Sections in Medical Microbiology & Immunology

- Chapter 1, pages 1-3
- Chapter 2, pages 4-14
  - The sections on endospores and synthesis of peptidoglycan will be covered in later lectures
- Chapter 3, pages 15-16
- Chapter 5, pages 23-24
Kingdom – *Prokaryotae*

Subkingdoms - *Archaebacteria* and *Eubacteria*
Bacteria are Named by Shape

- Cocci - spherical bacteria
  - Diplococci - two associated cocci (A)
  - Streptococci - chains of cocci (B)
  - Staphylococci - clusters of cocci (C)
  - Sarcinae - packet arising from alternating cell division planes (J)
Bacteria are Named by Shape

- **Bacilli** - rods (D)
  - Fusiform - tapered end (F)
  - Clavate or Coryneform - club shaped end
  - Filamentous (G)
  - Vibrios - comma shaped (H)
  - **Spirilla** - snakelike (I)
Bacteria are Named by Shape

- **Coccobacilli** - ovoid or ellipsoidal (E)
- **Spirochetes** - flexible envelopes and corkscrew appearance
Fig. 3-3. Arrangements of cocci based on the plane of cell division. A. Division in one plane produces diplococci or streptococci. B. Division in two planes produces a tetrad. C. Division in three planes produces a group of eight (sarcina) or clusters (staphylococci).
Internal Ultrastructure

**Envelope**
- **Flagella**
  - 6 proteins (~2 × 10^4 molecules/cell)
- **Pili**
  - 1 protein (~2 × 10^4 molecules/cell)
- **Outer membrane**
  - 50 proteins (~4 abundant, 10^4 molecules/cell)
  - 5 p-lipids (~5 × 10^5 molecules/cell)
  - 1 LPS (~9 × 10^9 molecules/cell)
- **Capsule**
  - 1 complex polysaccharide
- **Wall**
  - Peptidoglycan (1 molecule/cell)
- **Periplasm**
  - 50 proteins (~10^4 molecules/cell)
- **Cell membrane**
  - 200 proteins (~2 × 10^6 molecules/cell)
  - 7 p-lipids (~15 × 10^6 molecules/cell)

**Cytosol**
- 1,000 proteins (~10^6 molecules/cell)
- 60 tRNAs (~2 × 10^5 molecules/cell)
- Glycogen (variable)

**Polysomes**
- ~18,000 ribosomes/cell in 1,000 polysomes
- 35 proteins (~10^5 molecules, 1 of each per 70S ribosome)
- 3 tRNAs (5S, 16S, 23S; 56,000 molecules, 1 of each per 70S ribosome)
- 1,000 mRNAs (~1,400 molecules, 1 per polysome)

**Nucleoid**
- DNA (haploid chromosome, ~1 molecule)
Internal Ultrastructure

- **Nucleoid** - central condensed region that includes DNA
- **Intracytoplasmic granules** - inclusion bodies or metachromatic granules for storage of energy polymers such as glycogen
- **Ribosomes** - ribonucleoprotein complexes upon which protein synthesis occurs
Gram Positive versus Negative Bacteria

- The most important distinction among bacteria is whether they are gram positive or negative.
- This distinction is determined by the thickness of the bacterial cell wall.
- It is based on the Gram stain.
Gram Stain

1. Stain with crystal violet (purple) and iodine
2. Destain with acetone and alcohol
3. Counterstain with safranin (red)
Gram Stain

A. Gram stain

STEPS

1. Staining

2. Decolorization

3. Counterstain

Unstained
Purple
Blue
Red

Gram positive

Gram negative
Gram Positive Bacteria

Examples

- *Staphylococcus aureus*
- *Streptococcus pneumonia*
- *Clostridium botulinum*
Gram Positive Bacterium

Figure A1-2  Structure of the cell surface of a gram-positive bacterium.
Gram Positive Bacteria

- Cytoplasmic membrane
  - Phospholipid bilayer is similar to the cytoplasmic membrane of eukaryotic cells
  - Integral membrane proteins function in energy and transport
  - Bacteria *do not* contain sterols
Gram Positive Bacteria

- Peptidoglycan cell wall
  - Provides structure
  - Components of the cell wall are important in the inflammatory response
  - The thickness of the cell wall distinguishes gram positive from gram negative bacteria
    - The wall is about 40 layers thick in gram positive bacteria
    - The wall is only 1 layer thick in gram negative bacteria
  - Many antibiotics affect peptidoglycan synthesis
Gram Positive Bacteria

- Lipoteichoic acids
  - Present in all gram positive organisms
  - Phosphodiester linked glycerols or ribitols with a terminus in the membrane and extending through the peptidoglycan
  - Similar to lipopolysaccharides in gram negatives in that they promote negative surface charge
  - Teichoic acids are nontoxic, but they contribute to virulence
Lipoteichoic Acid
Gram Negative Bacteria

- **Examples**
  - *Neisseria gonorrhoea*
  - *Escherichia coli*
  - *Salmonella typhimurium*
Gram Negative Bacterium

Figure A1-3  Structure of the cell surface of a gram-negative bacterium.
Gram Negative Bacteria

- **Cytoplasmic membrane**
  - Similar to that of gram positive bacteria

- **Peptidoglycan cell wall**
  - Only about one layer thick
  - Does not retain the gram stain after treatment with ethanolic iodine
Gram Negative Bacteria

- **Periplasmic space**
  - Between the inner and outer membranes of gram negative bacteria
  - Contains the peptidoglycan layer and hydrolytic enzymes
  - Periplasmic binding proteins, which move nutrients through the space
  - Detoxifying enzymes such as β-lactamase, which inactivates penicillin
Gram Negative Bacteria

- **Outer membrane**
  - Only some molecules, mostly nonpolar, can diffuse through the outer membrane
  - Other molecules must enter through pores
  - This property is responsible for the native antibiotic resistance of gram negative bacteria (antibiotics can’t get through)
  - Contains lipopolysaccharide (LPS or endotoxin)
Lipopolysaccharide (LPS)
Lipopolysaccharide (LPS)

- **Lipid A**
  - Glucosamine disaccharide in a $\beta-1,6$ glycosidic linkage with fatty acid esterified to some hydroxyl groups
  - Responsible for the toxicity of LPS

- **Core oligosaccharide**
  - Short series of sugars linking lipid A to O antigen, contains keto-deoxyoctonoic acid and a heptose

- **Repeating or O antigen**
  - Repeating saccharide of variable length
  - Short or absent causes colonies to appear rough rather than smooth
  - Contains serologic determinants of endotoxin
How do molecules get through the outer membrane?

- **Pores** - Trimers of major outer membrane proteins or porins
  - Form pores through which solutes less than approximately 700 daltons in size can pass
  - Some antibiotics can pass through the pores in the outer membrane, which makes the bacteria sensitive to the antibiotic. However, resistance can result from a porin mutation.
Acid Fast Bacteria

- *Mycobacterium (tuberculosis and leprae)*
  - Usually stain positively with the gram stain because they have large amounts of waxes (mycolic acids) in their cell walls
    - The wax prevents stain from being washed out with acid
    - Walls also contain murein, polysaccharide and lipids
  - Distinguished by the acid fast stain
Acid Fast Stain

1. Stain with carbol-fuchsin (red) and heat
2. Destain with 3% hydrochloric acid and alcohol
3. Counterstain with methylene blue
Acid Fast Stain

B. Acid-fast stain

STEPS

1. Staining

2. Decolorization

3. Counterstain

Unstained
Purple
Blue
Red

Acid-fast
Figure 14–3. The Gram and acid-fast stains. Four bacteria and a PMN are shown at each stage. All are initially stained purple by the crystal violet and iodine of the Gram stain (A1) and red by the carbol fuchsin of the acid-fast stain (B1). Following decolorization, Gram-positive and acid-fast organisms retain their original stain. Others are unstained (A2, B2). The safranin of the Gram counterstain stains the Gram-negative bacteria and the background red (A3), and the methylene blue leaves a blue background for the contrasting red acid-fast bacillus (B3).
Cell wall-deficient bacteria

- Mollicutes
  - *Mycoplasma*
- L-forms or L-phase
  - Derived from gram-positive or gram-negative bacteria
  - Loss of peptidoglycan coat
  - Can be selected clinically by use of antibiotics
Characteristics of Bacterial Classes

- Spirochetes
  - Treponema

- Gram + Rods
  - Clostridium
    - Listeria
      - Staphylococcus
      - Streptococcus
  - Corynebacterium
  - Mycobacterium

- Gram + Cocci
  - Gram + Rods
  - Acid Fast

- Gram –
  - Escherichia, Shigella, and Salmonella
  - Yersinia
  - Vibrio
  - Legionella (doesn't counterstain)
  - Pseudomonas
  - Neisseria (diplococci)
  - Helicobacter

(all rods except Neisseria)
Capsule

- Polysaccharide coat found in both gram positive and gram negative organisms
  - slime - *weakly adherent*
  - microcapsule - thin coat
  - biofilm - growth within layers of polysaccharide
  - visualized by exclusion of India ink
  - Quellung reaction is used to serotype bacteria
Quellung Reaction

**Figure 23–1.** Electron micrograph of *Streptococcus pneumoniae*, type 1. The capsule has been reacted with type 1 Ab to accentuate its visibility (quellung reaction). (Mudd S et al: J Exp Med 78:327, 1943)
Capsule

- May contribute to virulence
  - Antiphagocytic
  - Interferes with complement
  - Growth in a biofilm prevents access of host cells or antibiotics
  - Each of these helps the bacteria to avoid host defenses
Pili

- **Sex pili**
  - Facilitate transfer of DNA between bacteria during conjugation
  - Serve as receptors for bacteriophage

- **Fimbriae or somatic pili**
  - Allow bacteria to adhere to various surfaces
  - Important in infection by some bacteria (*Neisseria gonorrhea*)

- Pili are shorter and thinner than flagellae
Flagella

- Appendages employed for motility
- Rotate clockwise or counterclockwise, depending on environmental stimuli
  - Counterclockwise move the bacterium in a straight line, towards attractants (positive chemotaxis)
  - Spirochetes have a flagellum-like structure wrapped around them, which facilitates gliding
Movement During Chemotaxis

Mechanism of movement during chemotaxis in *E. coli*. (a) An attractant causes a counterclockwise rotation of the flagella, which form a bundle to propel the cell forward. (b) A repellent causes the bundle to rotate clockwise, the bundle falls apart, and the bacterium tumbles.
Flagella

- Named by the location and number, which is characteristic of the bacteria
  - **Monotrichous** - single polar flagellum
  - **Peritrichous** - flagella all over surface
  - **Lophotrichous** - clusters of flagella at one end
  - **Amphitrichous** - clusters of flagella at both ends
Distribution of Flagella

Fig. 3-13. Flagellar arrangement. A. Monotrichous. Electron micrograph (× 24,000 before 50 percent reduction). B. Lophotrichous. Electron micrograph (× 30,000 before 59 percent reduction). C. Amphitrichous. D. Peritrichous. Electron micrograph (× 24,000 before 44 percent reduction). (Electron micrographs courtesy of W. Hodgkiss.)
Flagella Structure

- Filament
  - Composed of flagellin
- Hook
- Basal body
  - Motor-like apparatus
Flagella and Pili Structures

Figure 12
Comparison of the structure of the Gram-positive and Gram-negative cell envelopes, showing the major molecular components and their approximate dimensions. The region between the outer membrane and the cytoplasmic membrane of the Gram-negative envelope is called the periplasm.
Virulence Factors

- Factors that enhance the ability of the bacterium to cause infection
- Loss of a virulence factor can make the bacteria non-pathogenic
  - Leads to colonization and infection without disease
  - Carrier state (reservoir for disease-causing organisms)
- Restoration of a virulence factor can occur rapidly, leading to pathogenicity
Bacterial Growth

- Measurement of bacterial growth in culture
  - Scattering of light (absorbance at 600 nm)
  - Particle counting (electronic or microscopic)
  - Physical measurement of constituents, such as DNA or protein
  - Plating dilutions and counting colonies
Phases of Growth

- Growth of a culture diluted into fresh medium can be divided into 3 phases based on differences in growth rates
  - Lag
  - Exponential or Logarithmic
  - Stationary
Phases of Growth

![Graph showing the Phases of Growth]

- **Lag phase**: Initial period where cells are acclimating to their environment.
- **Exponential phase**: Period of rapid cell division.
- **Stationary phase**: Phase where cell division and death balance, resulting in a stable cell count.

**Cell mass** increases during the exponential phase, while **cell number** increases during both the exponential and stationary phases.
Instantaneous Growth Rate ($\alpha$)

\[
\frac{dM}{dt} = \alpha M \\
\frac{dM}{M} = \alpha \, dt \\
M_t = M_0 \, e^{\alpha t} \\
\ln \frac{M_t}{M_0} = \alpha t \\
\ln M_t = \ln M_0 + \alpha \, t
\]

- $M =$ Mass of bacteria
- $t =$ time of growth
- Plot the logarithm of the Mass against time in culture
  - straight line with slope equal to $\alpha$
Mean Generation Time

- **MGT or Doubling Time**
- The amount of time it takes for the population to double
- set $M_t = 2M_0$:
  \[
  \ln \frac{M_t}{M_0} = \ln 2 = \alpha \ dt_D
  \]
  \[
  t_D = \frac{1}{\alpha} \ln 2 = 0.69 \left(\frac{1}{\alpha}\right)
  \]
Exponential growth rate ($\mu$)

- Reciprocal of the mean generation time

\[ \frac{1}{t_D} = \mu = 1.45 \alpha \]
Examples of Growth Rate

- Mean Doubling Time
  - 20 minutes

- Exponential Growth Rate
  - 0.05 min$^{-1}$

- Instantaneous Growth Rate
  - 0.0345 min$^{-1}$
Temperature Optima Differ for Different Bacteria

- **Psychrophiles**: -5 to 30°C
  - *Listeria monocytogenes*, which has caused disease from inadequately pasteurized cheese
  - An outbreak in California in 1985 resulted in 142 infections and 18 adult deaths

- **Mesophiles**: 10 to 40°C
  - Pathogens that grow well at body temperature

- **Thermophiles**: 25 to 110°C
  - *Thermus aquaticus*, the source of Taq polymerase for PCR (Polymerase Chain Reaction)