Pharmaceutical microbiology

Chapter 2

By Dr. Mohammed Hussein Taleb

Lecture 4

08/10/2020

Pathogenesis of Infection Bacterial

1-Adherence (adhesion, attachment):

The process by which bacteria stick to the surfaces of host cells. After bacteria have entered the body, adherence is a major initial step in the infection process. The terms adherence , adhesion , and attachment are often used interchangeably.

2-Carrier:

A person or animal with asymptomatic infection that can be transmitted to another susceptible person or animal.

3-Infection

- Multiplication of an infectious agent within the body.
- Multiplication of the bacteria that are part of the normal flora of the gastrointestinal tract, skin, and so on is generally not considered an infection;
- on the other hand,
- Multiplication of pathogenic bacteria (eg, Salmonella species)—even if the person is asymptomatic—is deemed an infection

- Invasion The process whereby **bacteria, animal parasites, fungi, and viruses enter host cells or tissues and spread in the body**.
- Microbiota: **Microbial flora harbored by normal, healthy individuals**.
- Nonpathogen: A microorganism that does not cause disease; may be part of the normal microbiota.
- Pathogen: A microorganism capable of causing disease.
- Pathogenicity: The ability of an infectious agent to cause disease.
- Opportunistic pathogen: An **agent capable of causing disease only when the host's resistance is impaired (ie, when the patient is "immunocompromised").**

- **Toxigenicity**: The ability of a microorganism to produce a toxin that contributes to the development of disease.

- **Virulence:** The quantitative ability of an agent to cause disease. Virulent agents cause disease when introduced into the host in small numbers.
- Virulence involves **adherence, persistence, invasion, and toxigenicity** .

Identifying Bacteria That cause disease

- **Humans and animals have abundant normal microbiota that usually do not produce disease but achieve a balance that ensures the survival, growth, and propagation of both the bacteria and the host.**
- Some bacteria that are important causes of disease are **cultured commonly with the normal flora (eg, Streptococcus pneumoniae, Staphylococcus aureus).** Sometimes **bacteria that are clearly pathogens (eg, Salmonella serotype Typhi) are present, but infection remains latent or subclinical, and the host is a "carrier" of the bacteria.**

Transmission Of Infection

- Some **bacteria that commonly cause disease in humans exist primarily in animals and incidentally infect humans.**
- For example, **Salmonella and Campylobacter species typically infect animals and are transmitted in food products to humans.**
- For example, Y. pestis (plague) has a well-established life cycle in rodents and rodent fleas, and transmission by the fleas to humans is inadvertent;
- Bacillus anthracis (anthrax) lives in the environment, occasionally infects animals, and is transmitted to humans by products such as raw hair from infected animals

Bacterial Virulence Factors

- 1-Adherence Factors
- 2-Invasion
- 3-Toxins
- 4- Enzymes

Bacterial Virulence Factors

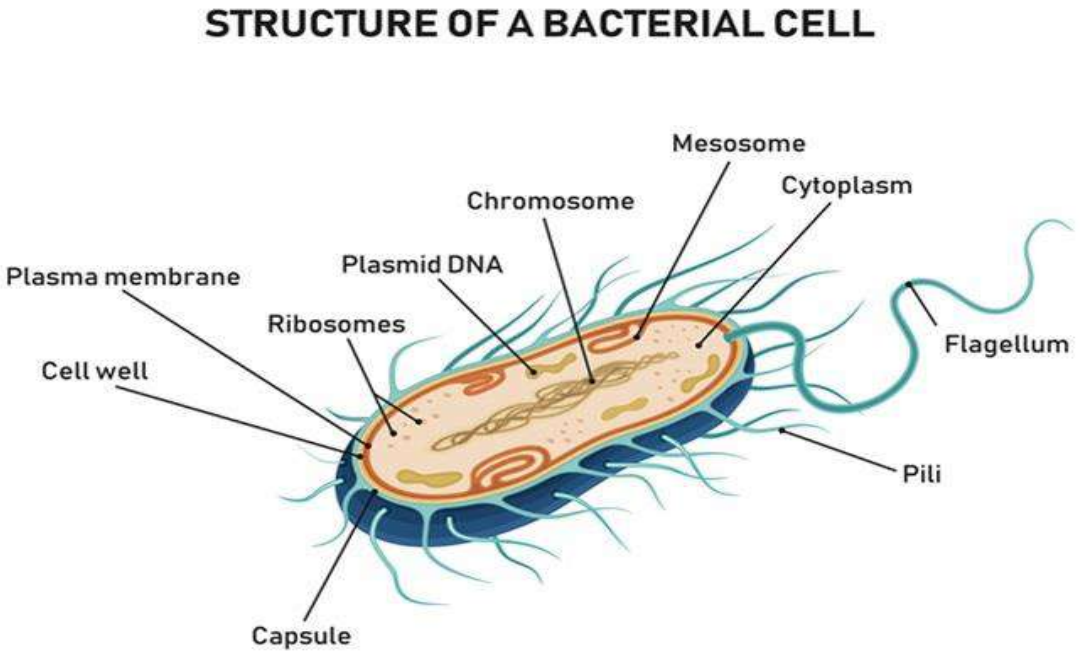
1-Adherence Factors

- When **bacteria enter the body of the host, they must adhere to cells of a tissue surface.** If they **did not adhere,** they would be **swept away by mucus and other fluids that bathe the tissue surface.**
- Adherence, which is only one step in the infectious process, is followed by **development of microcolonies** and **subsequent steps in the pathogenesis of infection**.
- The **interactions between bacteria and tissue cell surfaces in the adhesion process are complex**. Several factors play important roles, including
- **surface hydrophobicity**
- **net surface charge,**
- **binding molecules on bacteria (ligands),**
- **host cell receptor interactions.**

- **Bacteria** also **have specific surface molecules that interact with host cells.**

  Many bacteria have pili, (thick rodlike appendages) or fimbriae, (shorter "hairlike" structures) that extend from the bacterial cell surface and **help mediate adherence of the bacteria to host cell surfaces.**

- For example, some **E coli strains have type 1 pili, which adhere epithelial cell receptors**

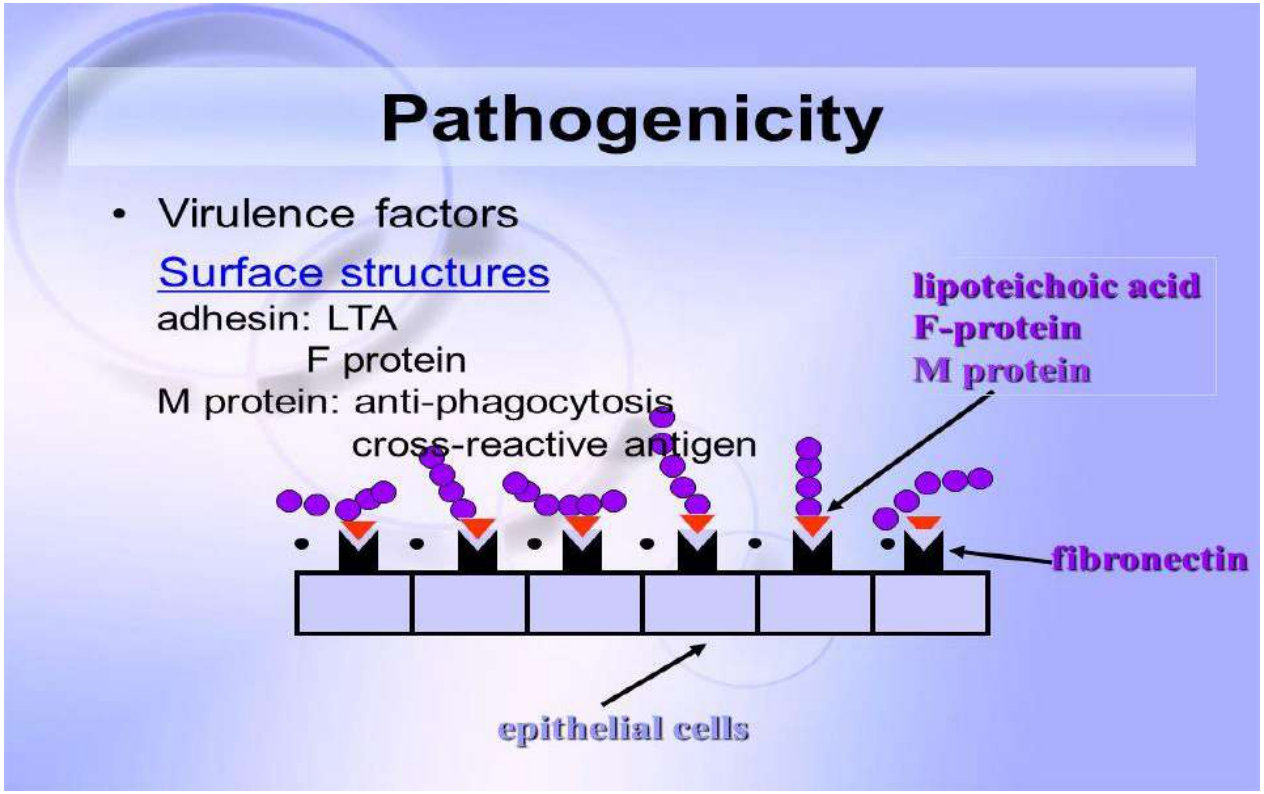


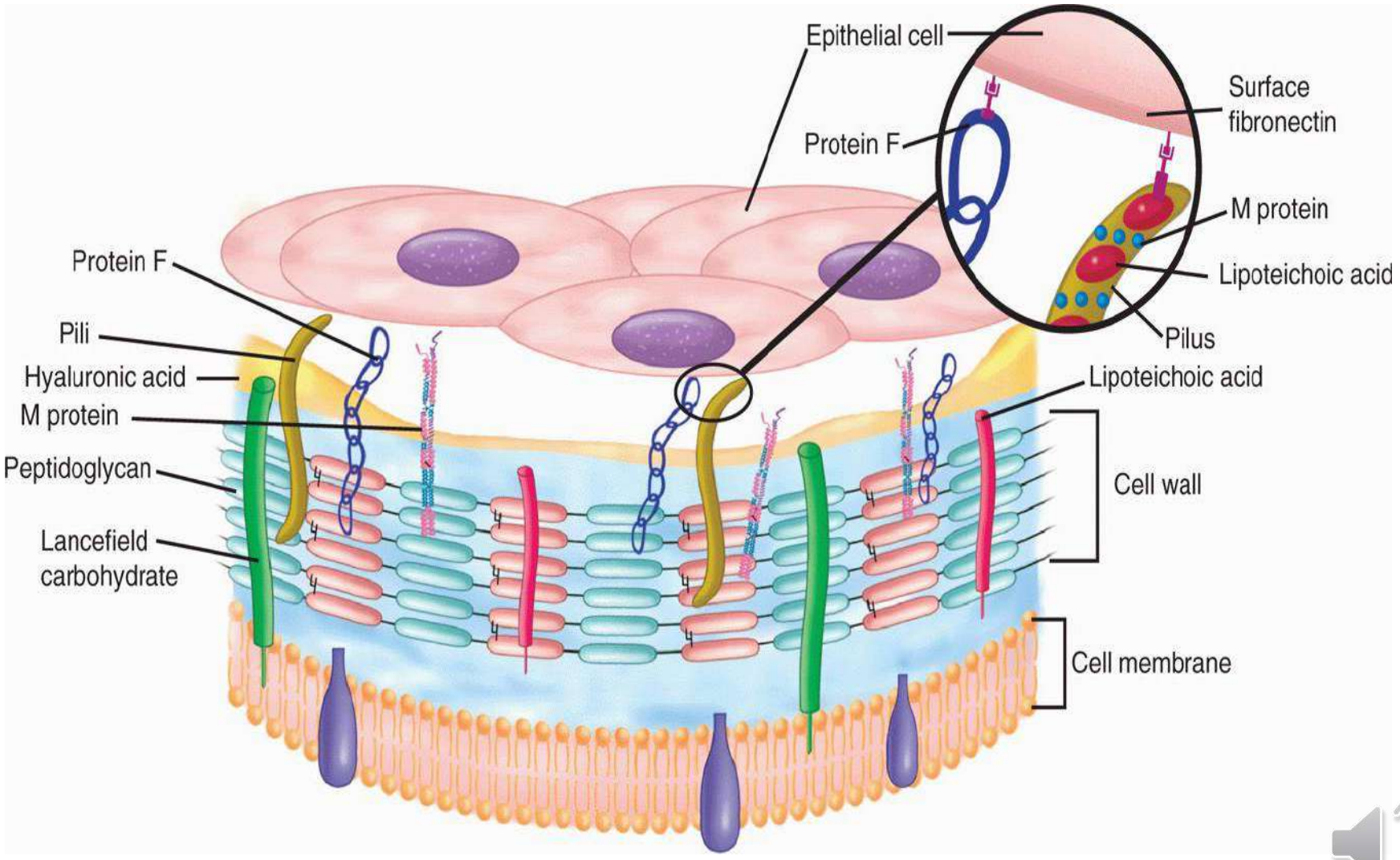
- Bacteria and host cells commonly have net negative surface charges and therefore repulsive electrostatic forces. These forces are overcome by hydrophobic and other more specific interactions between bacteria and host cells.

- In general, **the more hydrophobic the bacterial cell surface, the greater the adherence to the host cell.**
- Different **strains of bacteria within a species may vary widely in their hydrophobic surface properties and ability to adhere to host cells.**

- The E coli that cause diarrheal diseases have pilus (fimbriae)-mediated adherence to intestinal epithelial cells.
- The type of pili and specific molecular mechanisms of adherence appear to be different depending on the form of the E coli that induce the diarrhea.
- Other specific ligand-receptor mechanisms have evolved to promote bacterial adherence to host cells, illustrating the diverse mechanisms used by bacteria.
- Group A streptococci (Streptococcus pyogenes) also have hairlike appendages, termed fimbriae, that extend from the cell surface.

- **Lipoteichoic acid, protein F, and M protein** are found on the fimbriae. The **lipoteichoic acid and protein F cause adherence of the streptococci to buccal epithelial cells;** this adherence is **mediated by fibronectin**, which acts as the host cell receptor molecule. **M protein acts as an antiphagocytic molecule** and is a major virulence factor.




Epithelial cell
Surface fibronectin
Protein F
M protein
Lipoteichoic acid
Pilus
Protein F
Pili
Hyaluronic acid
M protein
Peptidoglycan
Lancefield carbohydrate
Lipoteichoic acid
Cell wall
Cell membrane

- Antibodies that act against the specific bacterial ligands that promote adherence (eg, pili and lipoteichoic acid) can block adherence to host cells and protect the host from infection.
- After adherence occurs, conformational changes in the host cell ensue that can lead to cytoskeletal changes allowing organism uptake by the cell.
- Sometimes changes in the adhesin molecule after attachment may trigger activation of virulence genes that promote invasion or that result in other pathogenic changes

2-Invasion

is the term commonly used to describe **the entry of bacteria into host cells, implying an active role for the organisms and a passive role for the host cells.**

In many infections, the bacteria produce virulence factors that influence the host cells, causing them to engulf (ingest) the bacteria.

- The host cells play a very active role in the process. Toxin production and other virulence properties are generally independent of the ability of bacteria to invade cells and tissues.
- For example, C. diphtheriae is able to invade the epithelium of the nasopharynx and cause symptomatic sore throat even when the C. diphtheriae strains are nontoxigenic.

- Invasion of Host Cells and Tissues For many disease-causing bacteria, invasion of the host's epithelium is central to the infectious process.
- Some bacteria (eg, Salmonella species) invade tissues through the junctions between epithelial cells. Other bacteria (eg, Yersinia species, N gonorrhoeae, Chlamydia trachomatis) invade specific types of the host's epithelial cells and may subsequently enter the tissue.

- When inside the host cell, bacteria may remain enclosed in a vacuole composed of the host cell membrane, or the vacuole membrane may be dissolved and bacteria may be dispersed in the cytoplasm. Some bacteria (eg, Shigella species) multiply within host cells, but other bacteria do not.

Pharmaceutical microbiology

Chapter 2

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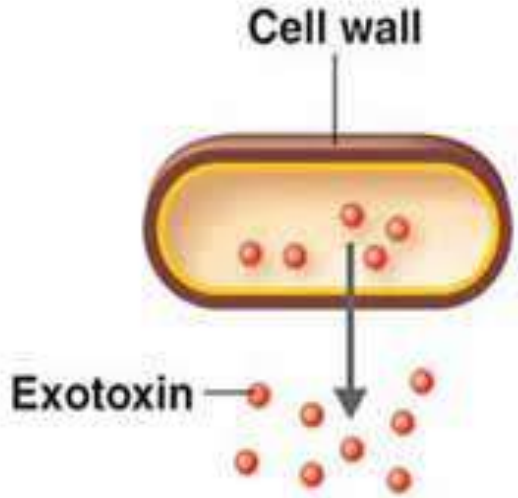
Lecture 5

11/10/2020

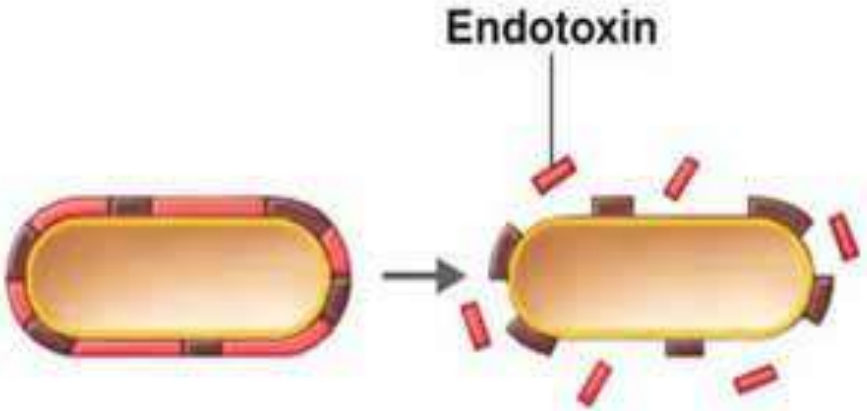
3-Toxins

- Toxins produced by bacteria are generally classified into two groups: **exotoxins and endotoxins.**
- Exotoxins are proteins that are most often excreted from the cell.
- However some exotoxins accumulate inside the cell and are either injected directly into the host or are released by cell lysis.
- Endotoxins are lipid molecules that are components of the bacterial cell membrane.

Differences Between Exotoxins and Endotoxins



(a) Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.



(b) Endotoxins are the lipid portions of lipopolysaccharides (LPSs) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A; see Figure 4.13c). The endotoxins are liberated when the bacteria die and the cell wall breaks apart.

Differences B/W exotoxin and endotoxin

	Exotoxin	Endotoxin
Species	Some species of both Gram-positive and Gram-negative bacteria	Most Gram-negative bacteria and *Listeria*
Protein Location	Proteins secreted from cell	Part of cell (lipopolysaccharide) that fragments off
Gene Location	Genes for exotoxin are in plasmid or bacteriophage	Genes for endotoxin are on bacterial chromosome
Toxicity	High toxicity	Low toxicity
Antigenicity	Highly antigenic (host forms antibodies called antitoxins)	Poorly antigenic
Vaccine	Vaccine available (formed from toxoids)	No vaccine available
Heat Stability	Heat labile	Heat stable
Example	Think cholera, tetanus, botulism	Think meningococcemia, sepsis

A. Exotoxins

- Many gram-positive and gram-negative bacteria produce exotoxins of considerable medical importance. Some of these toxins had major roles in world history.
- For example, tetanus caused by the toxin of *C. tetani* killed as many as 50,000 soldiers of the Axis powers in World War II; the Allied forces, however, immunized military personnel against tetanus, and very few died of that disease.
- Vaccines have been developed for some of the exotoxin-mediated diseases and continue to be important in the prevention of disease. These vaccines—called toxoids—are made from exotoxins, which are modified so that they are no longer toxic.

- Many exotoxins consist of A and B subunits. The B subunit generally **mediates**
- **1- adherence of the toxin complex to a host cell**
- **2- aids entrance of the exotoxin into the host cell.**
- The A subunit provides the toxic activity.
- Examples of some pathogenetic mechanisms associated with exotoxins are given below.

- Some strains of group A β-hemolytic streptococci produce pyrogenic exotoxin A that is similar to or the same as streptococcal erythrogenic toxin, which results in scarlet fever.
- Rapidly progressive soft tissue infection by streptococci that produce the pyrogenic exotoxin A has many clinical manifestations similar to those of staphylococcal toxic shock syndrome.
- The pyrogenic exotoxin A also is a super antigen that acts in a manner similar to TSST-1.

B. Exotoxins Associated with Diarrheal Diseases and Food Poisoning

- Exotoxins associated with diarrheal diseases are frequently called enterotoxins.
- V. cholerae has produced epidemic diarrheal disease (cholera) in many parts of the world.
- After entering the host via contaminated food or drink, V. cholerae penetrates the intestinal mucosa and attaches to microvilli of the brush border of gut epithelial cells.
- *V. cholerae,* usually of the serotype O1 (and O139), can produce an enterotoxin with a MW of 84,000.

- The toxin consists of two subunits—A, which is split into two peptides, A1 and A2, linked by a disulfide bond, and
- B. Subunit has five identical peptides and rapidly binds the toxin to cell membrane ganglioside molecules.
- Subunit A enters the cell membrane and causes a large increase in adenylate cyclase (AC) activity and in the concentration of cAMP.

- The **net effect** is rapid secretion of electrolytes into the small bowel lumen, with impairment of sodium and chloride absorption and loss of bicarbonate.
- Life-threatening massive diarrhea (eg, 20–30 L/day) can occur, and acidosis develops. The deleterious effects of cholera are due to fluid loss and acid–base imbalance;
- **Treatment,** therefore, is by electrolyte and fluid replacement.

- Some strains of *S. aureus* produce enterotoxins while growing in meat, dairy products, or other foods. In typical cases, the food has been recently prepared but not properly refrigerated.
- There are at least seven distinct types of the staphylococcal enterotoxin.

- After the preformed toxin is ingested, it is absorbed in the gut, where it stimulates vagus nerve receptors.
- The stimulus is transmitted to the vomiting center in the central nervous system.
- Vomiting, often projectile, results within hours. Diarrhea is less frequent. Staphylococcal food poisoning is the most common form of food poisoning. *S aureus* enterotoxins are super antigens.

C. LPS of Gram-Negative Bacteria

- **The LPS (endotoxin) of gram-negative bacteria are bacterial cell wall components that are often liberated when the bacteria lyse. The substances are heat-stable, have MWs between 3000 and 5000 (lipooligosaccharides, LOS) and several million (lipopolysaccharides) and can be extracted (eg, with phenol-water). They have three main regions.**
- Hypotension occurs early in gram-negative bacteremia or after injection of LPS.

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Lecture 6

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4- Enzymes

Many species of bacteria produce enzymes that are not intrinsically toxic but do play important roles in the infectious process. Some of these enzymes are discussed below.

- **Many bacteria produce tissue-degrading enzymes.** The best-characterized are **enzymes from *C. perfringens*** ،and, to a lesser extent, **anaerobic bacteria** **S aureus , and group A streptococci** .

- In addition to lecithinase, *C. perfringens* produces the proteolytic enzyme collagenase, which **degrades collagen the major protein of fibrous connective tissue, and promotes spread of infection in tissue.**

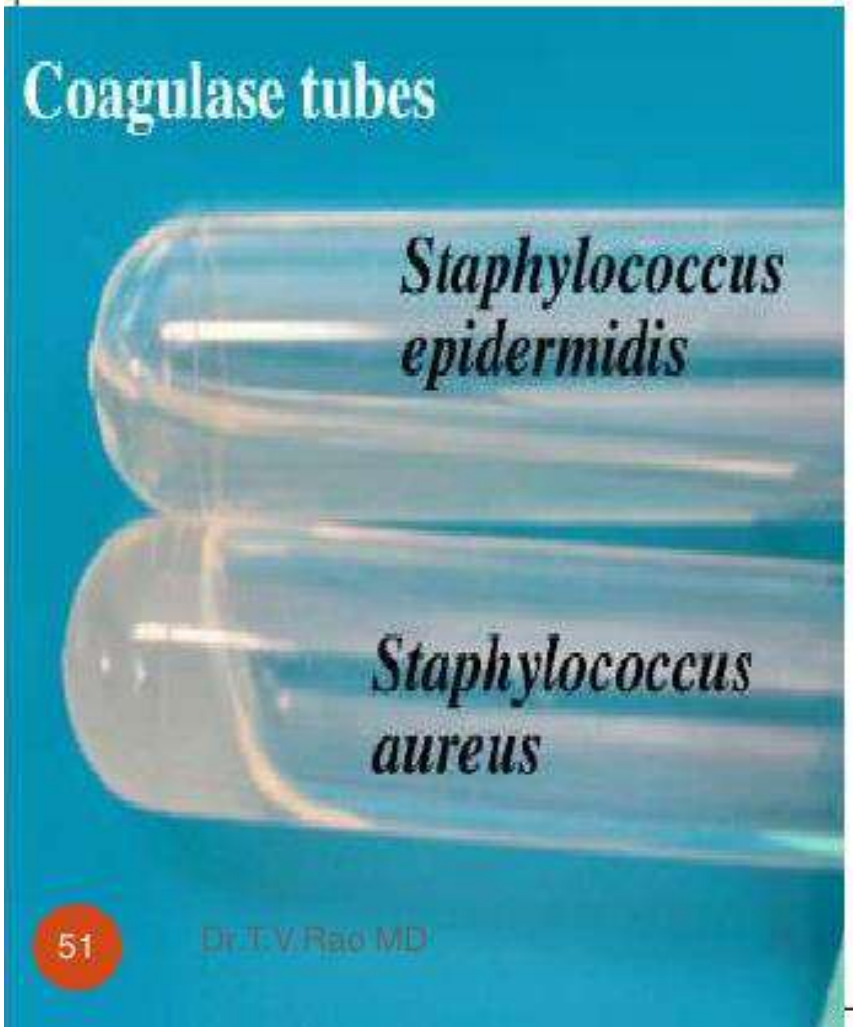
Some Extra Cellular Bacterial Proteins That Act As Invasins:

Invasin	Bacteria Involved	Activity
Hyaluronidase	Streptococci, staphylococci and clostridia	Degrades hyaluronic of connective tissue
Collagenase	*Clostridium*species	Dissolves collagen framework of muscles
Neuraminidase	*Vibrio cholerae*and *Shigella dysenteriae*	Degrades neuraminic acid of intestinal mucosa
Coagulase	Staphylococcus aureus	Converts fibrinogen to fibrin which causes clotting

- S. aureus produces coagulase, which works in conjunction with blood factors to coagulate plasma. Coagulase contributes to the formation of fibrin walls around staphylococcal lesions, which helps them persist in tissues.

- Coagulase also causes deposition of fibrin on the surfaces of individual staphylococci, which may help protect them from phagocytosis or from destruction within phagocytic cells.

Coagulase test

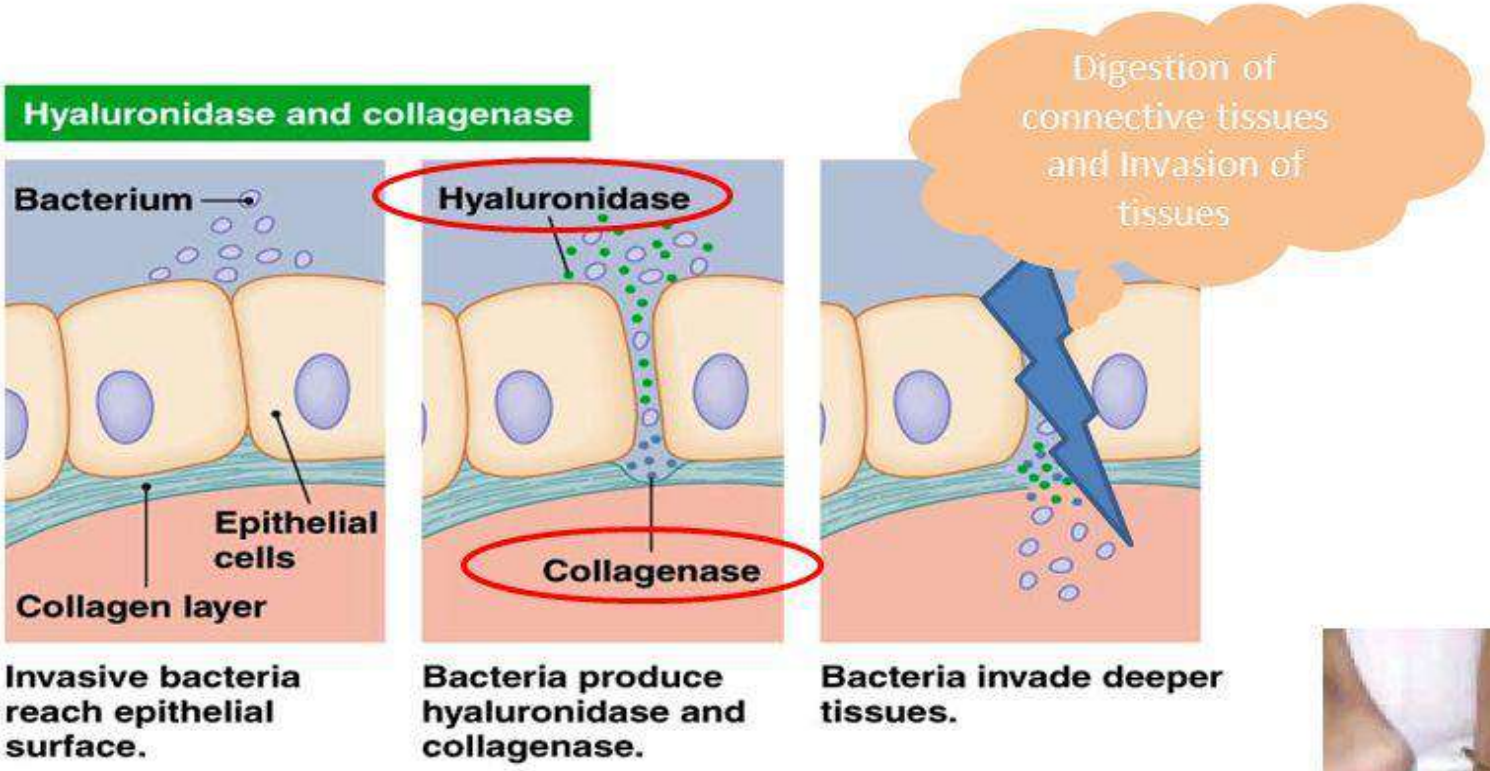


- Coagulase is an enzyme that clots blood plasma by catalyzing the conversion of a soluble protein (fibrinogen) to an insoluble protein (fibrin). This test is performed on Gram-positive, catalase positive species to identify the coagulase positive *Staphylococcus aureus*. Coagulase is a virulence factor of *S. aureus*. The formation of clot around an infection caused by this bacteria likely protects it from phagocytosis.

- **Hyaluronidases** are **enzymes that hydrolyze hyaluronic acid, a constituent of the ground substance of connective tissue.** They are **produced by many bacteria (eg, staphylococci, streptococci, and anaerobes) and aid in their spread through tissues.**

- **Many hemolytic streptococci produce streptokinase (fibrinolysin), a substance that activates a proteolytic enzyme of plasma.** This enzyme is **then able to dissolve coagulated plasma and probably aids in the rapid spread of streptococci through tissues.** **Streptokinase has been used in treatment of acute myocardial infarction to dissolve fibrin clots** .

(b) Hyaluronidase and collagenase



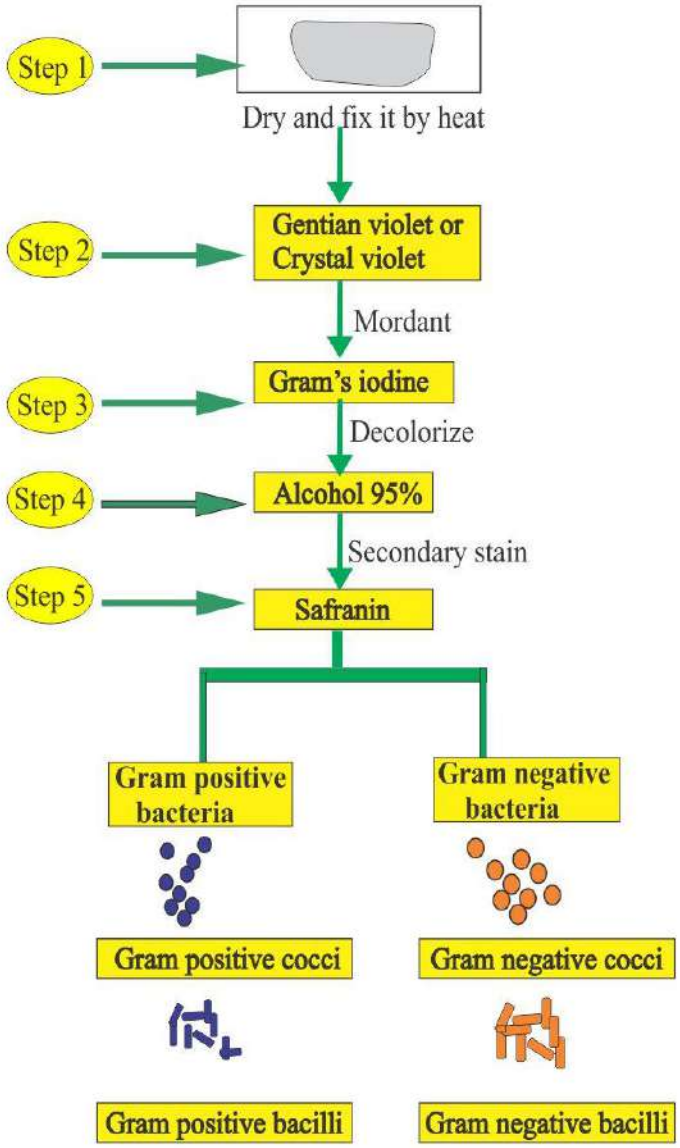
Hyaluronidase: is present in *Staphylococcus aureus* (Skin infections) *and Streptococcus pyogenes (Sore throat)*

Collagenase: is present in Clostridium perfringens (gas gangrene)

Gram stain or Gram staining

- Gram stain or Gram staining, also called Gram's method, is a method of staining used to distinguish and classify bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria. The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique.[1]
- Gram staining differentiates bacteria by the chemical and physical properties of their cell walls. Gram-positive cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain, crystal violet. Gram-negative cells have a thinner peptidoglycan layer that allows the crystal violet to wash out on addition of ethanol.

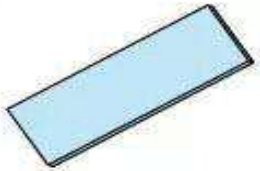
Gram stain



GRAM STAINING

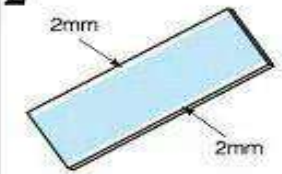
Flow Through Procedure

1



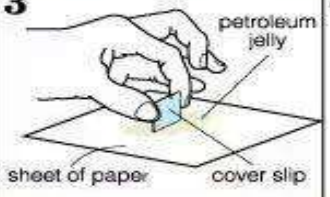
Wipe bottom of biofilm slide clean

2



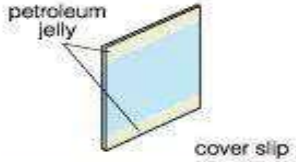
Clean top edges of slide about 2mm

3



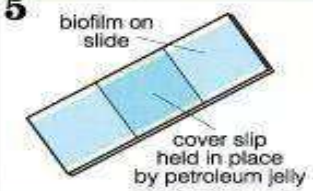
Build up a ridge of petroleum jelly on the top and bottom of a cover slip

4



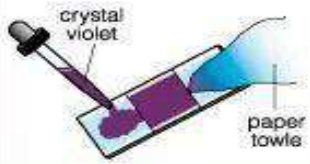
Cover slip with petroleum jelly

5



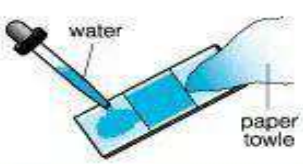
Biofilm on slide with cover slip

6



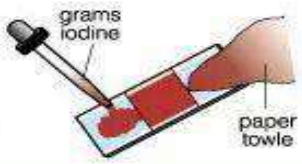
Add crystal violet-wait 30 sec.

7



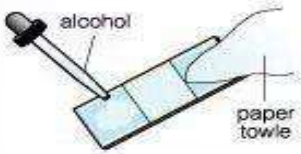
Wash with water

8



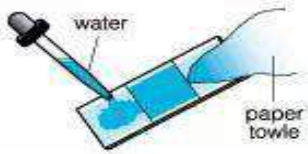
Add Grams Iodinet-wait 1.5 min.

9



Decolorize with alcohol

10

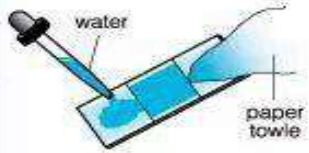


Wash with water

11

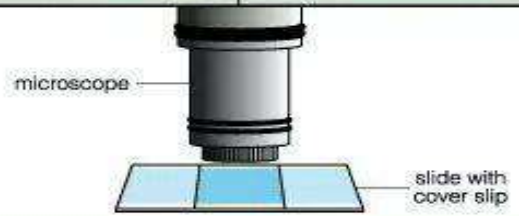
Stain with Safranin dye-wait 30 sec.

12



Wash with water

13



Examine under oil immersion through the cover slip

- They are stained pink or red the counterstain,
- [2] commonly safranin or fuchsine. Lugol's iodine solution is always added after addition of crystal violet to strengthen the bonds of the stain with the cell membrane. Gram staining is almost always the first step in the preliminary identification of a bacterial organism.

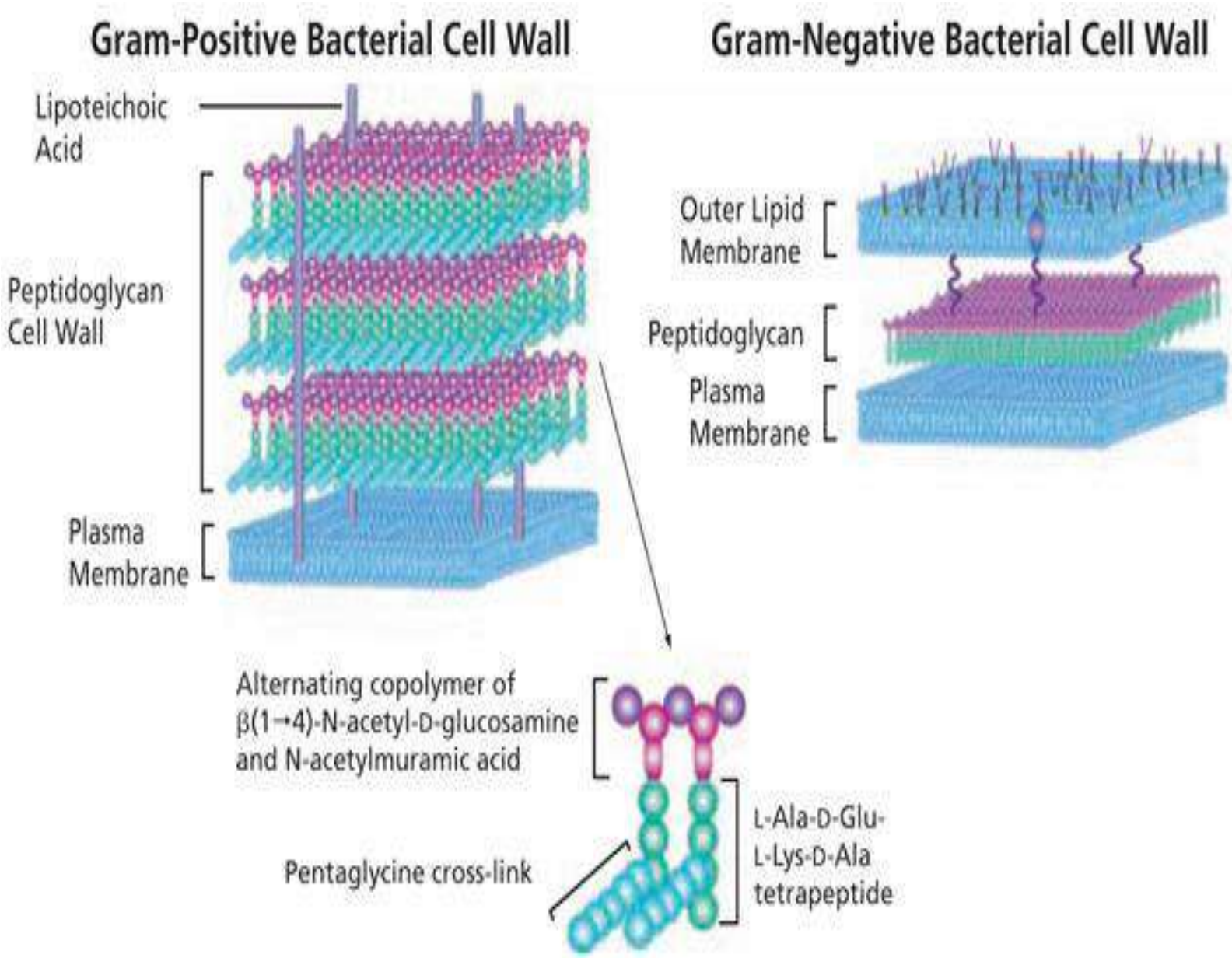
- While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique. This gives rise to gram-variable and gram-indeterminate groups.

- Gram stains are performed on body fluid or biopsy when infection is suspected.

- Gram stains yield results much more quickly than culturing, and are especially important when infection would make an important difference in the patient's treatment and prognosis; examples are cerebrospinal fluid for meningitis and synovial fluid for septic arthritis

Differences in colour B/w G+ve and G-ve

- Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin. There are four basic steps of the Gram stain:


Gram-Positive Bacterial Cell Wall
Gram-Negative Bacterial Cell Wall
Lipoteichoic Acid
Peptidoglycan Cell Wall
Plasma Membrane
Outer Lipid Membrane
Peptidoglycan
Plasma Membrane
Alternating copolymer of β(1→4)-N-acetyl-D-glucosamine and N-acetylmuramic acid
Pentaglycine cross-link
L-Ala-D-Glu-L-Lys-D-Ala tetrapeptide

Steps of staining

- Applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture. Heat fixation kills some bacteria but is mostly used to affix the bacteria to the slide so that they don't rinse out during the staining procedure.
- The addition of iodide, which binds to crystal violet and traps it in the cell
- Rapid decolorization with ethanol or acetone
- Counterstaining with safranin. Carbol fuchsin is sometimes substituted for safranin since it more intensely stains anaerobic bacteria, but it is less commonly used as a counterstain.