CHAPTER 9
THE CLINICAL SIGNIFICANCE OF BACTERIAL ANATOMY

WHY IS THIS IMPORTANT?

- Bacterial structures play a significant role in the five steps required for infection.

OVERVIEW
BACTERIAL CELLS

- Many of the structures of the bacterial cell play a role in the infectious disease process.
- Infection is a two way street.
  - The body has defenses.
  - The pathogen has structures to thwart those defenses.
- Mutations allow pathogens to evolve structures that can help with the requirements for infection.

THE BACTERIAL CELL WALL

- The bacterial cell wall is a protective barrier against:
  - Osmotic pressure changes
  - Other environmental stresses
- The cell wall is a complex meshwork structure composed of several parts.
- The cell wall is different in Gram-positive and Gram-negative bacteria.
THE BACTERIAL CELL WALL

- The primary structure of the cell wall is peptidoglycan which is composed of repeating sugar molecules.
  - N-acetyl glucosamine (NAG)
  - N-acetyl muramic acid (NAM)
- The meshwork is held together with small peptide chains.

BUILDING THE BACTERIAL CELL WALL

- The linking together of NAG and NAM subunits is facilitated by several enzymes.
- Cell wall construction mechanisms are targets for antibiotics.
BUILDING THE BACTERIAL CELL WALL

- There are three phases of peptidoglycan assembly of a new wall:
  - Cytoplasmic phase
  - Membrane-associated phase
  - Extra-cytoplasmic phase

BUILDING THE BACTERIAL CELL WALL: Cytoplasmic Phase

- NAG and NAM building-blocks are formed in the cytoplasm of the cell.
- The enzymes coded for by these genes attach 5 amino acids to each molecule of NAG and NAM.
- The cytoplasmic phase is an important target for antibiotics.
  - Antibiotics prevent the formation of the peptidoglycan subunits.

BUILDING THE BACTERIAL CELL WALL: Membrane Associated Phase

- Specific enzymes link the NAG and NAM subunits with the lipid portion of the bacterial cell plasma membrane.
- The first step in the cycle is the formation of a bond between peptidoglycan and the side of the plasma membrane facing the cytoplasm.
BUILDING THE BACTERIAL CELL WALL: The Extra-Cytoplasmic Phase

- Subunits are then moved from one side of the membrane to the other.
  - This is done by membrane associated enzymes.
  - It allows new components to be integrated into the growing wall.
  - It is an important target for antibiotics.

The last step in the formation of the meshwork is the binding together of the peptidoglycan layers.
- This is done with polypeptide chains.
- These connections give the wall many layers and an increased strength.
ADDITIONAL CELL WALL COMPONENTS

- Gram-positive bacteria have a more complex cell wall than Gram-negative bacteria.
- Gram-positive and Gram-negative cell walls have different additional components.
  - The Gram-positive cell wall is rich in peptidoglycan with multiple layers of meshwork.
  - The Gram-negative cell wall contains very little peptidoglycan.

ADDITIONAL CELL WALL COMPONENTS: Gram-Positive Bacteria

- In addition to many layers of peptidoglycan, the cell wall of Gram-positive bacterial cells also contain:
  - Teichoic acid
  - M protein
  - Mycolic acid.

ADDITIONAL CELL WALL COMPONENTS: Gram-Positive Bacteria

- Teichoic Acid
  - There are two forms
    - Wall teichoic acids – go part way through the wall.
    - Lipoteichoic acids – go completely through the wall and link to the plasma membrane.
  - Both forms protrude above the wall, which gives the bacterial cell a negative charge.
ADDITIONAL CELL WALL COMPONENTS: Gram-Positive Bacteria

- **M Protein**
  - This is a virulence factor.
  - It protrudes from the cell wall.
  - It is required for infection.
  - It is highly susceptible to mutations.

ADDITIONAL CELL WALL COMPONENTS: Gram-Positive Bacteria

- **Mycolic Acid**
  - This is found in the *Mycobacterium* species.
  - It consists of a waxy lipid incorporated into the cell wall.
  - It makes cells extremely resistant to environmental stress.
  - It acts as a barrier against antibiotics and host defenses.

ADDITIONAL CELL WALL COMPONENTS: Gram-Negative Bacteria

- The Gram-negative cell wall is more complex than the Gram-positive cell wall.
- Gram-negative bacteria only have a thin peptidoglycan layer.
  - They also have an outer membrane, known as the lipopolysaccharide layer (LPS).
- The LPS layer is composed of lipids, proteins, and polysaccharides.
- Lipoprotein molecules fasten the outer membrane to the peptidoglycan layer.
The outer membrane of Gram-negative bacteria has a unique outer layer.

- It is composed of lipopolysaccharides instead of the standard phospholipid molecules.
- It serves as a major barrier to the outside world for the Gram-negative cell.

It contains specialized proteins called porin proteins:

- These form a channel through the outer layer.
- This channel is responsible for passage of molecules and ions into and out of the Gram-negative cell.

The outer membrane also contains translocation protein systems.

- Some are found in the outer layer of the membrane and break down nutrients for transport.
- Some are found in the periplasmic space (the space between the outer membrane and the cell wall) and move substances out of the cell.
ADDITIONAL CELL WALL COMPONENTS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Gram-Positive Bacteria</th>
<th>Gram-Negative Bacteria</th>
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</thead>
<tbody>
<tr>
<td>Peptidoglycan</td>
<td>Thick layer</td>
<td>Thin layer</td>
</tr>
<tr>
<td>Teichoic acid</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Lipids</td>
<td>Very little</td>
<td>Lipopolysaccharide layer</td>
</tr>
<tr>
<td>Outer membrane</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Toxins</td>
<td>Exotoxins</td>
<td>Endotoxins</td>
</tr>
<tr>
<td>Sensitivity to antibiotics</td>
<td>Very sensitive</td>
<td>Moderately sensitive</td>
</tr>
</tbody>
</table>

CLINICAL SIGNIFICANCE OF THE BACTERIAL CELL WALL: Gram-positive bacteria

- Teichoic acid can cause colonization of the nasal epithelium.
- Lipoteichoic acid causes an inflammatory response.

CLINICAL SIGNIFICANCE OF THE BACTERIAL CELL WALL: Gram-Negative Bacteria

- The gram-negative cell wall is a complex structure involved in infection in many ways.
  - The outer layer is a barrier to antiseptics, disinfectants, and antibiotics.
  - Porin proteins exclude large molecules.
- The outer layer functions as endotoxin, with two parts having clinical relevance
  - Lipid A
  - O polysaccharide
CLINICAL SIGNIFICANCE OF THE BACTERIAL CELL WALL: Gram-Negative Bacteria

- Lipid A:
  - Anchors the lipopolysaccharide part of the outer layer
  - Is released when the cell dies.

- O Polysaccharides are:
  - Carbohydrate chains that are part of the outer layer
  - Variable from one bacterial species to another
  - Recognized by the adaptive immune response
  - Sometimes used as a diagnostic marker - *E. coli* O157: H7 is designated by O polysaccharide 157.

STRUCTURES OUTSIDE THE BACTERIAL CELL WALL

- There are five structures that can be found outside the cell wall.
- No bacterium has all five of these structures.
**STRUCTURES OUTSIDE THE BACTERIAL CELL WALL**

- Three structures are involved primarily with adherence (staying in)
  - Glycocalyx
  - Fimbriae
  - Pili

**THE GLYCOCALYX**

- The glycocalyx is a sticky substance composed of polypeptides, polysaccharides or both.
- It is produced in the cytoplasm and secreted to the outer part of the cell wall.
- It provides a protective element against environmental stress.
- It can be used for nutrition.

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**STRUCTURES OUTSIDE THE BACTERIAL CELL WALL**

- Two structures are involved primarily with motility (defeating host defenses and damaging the host)
  - Flagella
  - Axial filament
THE GLYCOCALYX

- If loosely attached to the wall, it is called a slime layer.
- If adhered tightly to the wall, it is called a capsule.
- Both variations give an adherence capability to the organism.

CLINICAL SIGNIFICANCE OF THE GLYCOCALYX

- Adherence is a major part of infection (staying in), especially in respiratory and urinary tract infections.
- The glycocalyx is a primary factor of adherence.

CLINICAL SIGNIFICANCE OF THE GLYCOCALYX

- The slime layer form is associated with some forms of dental decay.
- The capsule form:
  - Inhibits phagocytosis
  - Many organisms are not infectious without a capsule
  - Capsule genes can be transferred between organisms.
FIMBRIAE AND PILI

- Both are involved in adherence.
- Both appear as sticky projections which are shorter than flagella.
- They are found on Gram-negative organisms.
- Both are composed of pilin protein subunits.
- Genes for these can be transferred from one organism to another.
CLINICAL SIGNIFICANCE OF FIMBRIAE AND PILI

- Fimbriae and pili help pathogens fulfill the staying in requirement through adherence.
- Fimbriae are only used for adherence, particularly in the urinary and intestinal tracts.

CLINICAL SIGNIFICANCE OF FIMBRIAE AND PILI

- Pili can give bacteria motility in two ways
  - Twitching or gliding movements
  - Through extension and retraction of the pili.
- Pili are involved in the development of biofilms.

CLINICAL SIGNIFICANCE OF FIMBRIAE AND PILI

- They are a potential target for antibiotics.
- Pili use antigenic variation to change or mask their structure and avoid host defenses.
- They can form fragments (S pili) that bind antibody molecules and inactivate them.
CLINICAL SIGNIFICANCE OF FIMBRIAE AND PILI

- Pili are also clinically important because they facilitate the transfer of genetic material from one bacterial cell to another.
- Genes that have been transferred can be used for:
  - Antibiotic resistance
  - Toxin production
  - Defense against host defenses

AXIAL FILAMENTS

- Axial filaments are flagellum-like structures that are found on spirochetes.
- They wrap around the bacterial cell and are confined to the space between the plasma membrane and cell wall.
- They are used for motility and cause the entire organism to rotate like a corkscrew.

CLINICAL SIGNIFICANCE OF AXIAL FILAMENTS

- The corkscrew motion gives the bacterium the ability to bore through tissue (staying in).
- They allow organisms to get into the blood as well as other tissues (damage the host).
Flagella

- Flagella are used only for motility.
- They are long structures that extend far beyond the cell wall and even beyond the glycocalyx.
- They make it possible for bacteria to move from their point of origin to other places in the body.
  - *E. coli* can move from the large intestine, where it is harmless, to the urinary tract, where it can cause serious infection.

Structure of the Bacterial Flagella

- A flagella consists of the following three parts:
  - Filament
  - Hook
  - Basal body
STRUCTURE OF THE BACTERIAL FLAGELLA: Filament

- The filament is made of molecules of flagellin protein.
- They join to form a twisted helical chain of subunits.
- This gives the flagellum a hollow core.
- It is only seen in bacilli.
- It is flexible.

STRUCTURE OF THE BACTERIAL FLAGELLA: Hook

- The hook links the flexible filaments to the basal body.
STRUCTURE OF THE BACTERIAL FLAGELLA: Basal Body

- It is composed of paired ring structures that anchor the flagella to the cell.
- There are two different arrangements:
  - Gram-positive – uses only one pair of ringed structures fastened to the plasma membrane
  - Gram-negative – uses two pairs of ringed structures: one is fastened to the plasma membrane and one to the outer layer

FLAGELLA CONFIGURATIONS

- Monotrichous – one flagellum located at the end of the cell
- Amphitrichous – two flagella, one at each end of the cell
- Lophotrichous – two or more flagella located at the same end of the cell
- Peritrichous – flagella surround the entire cell.
CLINICAL SIGNIFICANCE OF THE FLAGELLA

- The flagella allow movement which allows:
  - Opportunistic infections
  - Escape from host defense (defeat of host defense)
  - Systemic infection (damage to the host)

STRUCTURES INSIDE THE BACTERIAL CELL WALL

- There are six major structures found inside the bacterial cell wall:
  - Plasma membrane
  - Nuclear region
  - Plasmids
  - Ribosomes
  - Inclusion bodies
  - Endospores

PLASMA MEMBRANE

- It is a delicate, flexible structure.
- It surrounds the internal cellular matrix and organelles.
- It provides a barrier between the inside and the outside of the cell.
PLASMA MEMBRANE

- It is involved in:
  - DNA replication
  - Generation of energy
  - Transport and secretion

PLASMA MEMBRANE: Structure

- The plasma membrane is a phospholipid bilayer.
  - Phospholipids in the two layers are arranged tail-to-tail.
- The head of a phospholipid is hydrophilic – loves water.
  - This region interfaces with water.
- The tail of a phospholipid is hydrophobic – hates water.
  - This region does not interface with water.
PLASMA MEMBRANE: Structure

- Proteins float in the plasma membrane.
- There are two basic types of membrane protein:
  - Peripheral proteins – found on either side of the membrane
  - Integral proteins – penetrate the membrane completely
    - Some form pores in the membrane.

ENERGY PRODUCTION AT THE PLASMA MEMBRANE

- ATP production occurs at the plasma membrane.
- The proteins associated with electron transport are located in the plasma membrane.

TYPES OF MEMBRANE TRANSPORT

- The plasma membrane regulates what enters the cell cytoplasm and what does not.
- There are three types of membrane transport:
  - Osmosis
  - Passive transport
  - Active transport.
OSMOSIS

- In osmosis, water chases the concentration of solutes.
  - If the solute concentration is greater outside the cell:
    - It allows water to leave the cell and results in plasmolysis (cell shrinks)
  - If the solute concentration is greater inside the cell:
    - It allows water to enter the cell and results in osmotic lysis (cell bursts).

OSMOSIS

- Cells that are placed in a hypotonic solution will undergo osmotic lysis.
- Cells that are placed in hypertonic solution will undergo plasmolysis.
- In an isotonic solution, the concentration is the same inside and outside the cell
  - This results in no change.

PASSIVE TRANSPORT

- There are two types of passive transport
  - Simple diffusion
  - Facilitated diffusion
PASSIVE TRANSPORT: Simple Diffusion

- Simple diffusion does not require ATP.
- It is based on the development of concentration gradients.
- Solutes move from regions of higher concentration to regions of lower concentration.

PASSIVE TRANSPORT: Simple Diffusion

- The higher the concentration gradient between two regions, the faster diffusion occurs.
- Diffusion slows down as equilibrium is reached.
- Simple diffusion only occurs with:
  - Lipid soluble molecules
  - Molecules small enough to pass through the membrane.

PASSIVE TRANSPORT: Facilitated Diffusion

- Facilitated diffusion does not require ATP.
- Molecules are brought across the plasma membrane by carrier proteins called permease proteins.
- Permeases achieve this by changing their three-dimensional shape.
- Molecules too large to fit into a permease are chopped into smaller pieces by enzymes secreted by the cell.
**ACTIVE TRANSPORT**

- Active transport requires ATP.
- Solutes are carried either into or out of a cell against the concentration gradient.
- It is the most common form of membrane transport.

**ACTIVE TRANSPORT**

- There are three types of active transport:
  - Efflux pumping
  - ABC transport systems
  - Group translocation
ACTIVE TRANSPORT: Efflux Pumping

- Efflux pumping involves proteins called the super family of transporters.
- It employs a revolving door mechanism - pumps bring in certain molecules and expel others at the same time.
- The energy source is the proton motive force of electron transport.
- It is efficient because two functions (intake and output) occur at the same time.

ACTIVE TRANSPORT: ABC Transport Systems

- ABC transport systems are very complex and involve several proteins.
- The molecule to be transported binds to a protein on the outside of the plasma membrane.
- It is handed off to a complex of proteins located in the plasma membrane.
- These proteins then transport the molecule into the cytoplasm.
ACTIVE TRANSPORT: Group Translocation

- Group translocation is unique to bacteria.
- It is very energy expensive and uses PEP instead of ATP.
- It helps make sure molecules stay inside the cell.
- An enzyme attaches a phosphate to the molecule, preventing the molecule from leaving the cell.

SECRETION

- The plasma membrane is also involved in secretion.
- Secretion is the movement of substances out of a cell.
- It involves several membrane proteins that act in specific sequence.
CLINICAL SIGNIFICANCE OF THE PLASMA MEMBRANE

- It is not a virulence factor but it is a primary target for antibiotics.
- Damage to the plasma membrane can:
  - Inhibit DNA replication.
  - Destroy the ability to produce energy.
  - Cause loss of membrane integrity and destruction of the cell.

THE NUCLEAR REGION

- Bacteria have no nucleus.
- The region where DNA is located is called the nuclear region.
- Bacteria usually have only one circular chromosome which contains all of the genetic information required by the organism.

PLASMIDS

- Plasmids are extra-chromosomal pieces of DNA that are separate from the main DNA structure.
- Some bacteria can carry more than one plasmid.
- Plasmids often carry genes for toxins and resistance to antibiotics.
- Plasmids can be transferred from one cell to another through pili during conjugation.
**CLINICAL SIGNIFICANCE OF DNA AND PLASMIDS**

- Because DNA is the genetic blueprint for the organism, any disruption or damage to it can be a lethal event.
- DNA is therefore a primary target for antibiotics
  - This type of therapy is routinely used for viral infections.
- Plasmids carry genes for toxins and antibiotic resistance.

**RIBOSOMES**

- Ribosomes are nonmembrane-enclosed organelles involved in protein synthesis.
- More active bacteria contain more active numbers of ribosomes.
- Each ribosome is composed of two subunits.
  - The subunits remain apart till messenger RNA is found.
- Protein synthesis (translation) occurs at the ribosome.
- Ribosomes in prokaryotes are different to those in eukaryotes.
CLINICAL SIGNIFICANCE OF RIBOSOMES

- The inhibition of protein synthesis is a lethal event so ribosomes are a major target for antibiotics.

INCLUSION BODIES

- Inclusion bodies are membrane-enclosed organelles used to store important materials.
- There are several types of inclusion bodies:
  - Those that store glycogen.
  - Metachromatic granules store phosphates in Corynebacterium species.
- Inclusion bodies have no clinical significance.

ENDOSPORES

- Endospores are formed through the process of sporulation.
- They form when a bacterium is exposed to great environmental stress.
- The process is restricted to Gram-positive rods.
  - There is one exception to this – Coxiella burnetii, which is a Gram-negative coccus.
ENDOSPORES

- They confer a type of dormancy on the cell.
- They are extremely resistant to heat, desiccation, toxic chemicals, UV irradiation, and antibiotics.
- Bacteria can survive for extraordinary lengths of time in the endospore state.

PROCESS OF SPORULATION

- Sporulation is a complex series of steps.
- The first step is replication.
- The second step is the sequestration of a copy of the chromosome.
  - This chromosome copy is surrounded by a septum.
- The third step is formation of the forespore.
  - Large amounts of peptidoglycan are deposited round the forespore.
- In the last step, the rest of the original cell deteriorates and degrades.
  - Bacterial genetic information is protected inside the endospores.
PROCESS OF SPORULATION

Germination

- Germination of the endospore back into a vegetative cell occurs when the environmental stress has subsided.
- The endospore accepts water molecules, swells, and cracks.
- The water activates metabolism and the cell begins to grow.

CLINICAL SIGNIFICANCE OF SPOROGENESIS

- If a bacterium is pathogenic when it undergoes sporogenesis, it will be pathogenic once it emerges.
- Endospores are resistant to almost all disinfectants and antiseptics.
- They are also resistant to antibiotics.
CLINICAL SIGNIFICANCE OF SPOROGENESIS

- Endospores are therefore a significant clinical problem.
- Endospores are also resistant to heat and can cause severe problems for the food industry, for example *Clostridium botulinum.*

<table>
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<tr>
<th>Structure</th>
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<th>Target for Antibiotics</th>
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<td>Glycolytic</td>
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*Note:* Microbiology: A Clinical Approach © Garland Science