Microbiology 101

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Learning Objectives

- Learn differences between gram positive and gram negative bacteria.
- Learn the differences in biology of prokaryotes and eukaryotes.
- Understand antimicrobial selection
- Understand common laboratory tests
- Understand MIC creep and ways to prevent it.
- 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.

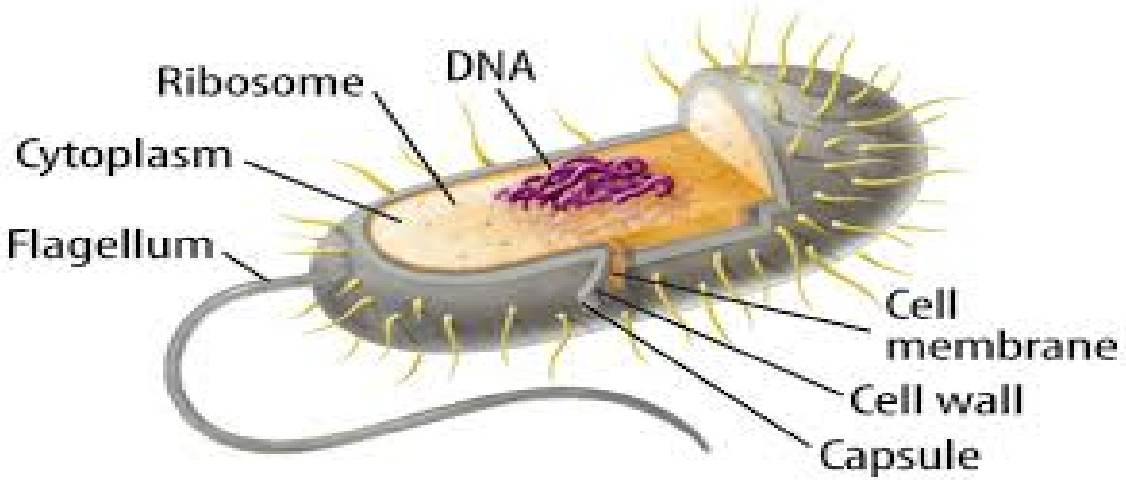
3 Domains of Microbiology

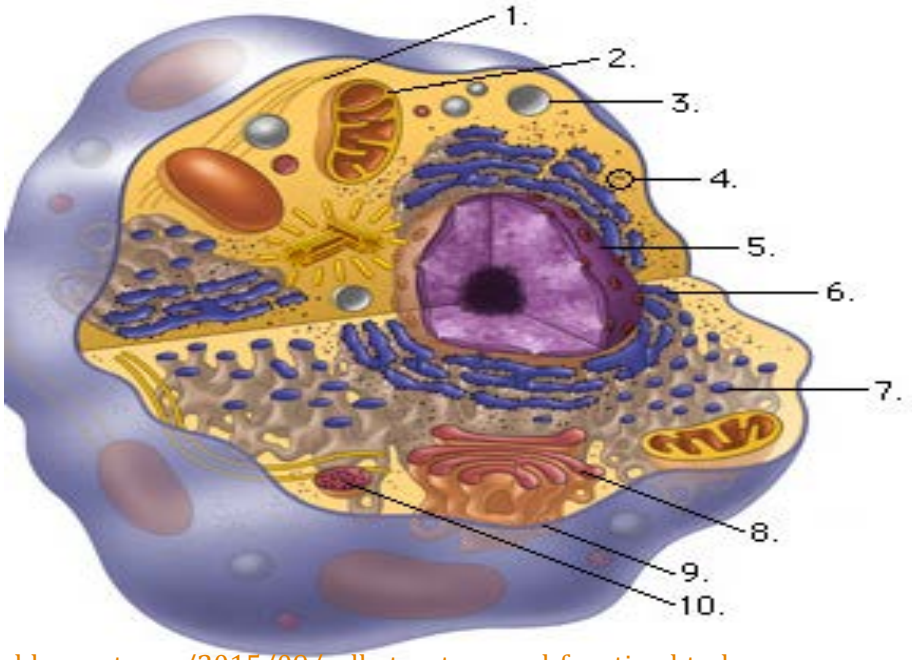
- Prokaryotes
  - **Bacteria**
  - **Archaea**
- Eukaryotes
  - **Eukarya**

Prokaryotes vs. Eukaryotes

- Simple
- Usually single celled organisms
- Contain no nucleus
- No membrane bound cell structures

- Complex
- Single or multi-celled
- Have a nucleus
- Contain a nuclear envelope
- Contain cell membrane-bound structures like mitochondria, golgi apparatus,etc.





http://7marshscience.blogspot.com/2015/09/cell-structure-and-function.html

http://www.phschool.com/science/biology_place/biocoach/cells/identify3.html

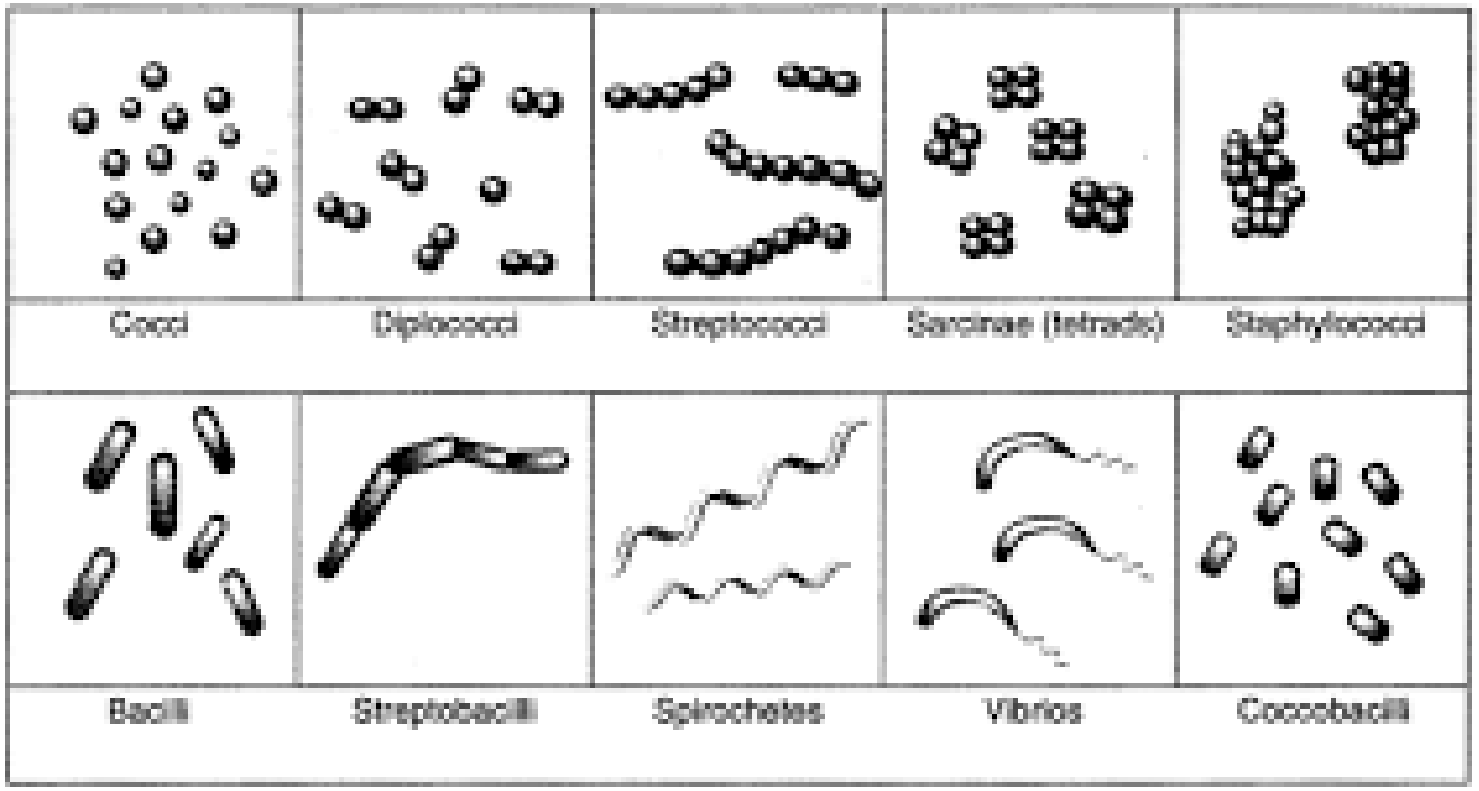
Prokaryotes: Bacteria

- 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

Prokaryotes: Bacteria

- 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
- Variety of cell shapes and arrangements

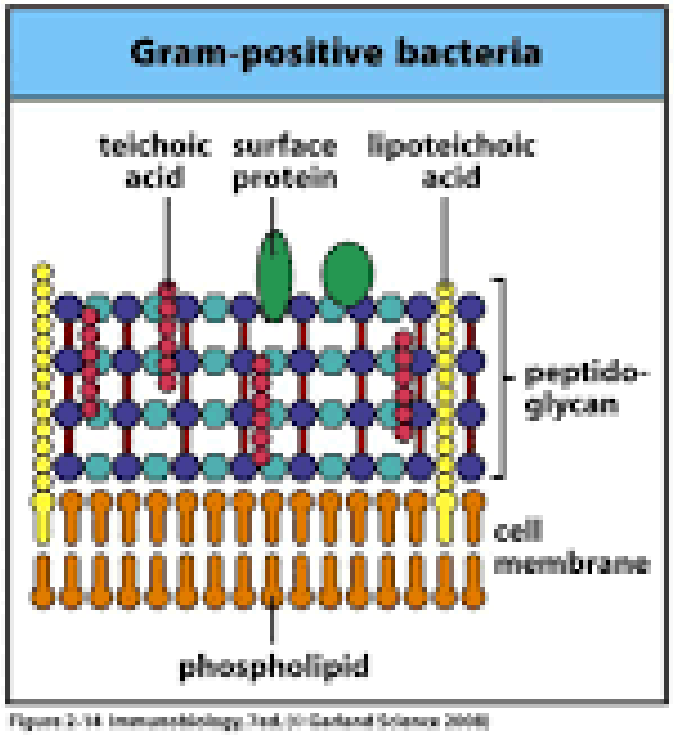
Prokaryotes: Bacteria



- http://www.keyword-suggestions.com/MSBncmFtIG9mIHNpbHZlcg/

Prokaryotes: Bacteria

- Gram positive
- Have a thick layer of peptidoglycan
- Generally susceptible to penicillins and cephalosporins
- Examples: *Staphylococcus aureus, Strephtococcus pneumoniae*



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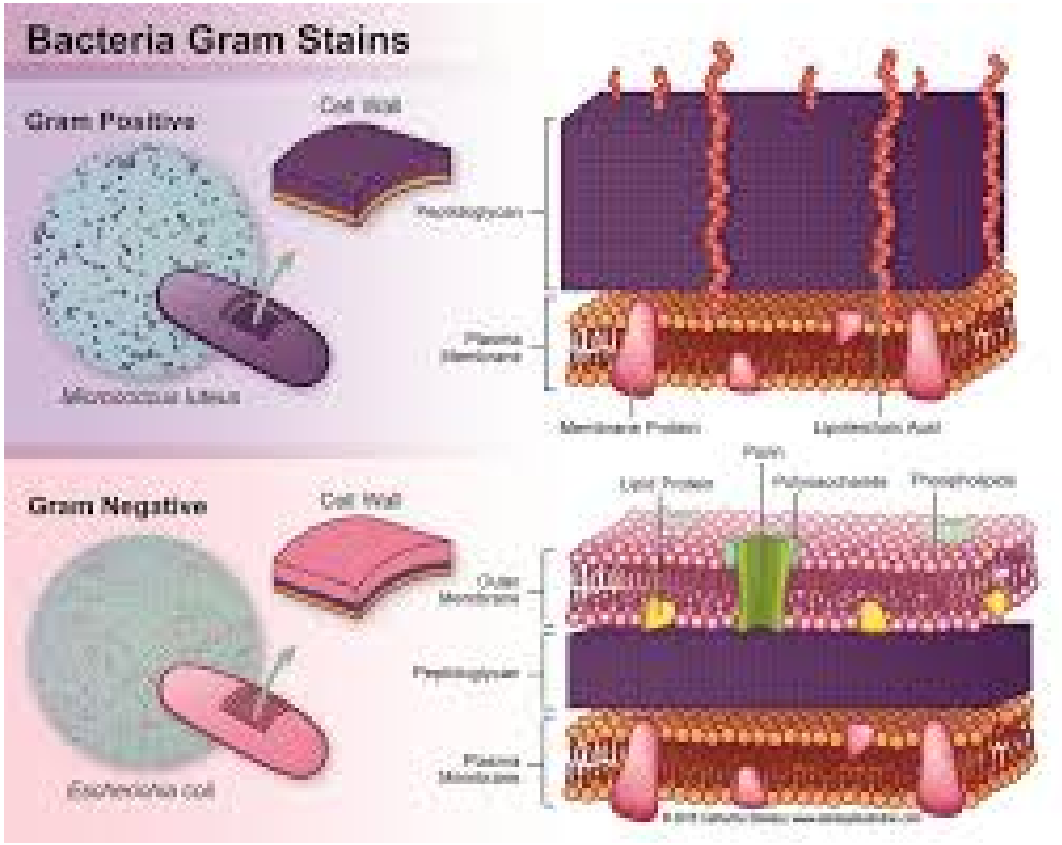

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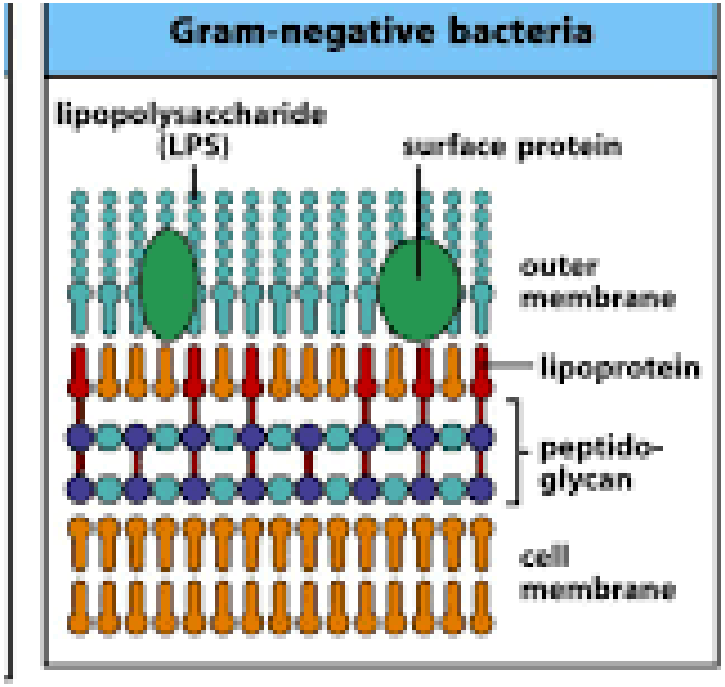
Prokaryotes: Bacteria

- Gram Negative
- Have small layer of peptidoglycan
- Contain additional layer called outer membrane
- Example: *Escherichia coli*, *Pseudomonas aeruginosa*



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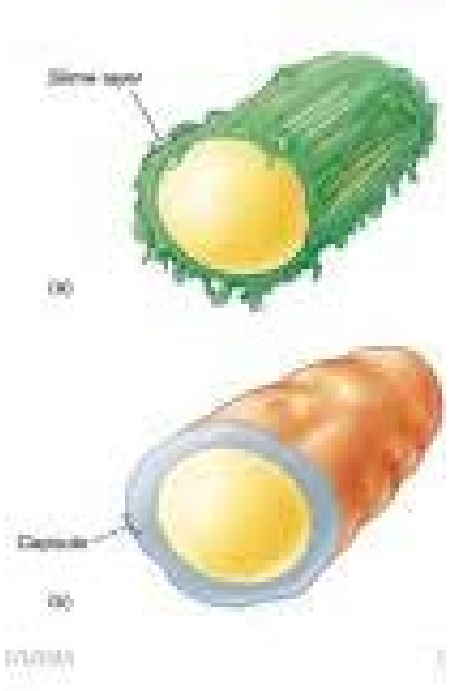
Prokaryotes: Bacteria

- May form another layer called a glycocalyx, which offers additional protection to cell.
- Helps avoid immune cell phagocytosis



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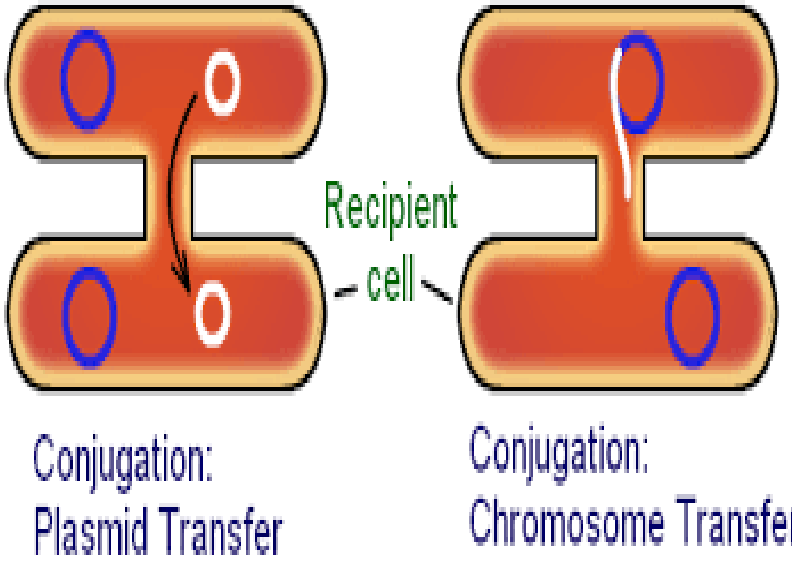
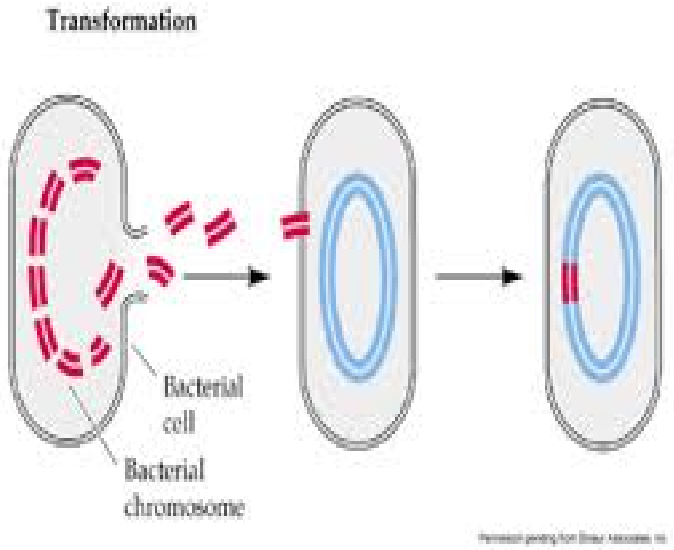
Prokaryotes: Bacteria

- 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

Prokaryotes: Bacteria

- 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





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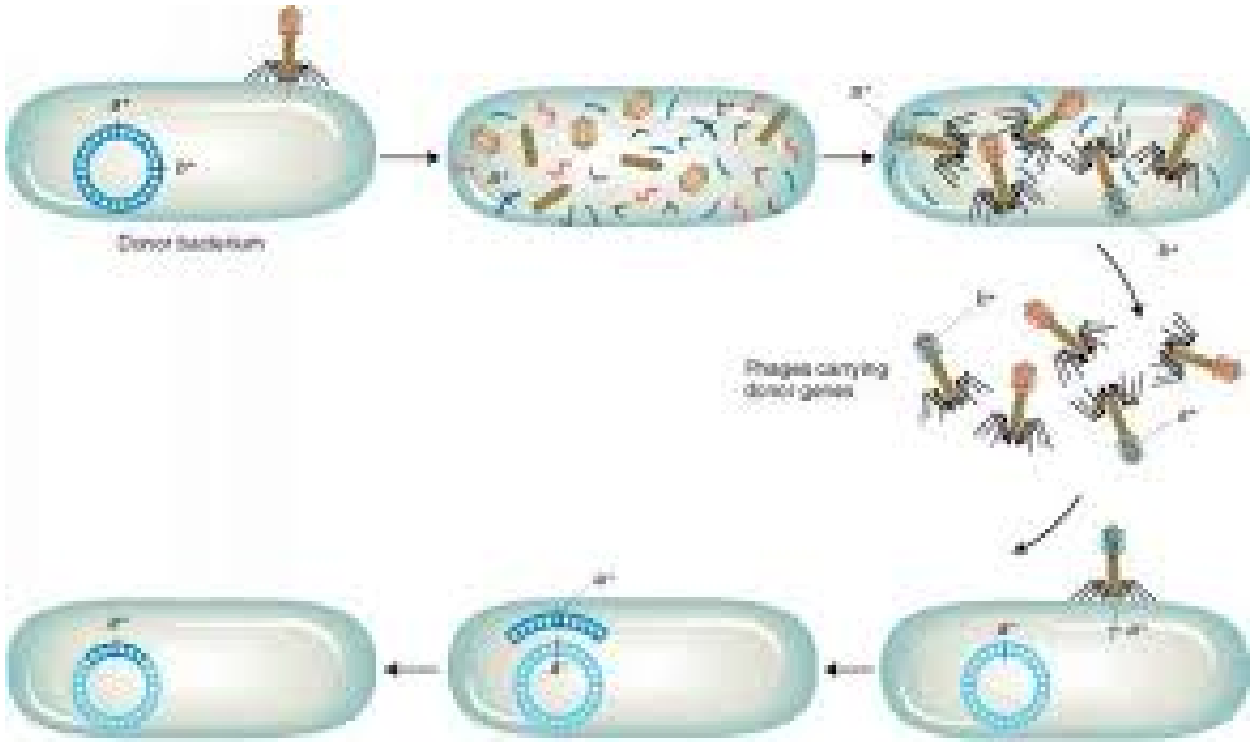


http://www.78stepshealth.us/transposable-elements/generalized-transduction.html

Prokaryotes: Bacteria

- 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 citin in cell walls
- Most people obtain fungal infections by accident

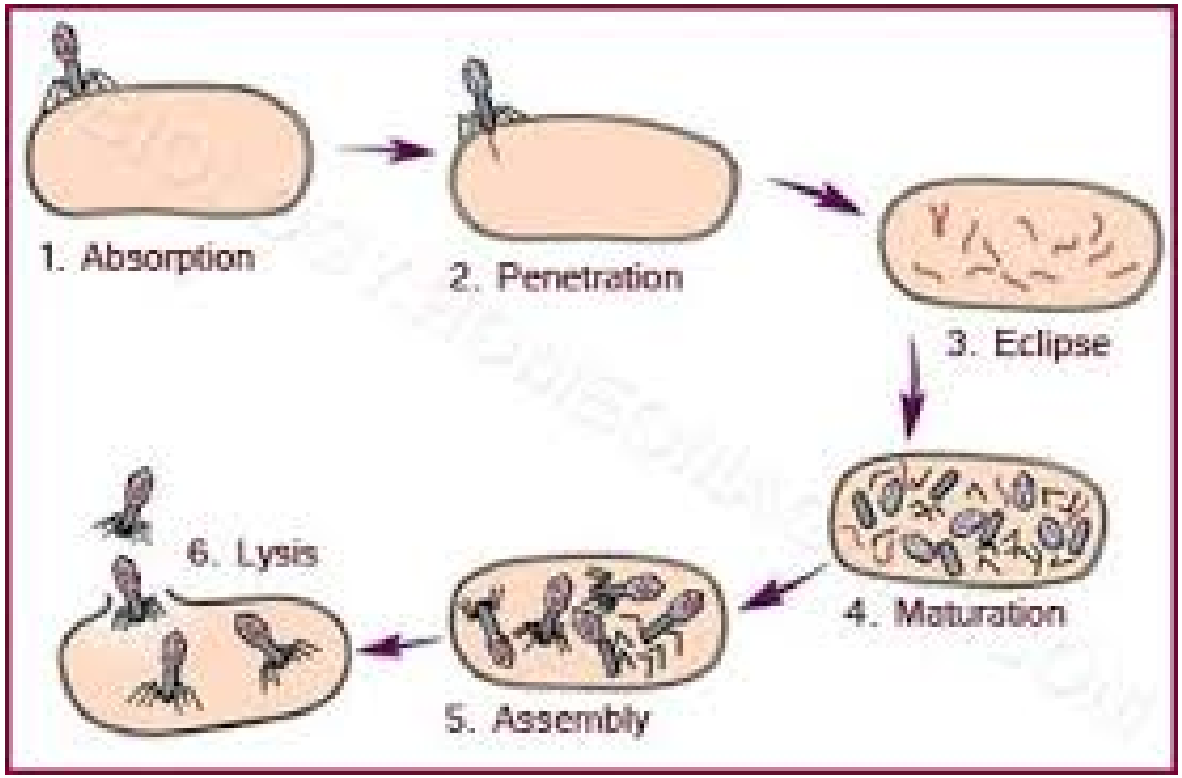
Eukaryotes- Fungi

- Yeasts
  - Single-celled organisms, reproduce by budding
  - Examples: *Candida* species & *Crytococcus 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

Viral Replication



http://biology.homeomagnet.com/viral-replication/

Viruses

- Viruses can be detected in a variety of ways:
  - Electron microscopy, enzyme linked immunosobent 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

- “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”

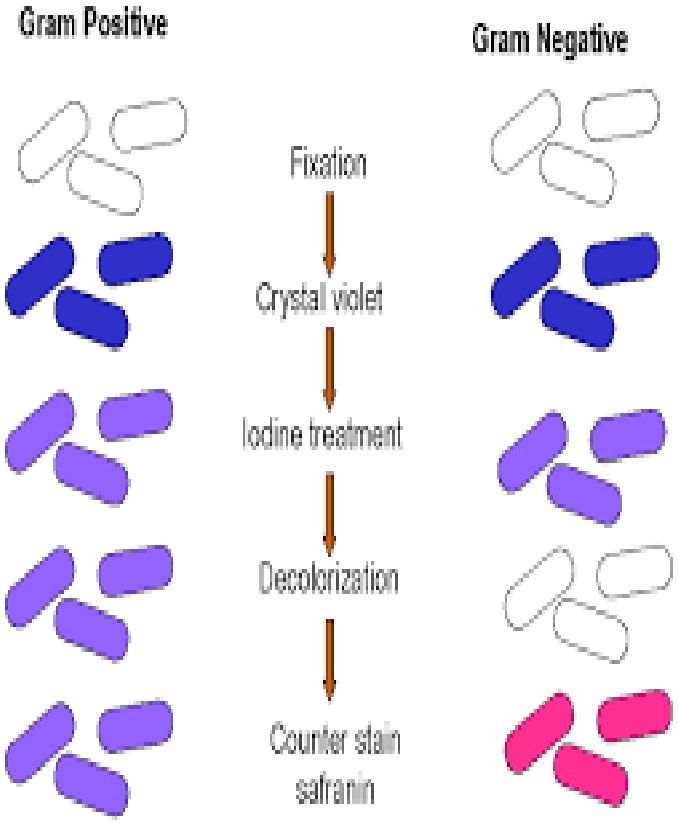
(APIC text of Infections Control and Epidemiology)

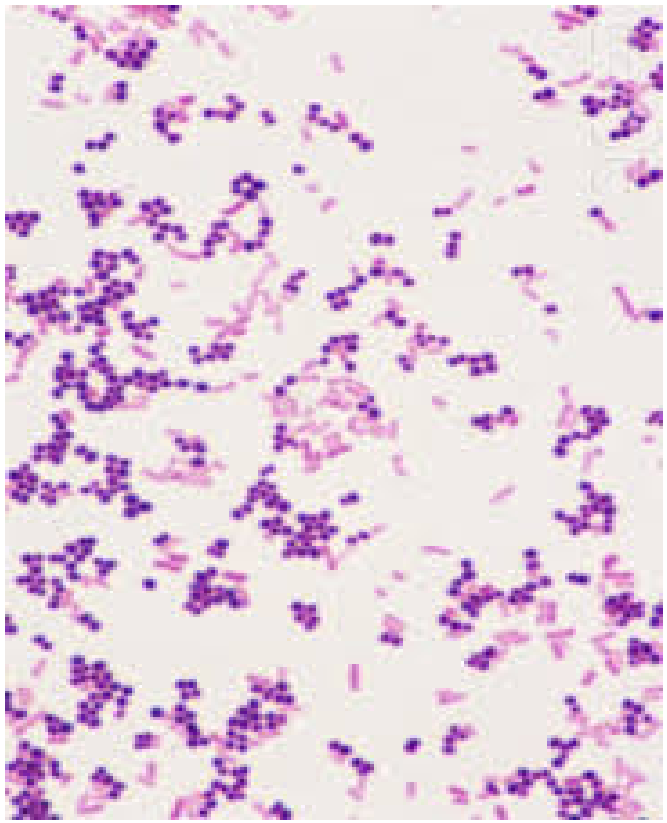
How do we find bacteria in patients?

- 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





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http://www.newhealthadvisor.com/Gram-Staining-Procedure.html

Other Ways to Identify Bacteria

- Acid-Fast Stain (Mycobacterium)
- Serotyping
- Bacteriophage typing
- Electrophoresis
- Plasmid analysis
- PCR
- Many others

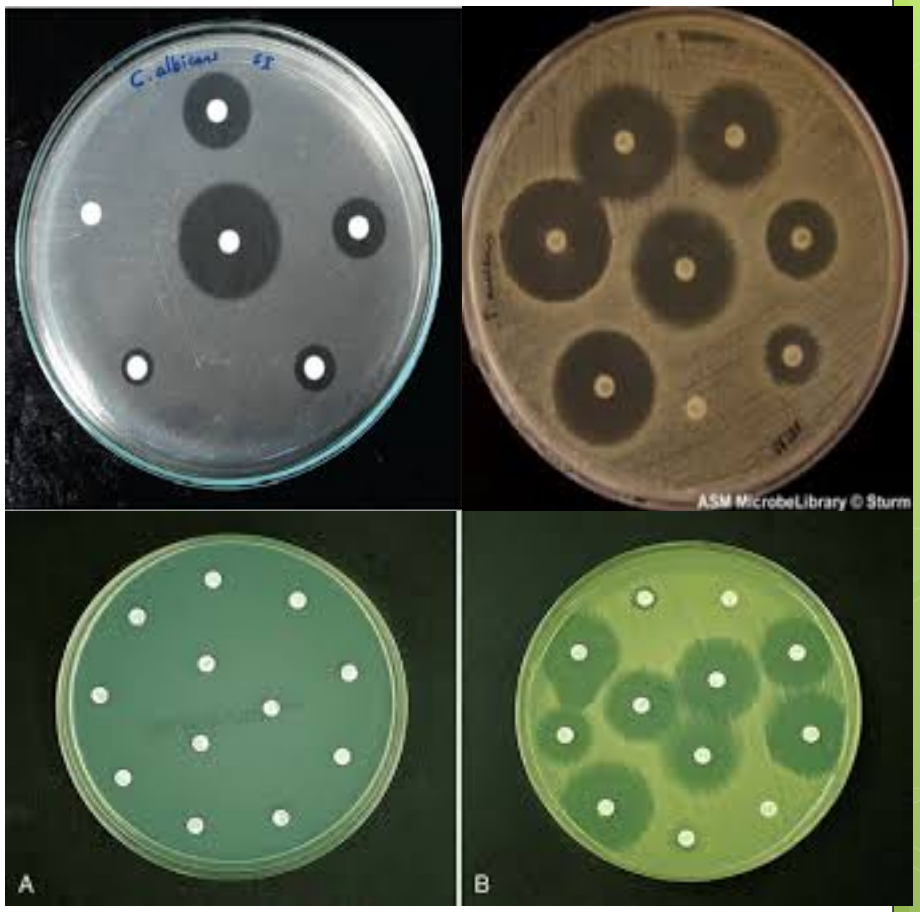
Antimicrobial Susceptibility

- Definition of Susceptibility:
- “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



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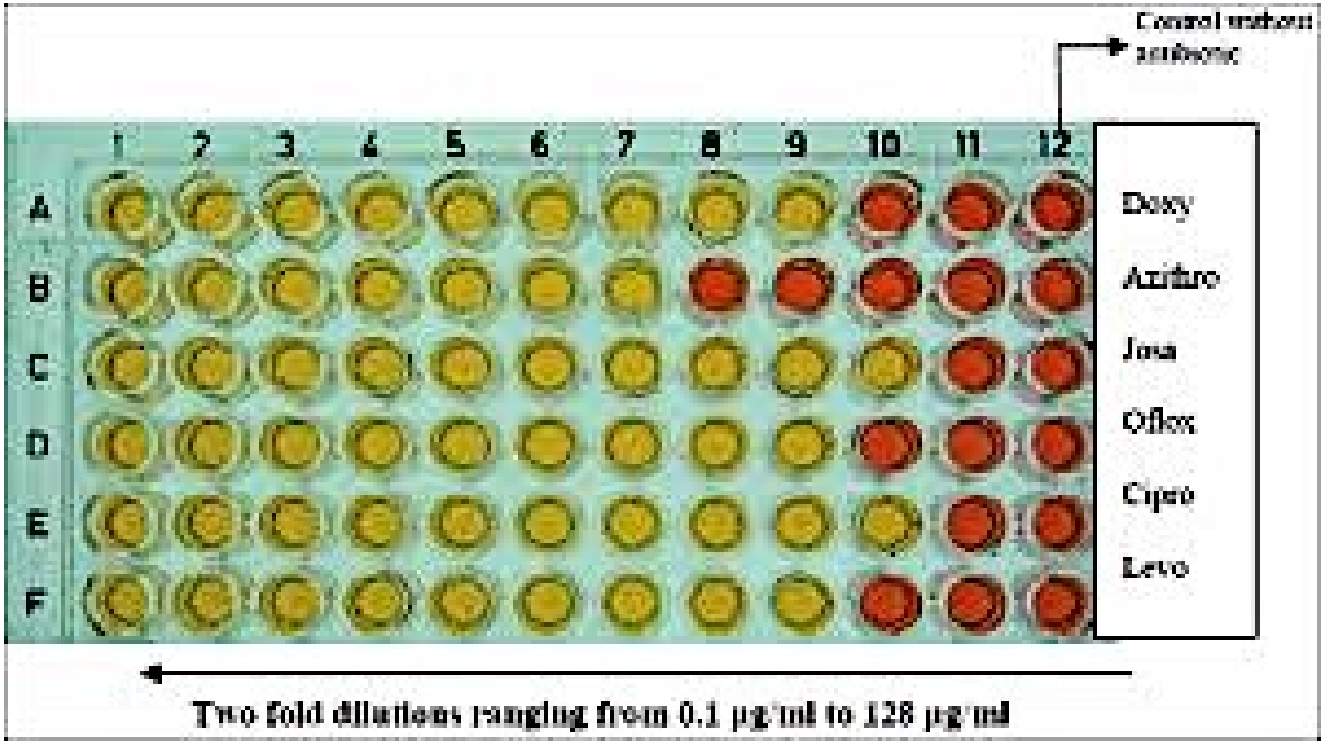
http://clinicalgate.com/laboratory-methods-and-strategies-for-antimicrobial-susceptibility-testing
https://quizlet.com/134218898/lab-test-2-flash-cards/

Antimicrobial Susceptibility-Testing

- Broth Dilution
  - Used to determined 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
  - Pic??



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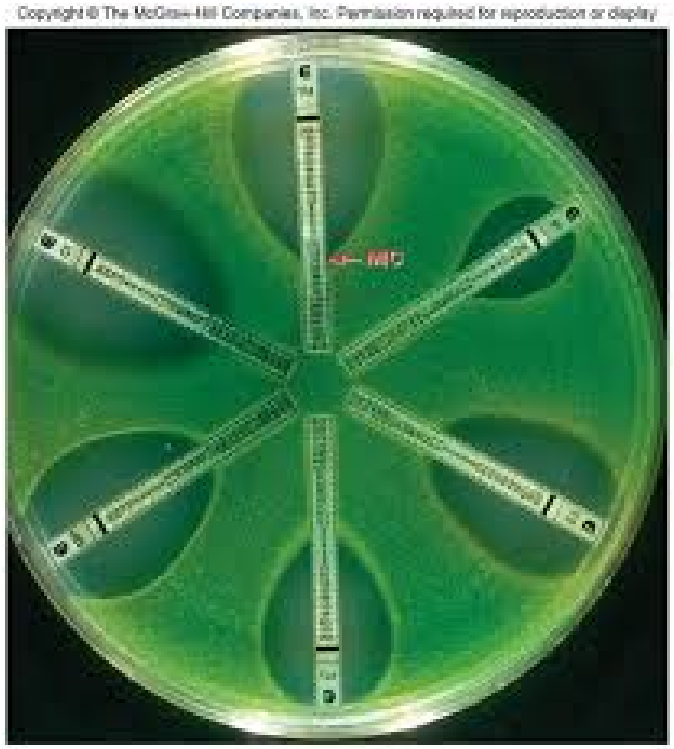
Antimicrobial Susceptibility-Testing

- 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



https://commons.wikimedia.org/wiki/File:Etest_Vancomycin_S_aureus.jpg
https://www.studyblue.com/notes/note/n/micro-lab-test/deck/6011931

Antimicrobial Susceptibility-Testing

- Synergy Testing-used to determine the inhibitory ability of combination of antibiotics
  - D-test with inducible clindamycin resistance
- β-lactamase Test- rapidly detects the enzyme β-lactamase that can be produced by bacteria
  - H. flu, Staph spp., Pseudomonas spp.

D-Test

http://aac.asm.org/content/49/3/1222.full

Antibiotic Resistance

- Mechanisms
  - Inactivation of antibiotic
    - β-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 Stewardship

- 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 complied in a region or state

Organism	Year	Number	% Susceptibility								
			Vancomycin	Gentamicin	Ciprofloxacin	Chloramphenicol	Erythromycin	Clindamycin	Tetracycline	Co-trimoxazole	Linezolid
MRSA	2007	1340	100	11	0	92	1	0	9	12	100
	2008	955	100	12	0	95	1	1	10	22	100
	2009	1283	100	8	1	94	1	1	22	50	100
MSSA	2007	1542	100	98	88	95	91	92	64	98	100
	2008	1423	100	98	90	94	88	88	64	99	100
	2009	1479	100	99	89	95	89	90	60	99	100

http://www.sirirajmedj.com/content.php?content_id=2552

Example:

- SD

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

- Association for Professionals in Infection Control and Epidemiology, Text of Infection Control and Epidemiology Chapter 24, p2-17
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