PHARMACEUTICAL MICROBIOLOGY and IMMUNOLOGY

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OBJECTIVES

- Bacterial Cell Structure
- Differences Between Gram Positive and Gram Negative Bacteria

Bacterial cell structure

- Bacteria are prokaryotic, single-celled, microscopic organisms.
- Bacterial cells vary in size depending on the species (1000-2000 nm in diameter)
- Even though bacteria are single-celled organisms, they are able to communicate with one another through a process called **quorum sensing**

Bacterial cell structure

- Bacteria display a wide diversity of shapes and sizes
- Most bacterial species are either spherical, called **cocci** (single:coccus) or rod-shaped, called **bacilli** (single:bacillus)
- Some bacteria, called **vibrio**, are shaped like slightly curved rods or comma-shaped
- Some of them are spiral-shaped, called **spirilla**, or tightly coiled, called **spirochaetes**.

Bacterial cell structure

Diplococcus: cocci arranged in pairs

Streptococcus: cocci arranged in chains

Tetrad: cocci arranged in squares of four

Sarcina: cocci in arranged cubes of eight

Staphylococcus: cocci arranged in irregular, often grape-like clusters

Bacterial cell structure

Bacillus: single bacilli

Streptobacillus: bacilli arranged in chains

Coccobacillus: oval and similar to a coccus

Vibrio: a curved or comma-shaped rod

Spirillum: a thick, rigid spiral

Spirochete: a thin, flexible spiral


Movie
time

Bacterial cell structure

Nucleoid:

- Bacteria do not have a membrane-bound nucleus
- The bacterial chromosome exists as a singular, covalently closed **circular molecule of double-stranded DNA**
- It is complexed with small amounts of proteins and RNA, but unlike eukaryotic DNA, is not associated with histones

Bacterial cell structure

Cytoplasm:

- The cytoplasm consists of approximately 80% water and contains enzymes that generate ATP directly by oxidizing glucose and other carbon sources
- It also contains some of the enzymes involved in the synthesis of peptidoglycan subunits
- Ribosomes, the DNA genome (nucleoid) and inclusion granules are also found in the cytoplasm

Bacterial cell structure

Ribosomes:

- Ribosomes are the sites in a cell in which protein synthesis takes place
- Bacterial ribosomes have a sedimentation rate of **70S** (measured in Svedberg units): their subunits have rates of **30S** and **50S**.
- Some antibiotics bind specifically to 70S ribosomes and inhibit bacterial protein synthesis. Those antibiotics kill bacteria without affecting the larger 80S ribosomes of eukaryotic cells and without harming the host

Bacterial cell structure

Plasmids:

- Plasmids are relatively small, circular pieces of double-stranded **extrachromosomal DNA**
- They are capable of autonomous replication and encode for many auxiliary functions that are not usually necessary for bacterial growth. For example; antibiotic resistance
- Plasmids may also transfer readily from one microorganism to another, and between species, thereby increasing the spread of resistance

Bacterial cell structure

Inclusion Granules:

- Bacteria occasionally contain inclusion granules within their cytoplasm
- These consist of storage material composed of carbon, nitrogen, sulphur or phosphorus
- Inclusion granules act as repositories of these nutrients when limitations occur
- For example; poly-β-hydroxybutyrate, glycogen and polyphosphate granules

Bacterial cell structure

Cell Envelope:

- Bacterial cells are covered by a **cell envelope** that is composed of a **cell membrane** and a **cell wall**
- The cell membrane is a phospholipid bilayer that regulates the transport of molecules into and out of the cell. This is the weak structure that would burst from the osmotic pressure without reinforcement
- The cell wall is the component of the envelope that provides that reinforcement.

Bacterial cell structure

Cytoplasmic Membrane:

- The cytoplasmic membrane is a fragile, phospholipid bilayer with proteins distributed randomly throughout
- These are involved in the various transport and enzyme functions associated with the membrane
- The cytoplasmic membrane serves many functions, including transport of nutrients, energy generation and electron transport; it is the location for regulatory proteins and biosynthetic proteins, and it acts as a semi-permeable selectivity barrier between the cytoplasm and the cell environment.

Bacterial cell structure

Cytoplasmic Membrane:

- Invaginations of the cytoplasmic membrane are referred to as **mesosomes**. Respiratory enzymes are located in mesosomes. Mesosome helps to synthesize and divide the DNA during bacterial cell division, or replication

- A major difference between prokaryotic and eukaryotic cells is that eukaryotes have sterols in their membranes (e.g. cholesterol) whereas prokaryotes do not

Bacterial cell structure

Cell Wall:

- The bacterial cell wall is an extremely important structure, being essential for the maintenance of the shape and integrity of the bacterial cell

- The primary function of the cell wall is to provide a strong, rigid structural component that can withstand the osmotic pressures caused by high chemical concentrations of inorganic ions in the cell

Bacterial cell structure

Cell Wall:

- Most bacterial cell walls have in common a unique structural component called peptidoglycan (also called murein); exceptions include the mycoplasmas.

- Peptidoglycan is a large macromolecule containing glycan (polysaccharide) chains that are cross-linked by short peptide bridges.

Bacterial cell structure

Cell Wall:

- The glycan chain acts as a backbone to peptidoglycan, and is composed of alternating residues of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG). They are connected by inter-peptide bridges.

- Bacteria can be divided into two large groups, on the basis of a differential staining method called Gram stain

Bacterial cell structure

Gram-positive Cell Wall:

- The cell walls of Gram-positive bacteria are quite **thick** and consists of between 60% and 80% **peptidoglycan**
- Gram-positive walls frequently contain acidic polysaccharides called **teichoic acids**
- In some Gram-positive bacteria **glycerol-teichoic acids** are bound to **membrane lipids** and termed **lipoteichoic acids**. During an infection, lipoteichoic acid molecules released by killed bacteria trigger an inflammatory response

Bacterial cell structure

Gram-negative Cell Wall:

- Gram-negative cell walls contain less peptidoglycan and a second membrane structure is found outside the peptidoglycan layer
- This outer membrane is composed of proteins, lipoproteins, phospholipids and lipopolysaccharide (LPS)
- LPS is an important molecule because it determines the antigenicity of the Gram-negative cell and it is extremely toxic to animal cells

Bacterial cell structure

Gram-negative Cell Wall:

- LPS molecule consists of three regions, namely Lipid A, core polysaccharide and O-specific polysaccharide (O-antigen).
- Lipid A is a lipid component of an endotoxin held responsible for the toxicity of Gram-negative bacteria.
- Lipid A is also responsible for the pyrogenic properties of Gram-negative bacteria (pyrogenic:producing heat, especially in the body; producing fever)

Bacterial cell structure

Gram-negative Cell Wall:

- Somatic O-polysaccharide antigens are detected by agglutination with specific antibodies. (agglutination is the clumping of particles)

- Although the outer membrane is relatively permeable to small molecules, it is not permeable to enzymes or large molecules.

Bacterial cell structure

Gram-negative Cell Wall:

- Indeed, one of the major functions of the outer membrane may be to keep certain enzymes that are present outside the cytoplasmic membrane from diffusing away from the cell.

- Moreover, the outer membrane is not readily penetrated by hydrophobic compounds and is, therefore, resistant to dissolution by detergents

Bacterial cell structure

Cell Wall:

- The region between the outer surface of the cytoplasmic membrane and the inner surface of the outer membrane is called the **periplasm**.

- Periplasm contains sugars, proteins, hydrolytic enzymes and transport proteins

Bacterial cell structure

Gram Stain:

- Essentially, the Gram stain consists of treating a film of bacteria dried on a microscope slide with a solution of crystal violet, followed by a solution of iodine; these are then washed with an alcohol solution

- After these procedure, in Gram-negative bacteria the cells lose the crystal violet-iodine complex and are rendered colorless, whereas Gram-positive cells retain the dye.

Bacterial cell structure

Gram Stain:

- Counterstaining with safranin is the last stage of this technique.
- Hence, under the light microscope Gram-negative cells appear pink while Gram-positive cells are purple
- These marked differences in response reflect differences in cell wall structure
- The Gram-positive cell wall consists primarily of a single type of molecule whereas the Gram-negative cell wall is a multilayered structure and quite complex


Movie
time

Bacterial cell structure

Gram-positive bacteria display these characteristics:

- **thick** peptidoglycan layer
- **teichoic acids** and lipoids are present, forming **lipoteichoic acids**, which serve as chelating agents, and also for certain types of adherence
- a much **smaller** volume of **periplasm** than that in Gram-negative bacteria

Bacterial cell structure

Gram-negative bacteria display these characteristics:

- a **thin** peptidoglycan layer is present
- has **outer membrane** containing **lipopolysaccharides** (LPS, which consists of **lipid A**, core polysaccharide, and **O antigen**) in its outer leaflet and phospholipids in the inner leaflet
- **porins** exist in the outer membrane, which act like pores for particular molecules

Bacterial cell structure

Gram-negative bacteria display these characteristics

- the cell wall of Gram-negative bacteria consists of a thin layer of peptidoglycan in the **periplasmic space** between the inner and outer lipid membranes
- between the outer membrane and the cytoplasmic membrane there is a space filled with a concentrated gel-like substance called **periplasm**
- if present, **flagella have four supporting rings** instead of two

Bacterial cell structure

- Lysozyme is a special enzyme found in **tears, saliva, sweat, and other body fluids**
- Lysozyme is capable of breaking the chemical bonds in the outer cell wall of the bacteria. Bacterial cell walls contain a layer of **peptidoglycan**, which is the specific site that lysozyme targets
- It destroys bacteria that attempt to enter our body through these passageways. It plays an important role in the prevention of bacterial infections

Bacterial cell structure

- When a Gram positive bacteria is exposed to lysozyme (or penicillin which blocks the peptidoglycan synthesis) will degrade the peptidoglycan layer resulting to complete removal of the cell wall producing a osmotically fragile wall-less spherical body called **Protoplast**

- When a Gram negative bacteria is exposed to lysozyme (or penicillin which blocks the peptidoglycan synthesis) it looses the peptidoglycan layer but retain the outer membrane leaving a less fragile spherical body called **Spheroplast**

Bacterial cell structure

- Cell wall of Gram positive bacteria is easily destroyed by the action of lysozyme. After digestion of peptidoglycan layer, Gram positive bacteria become **protoplast**

- Gram negative bacteria are refractory to lysozyme, because large protein molecule can't penetrate the LPS layer. After digestion of peptidoglycan layer, Gram negative bacteria become **spheroplast**

- If such cells are able to grow and divide, they are called **L forms.** L-forms are "**cell wall-deficient**" bacteria. L-form bacteria form **'fried egg-shaped'** colonies

Bacterial cell structure

- Some bacteria have an additional layer outside of the cell wall called the **glycocalyx**. This coating of macromolecules protects the cell and helps it adhere to surfaces

- A distinct, gelatinous glycocalyx is called a **capsule**, whereas an irregular, diffuse layer is called a **slime layer**

Bacterial cell structure

Capsule:

- Many bacteria secrete extracellular polysaccharides that are associated with the exterior of the bacterial cell. Not all bacterial species produce capsules. Capsule is located at outside of the cell wall

- It usually consists of **2% polysaccharides** and **98% water**, but can be composed of other materials (e.g., polypeptide (D-glutamic acid) in *Bacillus anthracis*)

- K antigen (capsular antigen)

Bacterial cell structure

Capsule:

- The capsule is considered a virulence factor because it enhances the ability of bacteria to cause disease (e.g. prevents phagocytosis)
- The capsule can protect cells from engulfment by eukaryotic cells, such as macrophages
- Capsules also help cells adhere to surfaces
- Capsules also contain water which protects the bacteria against desiccation

Bacterial cell structure

Flagella:

- Bacterial motility is commonly provided by flagella, long, helical shaped structures that project from the surface of the cell
- The filament of the flagellum is built up from multiple copies of the protein **flagellin**
- Where the filament enters the surface of the bacterium, there is a hook in the flagellum, which is attached to the cell surface by a series of complex proteins called the flagellar motor

Bacterial cell structure

Flagella:

- This rotates the flagellum, causing the bacterium to move through the environment
- **H antigen**: the antigen that occurs in the flagella of motile bacteria
- The numbers and distribution of flagella vary with bacterial species
- Some have a single, polar flagellum, whereas others are flagellate over their entire surface (peritrichous); intermediate forms also exist

Bacterial cell structure

Flagella:

Monotrichous bacteria have a single flagellum (e.g., *Vibrio cholerae*)

Lophotrichous bacteria have multiple flagella located at the same spot on the bacterial surfaces which act in concert to drive the bacteria in a single direction

Bacterial cell structure

Flagella:

Amphitrichous bacteria have a single flagellum on each of two opposite ends (only one flagellum operates at a time, allowing the bacterium to reverse course rapidly by switching which flagellum is active)

Peritrichous bacteria have flagella projecting in all directions (e.g., *E. coli*)

Bacterial cell structure

Flagella:

- Each flagellum consists of three distinct parts:

  - Filament – Hook - Basal Body.

- The filament lies external to the cell
- Hook is embedded in the cell envelope
- Basal Body is attached to the cytoplasmic membrane by ring-like structures

Bacterial cell structure

Flagella:

- Gram-positive bacteria have two basal body rings, one in the peptidoglycan layer and one in the plasma membrane.

- Gram-negative bacteria have four such rings: the L ring associates with the lipopolysaccharides, the P ring associates with peptidoglycan layer, the M ring is embedded in the plasma membrane, and the S ring is directly attached to the plasma membrane.

Bacterial cell structure

Fimbriae:

(singular) fimbria/(plural)fimbriae

- Fimbriae are structurally similar to flagella, but are not involved in motility
- They are straighter, more numerous and considerably thinner and shorter than flagella
- They are composed of fimbrillin protein

Bacterial cell structure

Fimbriae:

- Fimbriae are used by bacteria to adhere to one another and to adhere to animal cells and some inanimate objects

- Fimbriae act primarily as adhesins, allowing bacteria to attach to surfaces, including animal tissues in the case of some pathogenic bacteria, and to initiate biofilm formation

Bacterial cell structure

- **Adhesins** are cell-surface components or appendages of bacteria that facilitate adhesion or adherence to other cells or to surfaces, usually the host they are infecting or living in

- **Biofilm** is any group of microorganisms in which cells stick to each other and often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed of extracellular polymeric substances

Bacterial cell structure

Pili:

(singular) pilus/(plural)pili

- Pili are morphologically and chemically similar to fimbriae, but they are present in much smaller numbers (<10) and are usually longer.
- Proteins of pili are referred to as pilins
- Sex pilus holds two bacterial cells together and allows DNA to be transferred between them in a process called conjugation.