

## Microbiology 101

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### Learning Objectives:

1. Learn differences between gram positive and gram negative bacteria.
2. Learn the differences in biology of prokaryotes and eukaryotes.
3. Understand antimicrobial selection
4. Understand common laboratory tests
5. Understand MIC creep and ways to prevent it.
6. Have fun!

## Basic Microbiology

- ❖ What is microbiology?
  - According to APIC text of Infections Control and Epidemiology: “the field of microbiology includes the study of bacteria, fungi (molds and yeasts), protozoa, viruses and algae.”
  - As Infection Preventionists, you are going to encounter these organisms during your career.
- ❖ Three Domains of Microbiology
  - Prokaryotes: **Bacteria** and **Archaea**
    - ◆ Simple
    - ◆ Usually single celled organisms
    - ◆ Contain no nucleus
    - ◆ No membrane bound cell structures
  - Eukaryotes: **Eukarya**
    - ◆ Complex
    - ◆ Single or multi-celled
    - ◆ Have a nucleus
    - ◆ Contain a nuclear envelope
    - ◆ Contain cell membrane-bound structures like mitochondria, golgi apparatus, etc
- ❖ Prokaryotes: Bacteria
  - Very small, single-celled organism
  - Contain DNA in the form of a bacterial chromosome, which is circular
  - May contain plasmids, which are not necessary for bacteria but can be helpful for survival
  - Plasmids can carry genes for antibiotic resistance, toxin production, etc.
  - Bacteria can exchange plasmids to each other and incorporate into their chromosome
  - Complex cell wall structure

- Bacteria contain peptidoglycan in their cell wall
- Can have flagella, a long appendage for movement
- Can have fimbriae or pili which help with attachment
  - ◆ Gram positive bacteria
    - Have a thick layer of peptidoglycan
    - Generally susceptible to penicillins and cephalosporin
    - Examples: *Staphylococcus aureus*, *Streptococcus pneumoniae*
  - ◆ Gram Negative bacteria
    - Have small layer of peptidoglycan
    - Contain additional layer called outer membrane
    - Example: *Escherichia coli*, *Pseudomonas aeruginosa*
- May form another layer called a glycocalyx, which offers additional protection to cell.
  - ◆ Helps avoid immune cell phagocytosis
- Bacteria replicate by binary fission,
  - ◆ which is dividing one cell into two cells
  - ◆ Newly formed cells are called daughter cells
  - ◆ Generally it is very fast
    - E.coli can replicate very 15-20 minutes
    - Mycobacterium Tuberculosis replicates every 12-24 hours
- Bacteria are very good at mutations
  - ◆ Typically the daughter cells are exact copies of the original but mutations during replication (least common) or gene transfer (most common) from other bacteria changes the genome.
  - ◆ Transformation
  - ◆ Conjugation
  - ◆ Transduction
- ❖ Atypical Bacteria
  - Mycoplasma
    - ◆ Lack cell wall, only have outer plasma membrane
    - ◆ Resistant to antibiotics that attack the cell wall
    - ◆ Often require special lab testing
  - Chlamydiae
    - ◆ Bacteria that is obligate intracellular parasites
    - ◆ Only grow inside a host
  - Rickettsiae
    - ◆ Also obligate intracellular parasites

- ❖ Prokaryotes: Archaea
  - Live in extreme environments
    - ◆ Hot springs, salt lakes, Dead Sea
  - Do not contain peptidoglycan
  
- ❖ Eukaryotes
  - Humans are Eukaryotes
  - Microbes:
    - ◆ Algae
    - ◆ Fungi (Yeasts and molds)
    - ◆ Protozoa or Protists
  
- ❖ Eukaryotes- Fungi
  - Contain chitin in cell walls
  - Most people obtain fungal infections by accident
  - Yeasts
    - ◆ Single-celled organisms, reproduce by budding
    - ◆ Examples: *Candida* species & *Cryptococcus neoformans*
  - Molds
    - ◆ Consist of long, branching filaments called hyphae
    - ◆ Asexual or spore reproduction
    - ◆ Opportunistic infections
    - ◆ Examples: *Aspergillus* species
  
- ❖ Viruses
  - Non-living organisms, not cells, not a prokaryote or eukaryote
  - Also considered intracellular parasite
    - ◆ Grow within living cells and are dependent on hosts metabolic machinery to replicate
  - Contain DNA or RNA, in a single or double stranded formation
  - Complex reproduction cycle that involves: Attachment, Penetration, Replication, Maturation, and Release
  - Viruses can be detected in a variety of ways:
    - ◆ Electron microscopy, enzyme linked immunosorbent assay (ELISA), latex agglutination, DNA probes, PCR, etc.
    - ◆ Antibody detection methods, since they cause an immune response.
    - ◆ Difficult to culture
  - Testing IgG can help determine if a person has been exposed to a virus
    - ◆ Ex. Varicella (chicken pox)

## Clinical Microbiology

- ❖ Primary Goal of Clinical Microbiology
  - “identify the presence of pathogenic organisms in tissues, body fluids, excretions or secretions and to identify those pathogens species level based on morphological and biochemical properties”
  
- ❖ How do we find bacteria in patients?
  - First, prepare a slide of the sample for observation
    - ◆ Wet mount, heat fixed, etc.
  - Second, try and make a pure culture of the bacteria in question.
    - ◆ Typically done with the streak-plate method
  - Why a pure culture?
    - ◆ It's the only way to get reliable identification
  
- ❖ Other Ways to Identify Bacteria
  - Gram Stain- Primary Test
    - ◆ Where Gram Positive and Gram Negative terms come from.
    - ◆ Stepwise process of different stains which are: crystal violet, iodine, and safranin
    - ◆ Gram Positive bacteria stay purple or blue from the crystal violet/iodine complex because it stays in the peptidoglycan
    - ◆ Gram Negative bacteria have the outer membrane and the stain gets washed away with alcohol rinse, but keeps the pink safranin color
  - Acid-Fast Stain (Mycobacterium)
  - Serotyping
  - Bacteriophage typing
  - Electrophoresis
  - Plasmid analysis
  - PCR
  - Many others
  
- ❖ Antimicrobial Susceptibility
  - Def: “state or character of being susceptible”
  - Susceptibility helps determine which medication will help the patient the most to fight the infection
  - Antimicrobial Susceptibility- Testing
    - ◆ Disk Diffusion
      - Bacteria is plated on a plate
      - Disk coated in antibiotics are placed on the disc
      - The clear zone of inhibition is measured
      - CLSI guidelines- susceptible, intermediate, and resistant
    - ◆ Broth Dilution

- Used to determine the least amount of antibiotic necessary to inhibit the growth of the organism or the minimal inhibitory concentration (MIC)
  - Uses replication inoculation of the standardized suspension of bacteria in broth into a series of micro well containing antibiotics in different concentrations
  - The first well with no growth is the MIC
  - ◆ E-test
    - Combines both disk diffusion with ability to quantify the resistance provided by broth dilution
    - Often used with level of resistance is clinically important
    - Example: *Streptococcus pneumoniae* with penicillins/cephalosporins
  - ◆ Synergy Testing
    - Used to determine the inhibitory ability of combination of antibiotics
    - D-test with inducible clindamycin resistance
  - ◆  $\beta$ -lactamase Test
    - Rapidly detects the enzyme  $\beta$ -lactamase that can be produced by bacteria
    - H. flu, Staph spp., Pseudomonas spp.
- ❖ Antibiotic Resistance
- Mechanisms:
    - ◆ Inactivation of antibiotic
    - ◆  $\beta$ -lactamase producing bacteria
    - ◆ Low permeability of the bacteria to the antibiotic
    - ◆ Pumping antibiotics out of the cell after they have entered
    - ◆ Low binding affinity of antibiotics to the bacteria
  - MIC creep
    - ◆ MIC is growing, meaning its more difficult for the antibiotics to kill the bacteria or we need more antibiotic to kill the bacteria
    - ◆ This is a problem!! Can lead to treatment failures.
    - ◆ Causes antibiotics to be less effective and more prone for adverse drug reactions with higher concentrations
    - ◆ Example. Vancomycin resistant MRSA
  - Ways to Help Decrease Resistance
    - ◆ Use narrow spectrum antibiotics if susceptible
    - ◆ Create and Follow practice guidelines
    - ◆ Don't give antibiotics for viral infections
    - ◆ Be an advocate to patients, friends, family to tell them to finish course of antibiotics therapy , EVEN if they feel better
    - ◆ Vaccinations
    - ◆ Treat infection not colonization or contamination

❖ Antibiogram

- Chart or table that summarizes in percent how susceptible an organism was to an antibiotic
- Used to help determine which antibiotic to use in a known organism infections
- Helps find patterns of resistance
- Typically done in an institution and/or compiled in a region or state

❖ Final Thoughts

- Laboratories assist in the identification of an outbreak by confirming the organism and detecting unusual organisms and antimicrobial susceptibility patterns
- Clinical Microbiology plays an important role in the practice of infection prevention.

References:

1. Association for Professionals in Infection Control and Epidemiology, Text of Infection Control and Epidemiology Chapter 24, p2-17
2. Nester EW, Anderson DG, Roberts CE, and Nester MT. Microbiology, A Human Perspective. 7<sup>th</sup> Edition. New York: McGraw Hill; 2012.