing infection.

***Histoplasma capsulatum* and histoplasmosis**

I. General Properties

The environmental growth form consists of septate, branching hyphae that bear spores (2 - 6 μ m in diameter) called microconidia. Macroconidia (8 - 14 microns) with characteristic morphology (tuberculate) are also produced.

Yeasts are ovoid 1.5 - 2.0 μ m and are taken up by macrophages. The "capsular" appearance is an artifact of staining. *H. capsulatum* does not produce a capsule. Yeast is the tissue (37°C) growth form.

An important virulence property is the ability to grow within macrophages (see below).

H. capsulatum is widely distributed throughout the temperate, subtropical and tropical zones of the world. The organism is highly endemic in the Ohio and Mississippi Valley regions. Up to 90% of individuals living in endemic areas have a positive skin test.

Bird droppings may be a rich source of organisms.

II. Pathogenesis

Inhaled spores (microconidia) reach the small bronchioles or alveoli and germinate after 2 - 3 days. Yeasts proliferate within macrophages which migrate to the mediastinal lymph nodes, spleen and liver. Yeasts proliferate for 9 - 15 days prior to the onset of host cell mediated immune responses. Yeast cells are usually found intracellularly, but may be found extracellularly in cases of overwhelming histoplasmosis. Epithelial cells may also be infected, perhaps serving as a reservoir of infection.

The ability of yeasts to survive and replicate intracellularly within macrophages is a virulence property. The yeasts increase the pH of the phagolysosome by producing bicarbonate and ammonia, which inactivates host degradative enzymes. The organism may be spread widely throughout the body within macrophages prior to the onset of protective immunity.

III. Immunity

Histoplasma may infect an immune competent individual and survive inside of macrophages and epithelial cells. An intact fully functional immune system is necessary to prevent lethal disease. Mechanisms of immunity and control are an ongoing research area. Interactions between macrophages of the innate system and T cells of the adaptive system are critical for protection. TNF-alpha plays a critical role in these processes. IFN-gamma responses are also important.

IV. Clinical Features

Most infections are asymptomatic. Acute pulmonary histoplasmosis is a self-limited flu-like illness in immunocompetent hosts that usually goes unrecognized.

Chronic pulmonary histoplasmosis is an opportunistic disease often mistaken for tuberculosis. Structural defects in the lung allow fungal colonization in abnormal pulmonary spaces.

V. Epidemiology and Patient Populations of Special Interest

The most highly endemic areas include eastern United States (Ohio, Mississippi, and St. Lawrence River Valleys) and most of Latin America. In the soil, *H. capsulatum* exists as a mold in areas of high nitrogen content such as those with lots of bat and bird guano. HIV infected individuals are at risk in endemic areas and outbreaks are often associated with known reservoirs of the organism.

Immunocompromised patients are more at risk to developing symptoms after a primary infection. Re-activation of disease is also a common manifestation in immune compromised individuals. African histoplasmosis is an emerging infectious disease and found in central and western Africa between the latitudes 15 N and 10 S.

VI. Diagnosis

For disseminated histoplasmosis, a Wright stained smear of peripheral blood is almost always positive for intracellular yeasts within macrophages. The organism may also be isolated from blood cultures

Demonstration of organisms in infected tissue using a fungal stain and identification in laboratory cultures are used for diagnosis.

Organisms can be cultured on special media and may be isolated from blood cultures in disseminated histoplasmosis. Demonstration of dimorphism is necessary for identification along with the presence of tuberculate macroconidia in mold cultures.

A test for Histoplasma antigen in the urine is highly useful.

Serological tests are useful for monitoring progress of the disease; antibody titers decrease when the disease becomes inactive.

Skin testing is not used for diagnosis because of cross reactivity with *B. dermatitidis* and because it may interfere with serologic testing.

***Blastomyces dermatitidis* and blastomycosis (North American blastomycosis)**

1. General properties

At 37°C, growth occurs as large yeasts with characteristic morphology. The yeasts are 5 - 30 microns in diameter with **single buds** having a characteristic **broad base**. Environmental mold forms produce small pear shaped conidia.

The environmental distribution is difficult to define because of the lack of a specific, sensitive skin test. The organism is antigenically cross-reactive with *H. capsulatum*. Sporadic cases suggest that endemic regions surround the Mississippi and Ohio river basins and are also present in the Carolinas. Recreational activity in wooded areas along waterways is a major risk factor.

A high frequency of clinical disease is found following exposure of unexposed hosts to *B. dermatitidis*.

Blastomycosis is a common infection of dogs in endemic zones, and canine infections are often severe or lethal.

II. Pathogenesis

Humans and other mammals are primarily infected by inhalation of spores from hyphae in soil where *B. dermatitidis* is believed to dwell as a saprophyte. At 37°C, in the host, conidial spores transform into pathogenic yeast-phase cells, which multiply within the lung and may disseminate via the bloodstream and lymphatics to visceral organs. A surface protein, Bad1 (*Blastomyces* adhesin 1) is required for virulence. Bad1 has homology to invasin genes of Gram negative bacteria. Bad1 promotes uptake by macrophages. Yeast forms replicate readily in macrophages until the macrophages are activated by cytokines from T cells. Macrophages may carry the yeasts to other organs. Within several weeks, the host develops acquired cell mediated immunity with the appearance of DTH, proliferation of T cells in vitro, and circulating antibodies against fungal antigens. The onset of CMI leads to protection.

III. Immunity.

Inhaled conidia transform into yeast in the alveoli inducing an acute-inflammatory response that includes macrophages and neutrophils resulting in granuloma formation. Once a yeast, the fungus is generally resistant to phagocytosis and killing. Cell mediated immunity driven by antigen specific T-lymphocytes and lymphokine activated macrophages are key to control of the disease. The fungus produces a protein called BAD-1 that directly interferes with TNF- α function contributing to persistence and disease in vivo.

IV. Clinical Features

The acute primary pulmonary infection with *B. dermatitidis* may be asymptomatic or may produce an influenza-like syndrome. Acute infection may resolve spontaneously, but progressive disease involving the lungs and/or other organs develops in many patients.

The disease has a tropism for skin and bone.

V. Epidemiology and Patient Populations of Special Interest

Blastomyces commonly occurs along the river estuaries from Michigan to Mississippi in the USA and Canadian provinces bordering the Great Lakes. Scattered areas in Europe, Asia, Latin America and Africa exist. Hyperendemic areas include areas with warm moist soil that is often sandy and acidic or in woody areas. There is some overlap with the Histoplasma endemic areas. Occupational or recreational activity is associated with infection. African-American race and diabetes are risk factors for symptomatic disease. Dogs are highly susceptible to blastomycosis and can be sentinels at outbreak points. A recent ongoing outbreak is occurring in Wisconsin in the Hmong population for unknown reasons.

VI. Diagnosis

Diagnosis is made by the observation of thick-walled yeast cells with single broad-based buds in clinical specimens (either in KOH mounts or stained biopsy specimens) and by culture to demonstrate thermal dimorphism.

***Paracoccidioides brasiliensis* and paracoccidioidomycosis (South American blastomycosis)**

***Be aware of this organism for patients that have traveled to or are endemic to South America.** Ongoing research on this organism and the disease that it causes. Information is currently limited. There are many similarities in regards to pathogenesis and immunity with the other systemic mycoses.

I. General Properties

At 37°C, growth occurs as large yeasts with characteristic morphology. The yeasts are thick-walled with **multiple buds** having a characteristic **wagon wheel** appearance. Environmental mold forms produce small pear shaped conidia.

The fungus grows in the soil and is endemic in rural Latin America. Disease is limited to that region.

Summary of treatment of systemic fungal pathogens

Infections that are mild are likely to be cured without therapy. Amphotericin B or various azoles are used in severe cases. Immune compromised patients are at high risk for re-activation of disease and severe disseminated infections.

Opportunistic Fungal Pathogens

Learning objectives

- To name four genera of fungi that cause more than 90% of all reported fungal-related deaths.
- To describe the growth morphologies of each opportunistic fungal pathogen.
- To list risk factors that predispose humans to opportunistic fungal infections.
- To describe the major symptoms of disease caused by *C. neoformans*
- To describe a rapid method introduced in 2011 for detection of *C. neoformans* in spinal fluid or blood and the utility of the test for global health.
- To recognize the recent emergence of Cryptococcosis in the Northwestern United States
- To name encapsulated pathogenic fungi, the chemical composition of the capsule and its role in virulence.
- To name a cryptococcal pathogen that causes outbreaks in the Northwestern US.
- To distinguish *Aspergillus* sp. and *Mucor* sp. in histological stains of infected tissue.
- To define the major diagnostic test in use for invasive aspergillosis
- To define the key immunological protective mechanisms for each opportunistic fungal pathogen.
- To list differences between *Pneumocystis carinii* (recently renamed *Pneumocystis jirovecii*) and other fungi.
- To describe why *P. jirovecii* is considered to be a fungus and not an animal parasite.
- To memorize that *P. jirovecii* cannot be cultured.
- To describe the clinical features of PCP.
- To recognize the emerging impact of environmental mold on human health.

READING ASSIGNMENTS AND PREPARATION FOR LECTURE

Reading assignment: Levinson, Chapter 50 and Chapter 52 pp 427-428.

Reading assignment (for discussion in class): Notes from the Field: Fatal Fungal Soft-Tissue Infections after a Tornado --- Joplin, Missouri, 2011.

http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6029a5.htm?s_cid=mm6029a5_w

Video assignment (for discussion in class). Stuart Levitz, MD: Fungal Meningitis

<http://www.youtube.com/watch?v=H7DPdHTbeG8>

Overview

Most of the pathogens described in this section occur worldwide and cause the most severe diseases in immunocompromised hosts. However, there are exceptions that are noted below. The mortality of untreated cases in immunocompromised hosts approaches 100%. Antifungal therapy is almost always required. Specific therapeutic regimens are likely to change and thus will not be discussed in detail for each pathogen.

***Cryptococcus neoformans*, *C. gattii* and cryptococcosis**

I. General properties

Cryptococci are basidiomycetes that grow as yeast on agar media and in clinical specimens. A prominent polysaccharide capsule is a key distinguishing feature of cryptococci. The capsule is composed of glucuronoxylomannan (GXM). Mutants without capsules are not virulent. Organisms are urease positive and grow at 37°C as are all pathogenic fungi.

Cryptococci cause more life threatening infections than any other fungal pathogen. The primary disease caused by cryptococci is meningoencephalitis. The yearly global burden of cryptococcal meningitis is estimated to be nearly 1 million cases, with more than 620,000 deaths in sub-Saharan Africa. Mortality rates in AIDS patients are estimated to range from 15 to 20% in the United States and 55 to 70% in Latin America and sub-Saharan Africa, despite treatment¹.

Cryptococcus neoformans occurs widely in nature and grows abundantly in soil containing bird droppings (especially pigeon droppings). Immunocompromised hosts are infected.

Cryptococcus gattii is found in association with trees, and its geographical distribution is more limited. Both immunocompetent and immunocompromised hosts are affected. *C. gattii* is the cause of an outbreak of cryptococcosis that began in southwestern Canada and is spreading southward through the USA.

II. Pathogenesis

The most common route of transmission is inhalation of spores or desiccated yeast forms. Spores are released during the natural environmental lifecycle. The capsule is produced in copious amounts and is not efficiently eliminated by the host. The capsule prevents phagocytosis and other normal functions of host cells. Cryptococci also have phenol oxidases which use dopamine as a substrate to generate melanin. Melanin protects cryptococci from oxidative damage elicited by the host in response to infection.

If the host becomes immunocompromised, both local (within the lungs) and systemic spread can occur. However, the fungus has a preference for invading the central nervous system, and most clinical cases consist of meningoencephalitis (inflammation of the brain and meninges). Fungi enter the central nervous system (CNS) through the microcapillaries of the blood–brain barrier or by delivery within phagocytic cells.

III. Immunity

Protective immunity to *C. neoformans* is mediated by the integration of the innate and adaptive immune responses. Innate immune receptors that recognize *C. neoformans* include mannose receptors, CD14, CD18, and the Toll Like Receptors (TLRs). These receptors are expressed on cells of the innate immune system including macrophages, neutrophils, natural killer cells, monocytes and dendritic cells. Engagement of these receptors prompts the innate cells to secrete cytokines and chemokines which recruit additional phagocytes and T cells to the site of infection. Cell mediated immunity is a critical component for protection against *C. neoformans* and protection is mediated in part through induction of a Th1 immune response. The complement system also contributes to the immunity against these pathogens.

IV. Clinical Features

Chronic meningoencephalitis is the most common disease and is characterized by a slow, insidious onset. Symptoms include headache, stiff neck and body aches. Coughing and shortness of breath, from infection in the lungs may also occur. *C. neoformans* is likely to exist sub-clinically in urinary and respiratory tracts.

V. Epidemiology and Patient Populations of Special Interest

Human to human transmission is incredibly rare but has been recently reported. Acquisition of infection is most common through the respiratory tract. Traumatic inoculation has been reported as a primary cause of primary cutaneous cryptococcosis. Before the AIDS epidemic, cases of Cryptococcus were <1000 per year in the USA. HIV infections account for more than 75% of the predisposing factors for cryptococcosis. Diagnosis of cryptococcosis with unknown predisposing risk factors should always suggest an evaluation for HIV infection. The risk for Cryptococcosis occurs late in HIV infection when the CD4+ lymphocytes are less than 100/mm³. The vast majority of cryptococcosis in AIDS patients is caused by *C. neoformans*. *C. gattii* infections have been reported to occur in otherwise immune competent patients, particularly in sub-tropical areas. An outbreak of *C. gattii* infections has occurred on Vancouver Island in British Columbia, and case reports now extend to the west coast of the USA.

VI. Diagnosis and Treatment

The presence of capsular antigen in CSF, plasma or urine provides a rapid method for definitive diagnosis. Until recently the format for this test has been the latex agglutination assay, however a new “dipstick” test (Lateral Flow Assay) has been developed that rapidly allows diagnosis <http://www.immy.com/products/cryptococcus/>.

Encapsulated organisms may be visualized in CSF using an India ink wet mount, however this is less sensitive and specific than capsular antigen detection. Encapsulated organisms may also be identified by the mucicarmine, PAS and methanamine stains of infected tissue.

C. neoformans and *C. gattii* can be differentiated utilizing CGB Agar. *C. gattii* cultures turn CGB agar blue.

The antifungal drugs flucytosine, amphotericin B, and the triazoles fluconazole and itraconazole are key drugs in the treatment of these infections.

Cryptococci may also be cultured, but this is not commonly performed in clinical laboratories.

Aspergillus and aspergillosis

I. General Properties

Aspergillus fumigatus and *Aspergillus flavus*, the two major causes of aspergillosis, grow in mycelial (mold) form only and thus are **not** dimorphic. In cultures, *Aspergillus* species form aerial hyphae which are septate and have characteristic stalk-like conidiophores bearing brush-like conidiospores which are easily airborne. The organism is ubiquitous in the environment, producing large numbers of airborne spores. When growing in host tissue, Aspergilli produce septate hyphae with V-shaped branching.

It is estimated *Aspergillus* species cause over 200,000 life-threatening infections/yr worldwide².

Inhalation of spores of *Aspergillus* species causes invasive infections in individuals with impaired immune function, in particular those with lower than normal numbers of neutrophils, solid organ transplant recipients, and patients on immunosuppressive therapies, such as high-dose corticosteroids. An emerging risk group includes patients with chronic obstructive pulmonary disease (COPD; also called emphysema).

A. fumigatus is also a ubiquitous aeroallergen. Globally, millions of susceptible individuals develop pulmonary and nasal allergies to *A. fumigatus* and other airborne fungal particles³. A Th2 type disease associated with *A. fumigatus* is called Allergic Bronchopulmonary aspergillosis. It can affect up to 20% of cystic fibrosis patients and 5 to 15% of Asthma patients.

II. Pathogenesis

Ubiquitous airborne spores (conidia) land on exterior surfaces or in the lungs of susceptible hosts. Germ tubes emerge from conidia and colonize cavity lesions, filling the spaces with hyphae. In invasive aspergillosis in susceptible hosts that lack normal numbers of neutrophils, the fungal hyphae grow rapidly and penetrate human tissue. Immediate and aggressive antifungal therapy is required to save the patient. Dissemination to other organs is often observed. Classic virulence factors have not been defined and virulence is thought to be multifactorial.

III. Immunity

Cells of the innate immune system are critical in providing immunity to *Aspergillus* infections. Alveolar macrophages and neutrophils are responsible for rapidly clearing inhaled fungal spores from the respiratory tract, primarily through oxidative mechanisms. Pattern recognition receptors (PRRs) that recognize *A. fumigatus* spores as they swell and expose Beta 1,3 glucan include Dectin-1 and the Toll Like Receptors. A soluble PRR, Pentraxin 3 has recently been recognized as a critical molecule for host defense against *A. fumigatus*. An emerging role for the IL-1 family of cytokines in resistance and immunopathology to *A. fumigatus* is clinically important. A resistance response to *A. fumigatus* also involves the adaptive immune response with a Th1 response stimulating protection and resistance to the fungus.

IV. Clinical Features

Invasive aspergillosis may present with symptoms of fever, coughing, chest pain, and shortness of breath.

Growth within cavities of the lung that occur after other diseases or trauma such as tuberculosis is seen. Aspergilloma, or “fungus ball” is a common finding in chronic aspergillosis².

Symptoms of allergic bronchopulmonary aspergillosis (ABPA) may include wheezing and coughing.

It is increasingly recognized that the disease spectrum associated with *Aspergillus* spp. is broad and of significant clinical relevance.

V. Epidemiology and Patient Populations of Special Interest

Human to human transmission is not part of the considerations for *A. fumigatus* infection. Spores are inhaled from environmental sources into the respiratory tract. Traumatic inoculation has been reported. Invasive aspergillosis (IA) most often occurs in the context of hematopoietic stem cell transplantation (HSCT) and hematologic malignancies. It occurs also in solid organ transplantation. A patient population with significant risk for IA are Chronic Granulomatous Disease (CGD) patients. IA can occur in the context of HIV/AIDS, but this normally occurs at very late stages of the HIV infection. IA is increasingly observed in patients being treated for graft vs. host disease. ABPA can also occur in the context of Cystic Fibrosis and COPD though development of IA in these patients is rare.

VI. Diagnosis and Treatment

Diagnosis may be made by biopsy of infected material exhibiting characteristic hyphal morphology. A serum test for the fungal antigen galactomannan is available however, it is about 80% sensitive for the detection of this disease in neutropenic patients who are not taking antifungal drugs but only about 20% sensitive if the patients are taking these drugs.

Eosinophilia and IgE antibodies specific for *Aspergillus* antigens may be used to diagnose ABPA.

The antifungal drug of choice to treat *A. fumigatus* infections is Voriconazole. Amphotericin B and other triazoles are also utilized. The echinocandins are currently used as salvage therapy.

***Mucor, Rhizopus* and mucormycosis, zygomycosis and phycomycosis**

I. General Features.

Organisms grow in mycelial (mold) forms only and thus are not dimorphic. Unlike *Aspergillus* however, hyphae in this group of organisms are not septate. Instead, hyphae are coenocytic, without cytoplasmic compartmentalization. They form right angle branching (see Figure 50-8 in Levinson).

It is estimated that over 10,000 cases of life-threatening mucormycosis infections/yr occur worldwide³.

The source, transmission, spread and clinical manifestations are similar to those for *Aspergillus*. Increased susceptibility occurs in the setting of diabetic ketoacidosis causing rhinocerebral infections. Spores land in the sinuses, germinate and rapidly invade tissue. These infections are particularly difficult to treat given their inherent resistance to many antifungal agents and rapid growth leading to fast tissue damage and host mortality. With the emergence of azole prophylaxis therapy in many major transplant centers, the incidence of breakthrough mucormycoses is increasing.

II. Pathogenesis

Pathogenesis mechanisms of this group of fungi are poorly understood. The general pathogenesis is similar to the *Aspergilli* in terms of spores germinating into hyphae that can penetrate tissue, cause damage, and disseminate widely throughout the body.

III. Immunity

Little is known about the host factors essential for protection against these organisms. It is clear that an intact innate and adaptive immune system is important.

IV. Clinical Features

Pulmonary infections are very similar to *Aspergillus* infections. The most common presentation of these infections is acute rhinocerebral zygomycosis. This infection presents as acute sinusitis mimicking bacterial sinusitis with fever and headache located in the frontal or retroorbital regions. Dissemination is often a common occurrence with these infections.

V. Epidemiology and Patient Populations of Special Interest

Zygomycetes have a wide geographic distribution. Person to person transmission is rare. Infections typically are acquired through inhalation of spores or blunt force trauma. A special patient population at risk for Zygomycoses are diabetes mellitus patients with ketoacidosis. Hematopoietic stem cell transplantation (HSCT) and hematologic malignancies are also commonly associated with these infections. It also occurs in solid organ transplantation. Deferoxamine therapy in otherwise immune competent individuals is also a risk factor.

VI. Diagnosis and Treatment

Tissue biopsy and microscopic examination remain the gold standard for diagnosis. On histology, the hyphae are broad and ribbon like often lacking septa. Aggressive surgical debridement coupled with amphotericin B is often the primary treatment strategy employed as many of these species are resistant to triazole antifungals. New derivations of the triazoles, such as Posaconazole hold great promise.

***Pneumocystis carinii* (*Pneumocystis jiroveci*) and Pneumocystis pneumonia (PCP)**

I. General Properties

MAJOR POINT. Human forms **cannot** be cultured and cannot be grown in animals. This is an obligate pathogen.

The organism exists as cysts that are 5 - 8 microns in diameter that contain sporozoites that are released when the cyst ruptures. The sporozoites mature to pleomorphic trophozoite forms 2-5 microns in diameter.

It is estimated that over 400,000 cases of life-threatening Pneumocystis infections/yr occur worldwide³.

Specific pathogens exist for each animal species. Isolates from animals and humans are distinct. Each type of animal has "it's own Special Form (*sf*) of *P. carinii* that does not infect other species. Animals are not a reservoir of human disease. The human form has recently been assigned the name *Pneumocystis jiroveci*. It exists worldwide. The presence of specific antibodies in over 70% of normal hosts by the age of 5 indicates that asymptomatic infections are common.

Pneumocystis is thought to have coevolved with its mammalian host, with gradual differentiation into host-specific species and loss of its ability to survive independently.

Although *P. carinii* (*jiroveci*) is now classified as a fungus, prior to the advent of molecular biology, this organism was believed to be an animal parasite because:

- A. Morphology is similar to cysts and trophozoites of the phylum Apicomplexa, which are around parasites relative fragility of trophozoite cell wall.
- B. Susceptibility exists to certain antiparasitic agents such as pentamidine, and trimethoprim/sulfamethoxazole.
- C. Lack of susceptibility to Amphotericin B.
- D. Absence of ergosterol and presence of cholesterol and other sterols in cell membranes.

The genetic, structural, and biochemical evidence for classification as a fungus is:

- A. rDNA and other sequences establish a fungal taxonomy.
- B. Presence of translation elongation factor 3 (EF3) a protein found only in fungi.
- C. Presence of thymidylate synthase and dihydrofolate reductase on two separate genes as is found for fungi.
- D. Presence of cell wall chitin, glucan and mannoproteins typical of fungi.

PCP is associated with the early recognition of AIDS prior to the discovery of HIV and with the introduction of immunosuppressive therapies. It is currently a major cause of morbidity and mortality in those infected with HIV and likely contributes to deterioration in COPD³.

II. Pathogenesis

Loss of CD4+ immunity leads to susceptibility. Transmission occurs via aerosols from patients with pneumonia or from early-life contact with family or community members who carry the organism in their lungs⁴.

Inhaled cysts produce an inflammatory response consisting primarily of plasma cells, resulting in a frothy exudate that blocks oxygen exchange leading to plasma cell pneumonia.

III. Immunity

Host immunity to *Pneumocystis* involves complex interaction between alveolar epithelial cells, CD4+ T cells, alveolar macrophages, neutrophils, and soluble mediators. CD4+ T cells are critical mediators that stimulate recruitment and activation of monocytes and alveolar macrophages. Their absence or functional impairment is a defining risk factor for *Pneumocystis* infection as exhibited by AIDS patients with CD4 counts less than 200 cells per microliter. Uptake and killing of the fungus is primarily mediated by alveolar macrophages through pattern recognition receptors. Severe *Pneumocystis* infection, however, can stimulate a hyperactive immune response that can cause significant host damage. This immunopathogenesis is stimulated by increases in CD8+ T cell mediated neutrophilic lung inflammation.

IV. Clinical Features

PCP is characterized by a sudden onset of fever, nonproductive cough, dyspnea and tachypnea. Chest X-ray shows diffuse interstitial pneumonia.

Oxygen uptake is impaired resulting in hypoxemia and often necessitating oxygen therapy.

V. Epidemiology and Patient Populations of Special Interest

PCP has been diagnosed in individuals world-wide. Airborne transmission is well documented and it is now clear that both human and environmental sources of infection exist. Evidence suggests that people are exposed very early to *Pneumocystis* and repeated cycles of acquisition and immune clearance occur in immune competent individuals. Reactivation of latent infections may occur, but the bulk of infections are new. Patient to patient transmission is possible! Defects in CD4+ T lymphocytes are the primary risk factor for PCP infection. CD4+ counts less than 200 cells/ μ L are an important risk factor. Defects in B cells and antibody production may also predispose individuals to PCP. Therapies with monoclonal antibodies such as adalimumab, infliximab, etanercept are also a risk factor for PCP. The largest increase in PCP incidence occurred concomitant with the AIDS epidemic. Clusters of PCP cases were one of the first indicators of the HIV epidemic. Other patients on immunosuppressive therapies are also at risk for PCP.

VI. Diagnosis and Treatment

Demonstration of the organism in infected material using the methanamine silver stain or fluorescent stain with specific antibodies provides a definitive diagnosis. PCR based tests may also be useful. Chemoprophylaxis is widely used for preventing PCP in patients with CD4+ T cell counts in the at risk level. Primary prophylaxis in HIV infected adults involves use of Trimethoprim-sulfamethoxazole (TMP-SMX). TMP-SMX also remains the drug of choice for treatment of PCP.

VII. Prevention

Keep immunosuppressed patients away from infected hosts.

Other Impacts of Fungi on Human Health: Environmental Mold

I. General Properties

There has been significant interest and much attention to the question whether fungi found in building environments contributes to disease. While this remains a controversial issue, it is now generally well accepted that high levels of mold in a living environment are to be avoided. The Center for Disease Control (CDC) has a web-page with information regarding environmental mold and human health concerns: <http://www.cdc.gov/mold/>.

Common indoor molds include *Cladosporium*, *Penicillium*, *Alternaria*, and *Aspergillus* spp. It is clear that some people are sensitive to mold and mold products. While still undefined,

health problems from the presence of mold or mycotoxins in living environment is a real and emerging consideration for the clinician.

II. Pathogenesis

Inhalation of mold spores, particles, and/or products produced by molds such as mycotoxins and other so called “secondary metabolites” produced by the fungi. These are not infections but rather immune mediated responses to the mold and or their products. Some mycotoxins are highly toxic if ingested.

III. Immunity

Rather than immunity to infection, disease in this context is most often a hypersensitive reaction to the mold and/or its products. Therefore, these conditions are often associated with Th2 like immune responses.

IV. Clinical Features

Mild symptoms include nasal stuffiness, eye irritation, wheezing, or skin irritation. People with severe allergies have more extreme responses that may include fever and shortness of breath. People with chronic lung diseases and Asthma may be at higher risk for adverse effects from environmental mold exposure.

V. Epidemiology and Patient Populations of Special Interest

Indoor environments with high levels of moisture are associated with poor air quality and high levels of mold spores and mold products. Lower socioeconomic populations can be at higher risk for health problems from environmental mold due to their housing conditions and living environments. For example, a recent report on poor housing conditions for Canada’s First Nations populations highlighted the importance of housing conditions, mold growth, and health.

SUMMARY OF PROPERTIES OF SOME IMPORTANT BACTERIAL EXOTOXINS
(also see tables 7-10 to 7-13 in Levinson)

<u>BACTERIUM</u>	<u>TOXIN</u>	<u>EFFECTS</u>	<u>MECHANISM</u>	<u>COMMENTS (VACCINE, ANTITOXIN, ETC.)</u>
<i>Clostridium botulinum</i>	botulinum toxin (several immunological types)	neurotoxin causing flaccid paralysis (a protease)	acts at myoneural junction, blocks release of the transmitter acetylcholine (cleaves releasing protein)	antitoxin (specific for type of toxin) (passive immunity)
<i>Clostridium tetani</i>	tetanus toxin (one immunological type)	neurotoxin (spastic paralysis) (a protease)	blocks normal post-synaptic inhibition; blocks release of inhibitory transmitter glycine (cleaves releasing protein)	excellent toxoid for active immunization; antitoxin for wounds in unimmunized persons
<i>Clostridium perfringens</i>	alpha toxin	gas gangrene; necrosis, cytolytic, lethal	lecithinase (disrupts cell membranes)	antitoxin available but effectiveness is in doubt
<i>Clostridium difficile</i>	A. enterotoxin B. cytotoxin	watery diarrhea kills cells (forms pseudomembranes)	Toxin B is cytotoxic to enterocytes (depolymerize actin filaments)	both toxins are involved in antibiotic-associated pseudomembranous enterocolitis
<i>Corynebacterium diphtheriae</i>	diphtheria toxin	kills cells; pseudomembranes in throat; myocarditis	inhibits protein synthesis (ADP ribosylates elongation factor 2)	excellent toxoid for prevention (toxoid is also used in the Shick test for diagnosis)
<i>Pseudomonas aeruginosa</i>	exotoxin A	kills cells	like diphtheria toxin	
<i>Staphylococcus aureus</i>	alpha toxin	hemolytic, leukocytic; paralysis of smooth muscle; local necrosis	lytic pores in membrane with loss of low molecular weight substances	responsible for beta (complete) hemolysis on blood agar plates
<i>Staphylococcus aureus</i>	enterotoxin	food poisoning (emesis, diarrhea)	a superantigen (see below)	present only in some <i>S. aureus</i> strains
<i>Staphylococcus aureus</i>	toxic shock toxin	fever, rash, shock	a superantigen, binds to class II-MHC protein induces IL-1 and IL-2, TNF	present only in some <i>S. aureus</i> strains
<i>Staphylococcus aureus</i>	exfoliatin (scalded skin syndrome)	desquamation (cleaves skin desmosome protein)	a protease	present only in some <i>S. aureus</i> strains
<i>Streptococcus pyogenes</i>	streptolysin O	lyses red blood cells, etc. (Beta hemolysis)	Toxin oligimers form membrane pores	anti-streptolysin O (ASO) test used in diagnosis of rheumatic fever
<i>Streptococcus pyogenes</i>	erythrogenic toxin	rash (scarlet fever)	also a superantigen	produced only by strains lysogenized with phage carrying toxin gene
<i>Streptococcus pyogenes</i>	pyrogenic exotoxin A	streptococcal toxic shock syndrome	a superantigen (like staphylococcal TSST)	related to Erythrogenic toxin; only produced in some strains
<i>Vibrio cholerae</i>	cholera toxin	massive diarrhea	activation of adenylate cyclase	acts by ADP ribosylation
<i>Escherichia coli</i> (Enterotoxigenic strains)	1. heat labile (LT) enterotoxin 2. heat stable (ST) enterotoxin	diarrhea diarrhea	identical to cholera toxin acts on guanylate cyclase	only some <i>E. coli</i> strains produce toxins or are pathogenic
<i>Escherichia coli</i> (Enterohemorrhagic strains)	cytotoxin (Verotoxin)	bloody diarrhea (hemorrhagic colitis) kidney damage	like Shigella toxin (Shiga toxin)	vehicle is undercooked hamburger, other foods contaminated with cow feces
<i>Shigella dysenteriae</i>	cytotoxin (Shiga toxin)	kills cells	inhibits protein synthesis (inactivates the 60S ribosome) (the 28S rRNA site)	exact role in dysentery is unclear
<i>Bordetella Pertussis</i>	Pertussis toxin	whooping cough	activation of adenylate cyclase	acellular vaccine (mainly inactivated toxin)
<i>Bacillus Anthracis</i>	1. edema factor 2. lethal factor 3. protective antigen	1. tissue edema 2. inhibits cell division 3. binds to cells	1. adenylate cyclase 2. protease 3. binding subunit for 1 and 2 (AA-1)	acellular vaccine contains protective antigen (#3)

REVIEW OF MANY OF THE COMMONLY USED
VACCINES FOR BACTERIAL DISEASES
(also see table 12-1 in Levinson for a more complete listing)

<u>BACTERIUM</u>	<u>DISEASE</u>	<u>DESCRIPTION OF VACCINE</u>
<i>Mycobacterium tuberculosis</i>	tuberculosis	live-attenuated (BCG) (not used in U.S.)
<i>Bordetella pertussis</i>	whooping cough	purified protein (part of DTaP vaccine)
<i>Salmonella typhi</i>	typhoid	killed bacteria or live attenuated
<i>Vibrio cholerae</i>	cholera	killed bacteria, or live attenuated (not very effective)
<i>Neisseria meningitidis</i>	meningitis	capsular polysaccharides A and C (two of the most common antigenic types) (Y and W also available)
<i>Haemophilus influenzae</i>	meningitis	capsular polysaccharide type b conjugated to carrier protein (Hib)
<i>Streptococcus pneumoniae</i>	pneumococcal pneumonia	the 23 most common antigenic types of polysaccharide capsule (not suitable for children) The 8 most common antigenic types of polysaccharide capsule, each conjugated to a highly immunogenic protein (used as a pediatric vaccine).
<i>Clostridium tetani</i>	tetanus	toxoid (part of DTaP vaccine)
<i>Corynebacterium diphtheriae</i>	diphtheria	toxoid (part of DTaP vaccine)

REVIEW OF SPORE-FORMING ABILITY IN PATHOGENIC BACTERIA

1. Recall that the spore is a non-multiplying stage that is markedly resistant to adverse conditions such as heating and drying. You need to know three classes of spore-formers.
 - a. All members of the genus *Clostridium* (Gram-positive, obligate anaerobic, rods) form spores.
 - b. All members of the genus *Bacillus* (Gram-positive, aerobic, rods) form spores.
 - c. *Coxiella burnetii* (obligate intracellular rickettsial organism) forms a stable extracellular stage that is termed “spore-like” but sometimes simply called a spore.

REVIEW OF RELATIONSHIP OF PATHOGENIC BACTERIA TO OXYGEN

Recall that bacteria can be classified as obligate anaerobes, obligate aerobes, or facultative anaerobes. You should know the obligate anaerobes for two reasons:

(1) because they cause infections in anatomical sites that can be rendered anaerobic by compromising the blood supply or by the consumption of oxygen by aerobic or facultative bacteria and (2) because they require care in obtaining the diagnostic specimen and in culturing it under anaerobic conditions.

The obligate anaerobes considered in this course are:

1. *Clostridia* (gram positive rods, the only obligate anaerobes that produce spores)
 - a. *Cl. tetani*
 - b. *Cl. botulinum*
 - c. *Cl. perfringens*
 - d. *Cl. difficile* (There are other, less common species of *Clostridia*)
2. *Bacteroides* (gram negative rods)
 - a. *Bacteroides fragilis* (penicillin resistant) (prominent in large intestine)
 - b. Other *Bacteroides* species
3. *Fusobacterium* (gram negative rods)
4. *Actinomyces* (the actinomycetes are gram positive filamentous branching rods)
5. *Peptostreptococcus* (gram positive cocci related to streptococci)

REVIEW OF THE INTRACELLULAR STATUS OF MICROORGANISMS

1. Obligately intracellular bacteria: These bacteria can multiply only within host cells.
 - a. All chlamydia
 - b. All rickettsia
 - c. *Mycobacterium leprae*
2. Facultatively intracellular bacteria: These bacteria can be grown on artificial media in the laboratory. However, in natural infections they multiply within host cells for prolonged periods during the course of disease. These facultative intracellular bacteria may be protected against some components of the host immune response when they are within host cells. In addition in their intracellular location, these bacteria will be protected from those antibiotics that do not readily penetrate host cells. Thus, an antibiotic effective *in vitro* may not be effective *in vivo*.
 - a. *Mycobacterium tuberculosis* (and probably all other pathogenic mycobacteria)
 - b. *Salmonella typhi* (other *Salmonella* species and the *Shigella* organisms also have an important intracellular phase of growth during infections. However, these cause less severe disease)
 - c. *Listeria monocytogenes*
 - d. All *Brucella* species
 - e. *Legionella pneumophila*
 - f. *Yersinia pestis*
 - g. *Francisella tularensis*
3. Predominantly extracellular bacteria: Although the above list of facultative intracellular bacteria may not be complete, you can make the simplifying assumption that all other pathogenic bacteria are predominantly extracellular. When these bacteria are found within cells, they have been phagocytosed and their intracellular existence will be brief. They will either be destroyed by the phagocyte or will, themselves, destroy the phagocyte and be released to grow in the extracellular fluid. Note that the *Neisseria* are predominantly extracellular bacteria. Do not be confused by the diagnostic criterion that requires intracellular gram negative diplococci to identify *Neisseria gonorrhoeae* in a stained slide from a urethral swab.
4. Predominantly intracellular fungi: *Histoplasma capsulatum*