TN Department of Health

Clinical Microbiology

A Refresher

Melphine M. Harriott, PhD, D(ABMM),MLS(ASCP),SM[CM], Clinical Microbiologist | May 9, 2025

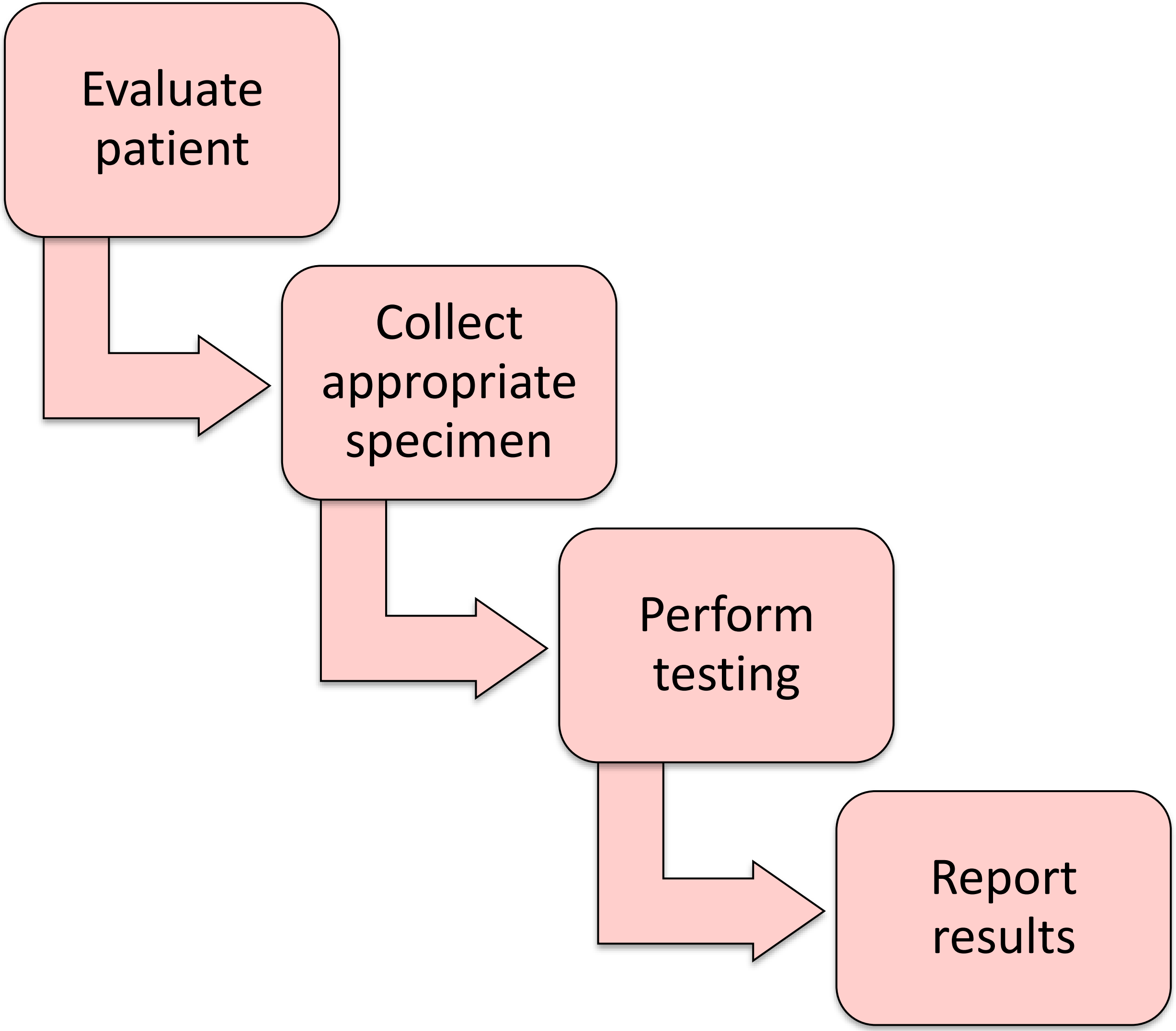
Objectives

- Explain the infectious diseases diagnostic testing process, including appropriate specimen collection.
- Describe the bacterial/fungal culture and antimicrobial susceptibility process.
- Define beta-lactamases and discuss how they contribute to bacterial resistance.

Outline

- Basics of microbiology laboratory testing
- Bacterial/fungal cultures
- Antimicrobial susceptibility testing
- Antimicrobial resistance

Basics of Microbiology Laboratory Testing


Evaluate patient
Collect appropriate specimen
Perform testing
Report results

Specimen Collection

- Most important part of the testing processing
- Good test results begin with a good specimen
- Includes:
  - Collection of specimen
  - Storage of specimen
  - Transport of specimen

Specimen Collection: Appropriate Specimen

- The specimen collected from a patient depends on clinical symptoms
  - Blood
  - Cerebrospinal fluid (CSF)
  - Respiratory
    - Throat
    - Sputum
    - Bronchial washings
  - Eye
  - Ear
  - Wound / Tissues
  - Urine
  - Genital specimens
  - Stool

Sterile vs. non-sterile site

Specimen Collection: Important Factors

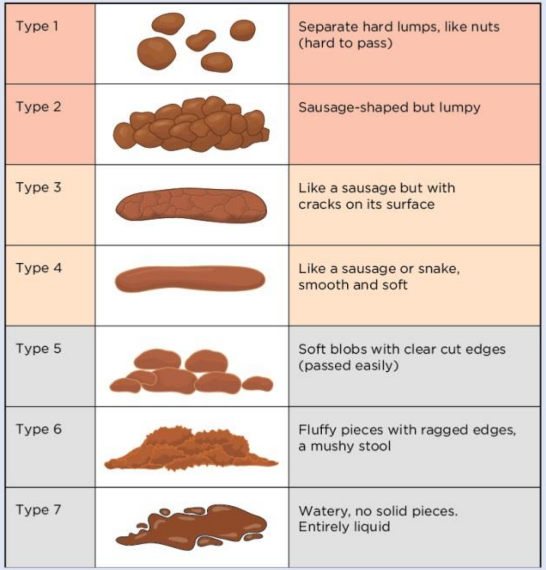
- Timing of collection
- Amount of material
- Contamination
  - Normal microbiota or other contamination
- Labeling
- Storage
- Transportation

Case in Point: *C. difficile*

- Collect specimens only on symptomatic patients
  - Colonization vs. Infection

- Collect stool
  - Unformed, liquid stool
  - Patient should not be on laxatives
  - Collect in approved sterile container based on lab guidance
  - Transport to lab within designated time and temperature

Fig 1. **Bristol Stool Chart**



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Infectious Diseases Diagnostic Tests

- Microscopy
- Culture
- Molecular methods
- Immunological analysis

Microscopy

- Can be performed directly from patient specimen or from a culture
- Mainly used for bacteria, fungi and parasites
  - Rarely used for viruses
- Various stains may be used to visualize the microorganism
  - Example: Gram stain

Culture

- Mainly used for bacteria, fungi
- Rarely used for viruses
  - Viruses are obligate intracellular pathogens
  - Need host cells to grow; must grow on live cells in the laboratory
- Some parasite can be cultured but it's rarely performed

Molecular Methods

- Can be performed directly from patient specimen or from a culture
- Used for bacteria, fungi, viruses and parasites

- Includes:
  - DNA probes
  - NAAT (nucleic acid amplification test)
  - PCR (polymerase chain reaction)
  - 16s
  - Sequencing

Immunological Analysis

- Evaluation of
  - Antigen - protein on microorganism
  - Antibody - immune response to microorganism
    - IgM antibody – produced during acute phase of disease
    - IgG antibody – produced later on during the disease process; can persist for life
- Examples test methods
  - ELISA, EIA, lateral flow

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Immunological Analysis

- Evaluation of
  - Antigen - protein on microorganism
  - Antibody - immune response to microorganism
    - IgM – acute response
    - IgG – chronic or resolved infection

- Used for bacteria, fungi, viruses and parasites

Bacterial/Fungal Culture

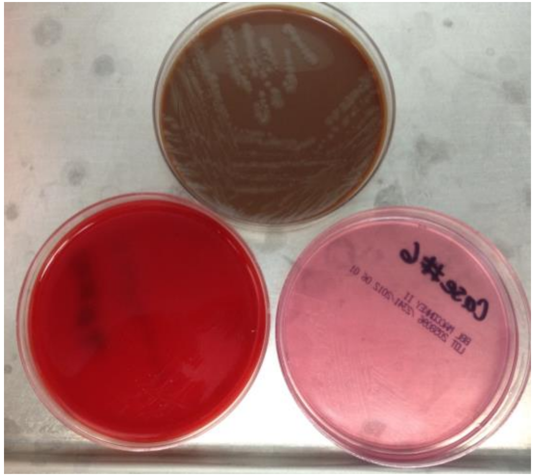
Culture

- The process of growing microorganisms by taking bacteria from the infection site and growing them in the artificial environment of the laboratory

- Must recreate body environment in the lab including
  - Temperature
  - Atmosphere
  - Nutrients

Culture Media

- Provide the nutrients the organism needs to grow outside the body



Harriott, 2012

Culture Process

- Process patient specimen
- Choose correct media plates for specimen type and the organism that may be causing the infection
- Inoculate media with patient specimen
- Incubate at correct temperature, atmosphere and for the correct amount of time

Culture Incubation Time

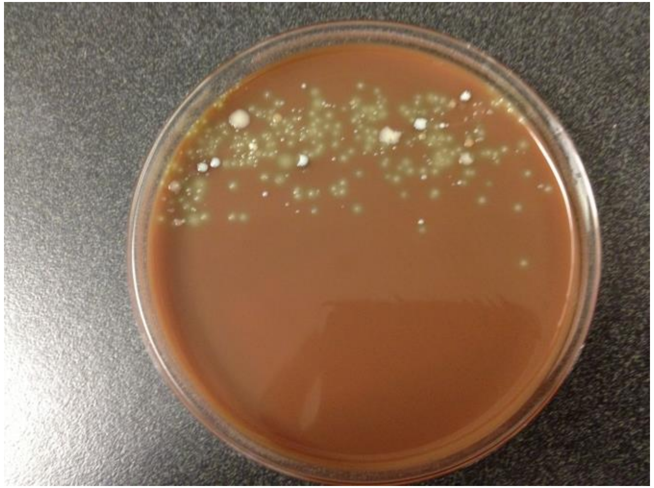
- Appropriate time
  - Varies for specimen and organism
    - 1 -2 days for most bacteria
    - 3 weeks for fungi
    - 6 weeks for mycobacteria

- After predetermined incubation time, remove plates and identify any organisms growing

Identification of Growth

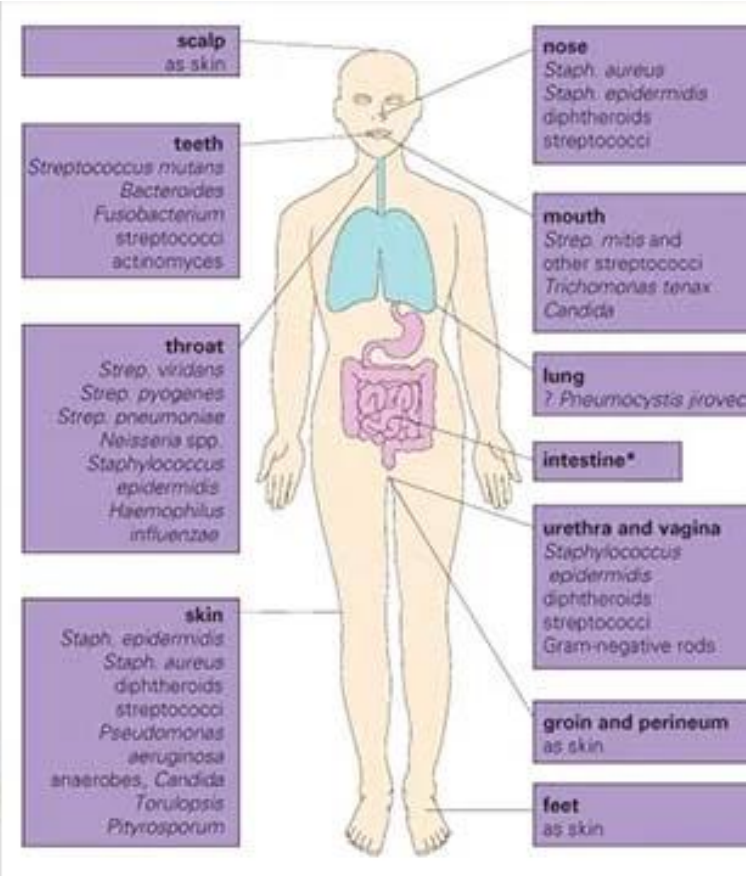
- Evaluate plates after designated incubation period for growth of pathogens
- Must distinguish pathogen from microbiota

Harriott, 2013



Harriott, 2013

Normal Flora



https://microbenotes.com/normal-human-microbiota/

Identification of Growth

- If a potential pathogen is suspected, then further testing is warranted
- Type of tests
  - Phenotypic
    - Evaluation of the expression of genetic characteristics
  - Genotypic
    - Use of molecular techniques to evaluate RNA or DNA from colony growing on plate

Phenotypic Identification

- Evaluation of colonies
- Biochemical testing
  - Mostly automated
- MALDI-TOF
  - Mass spectroscopy

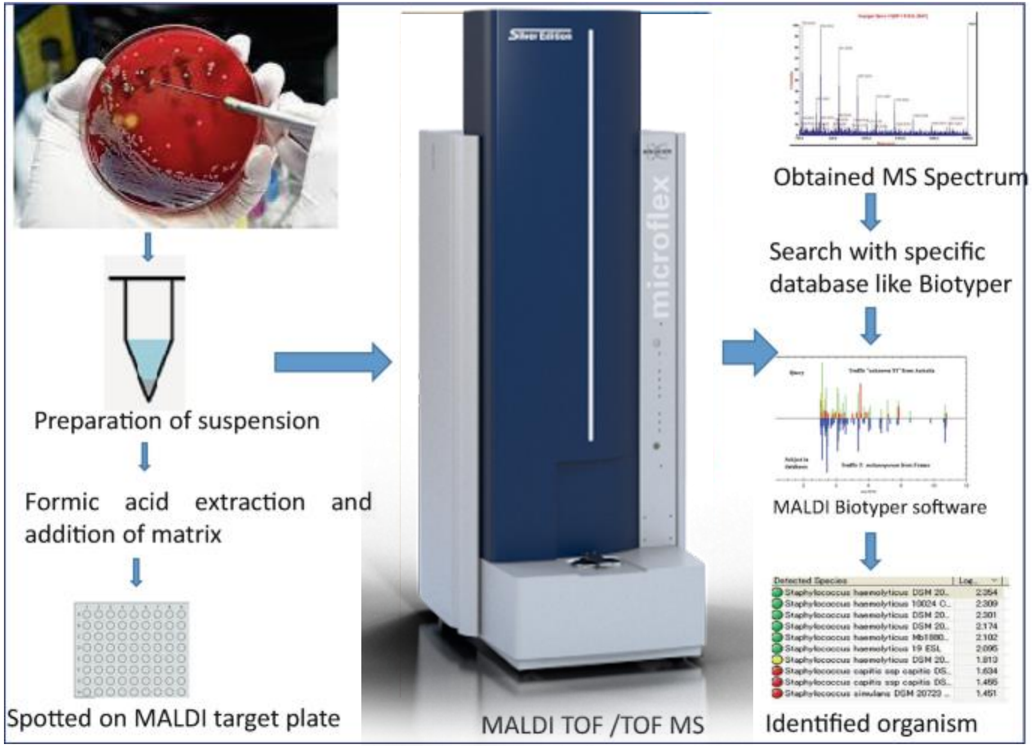
Staphylococcus aureus

Harriott, 2012

Harriott, 2012

Harriott, 2004

MALDI-TOF



Mandal, S.M., Paul, D. (2022). MALDI-TOF MS for Bacterial Identification. In: Automation and Basic Techniques in Medical Microbiology. Springer, New York, NY. https://doi.org/10.1007/978-1-0716-2372-5_6

Total time varies – average 3-7 days

Example workflow

Evaluate 24-72 h bacterial culture

Microscopic evaluation:
Gram Stain bacteria from culture plates

Macroscopic Evaluation:
Evaluate growth on plates (e.g. what plates are the bacteria growing on; hemolysis?)

Genotypic and Phenotypic Identification:
Choose and perform appropriate identification tests

Identify genus and species

Perform susceptibility Testing

Blood Cultures

Clinical signs/symptoms

Blood cultures drawn

- Typically, 2 sets drawn 10-15 minutes apart
- 1 set = 2 bottles (aerobic and anaerobic)
- Each set will have a different accession number
    - So, 2 bottles will have the same accession number

- Bottles received in the lab and placed on the machine (analyzer).
- Bottles remain on the machine for 5 days.
- Bottles that stay negative after 5 days are reported as "No Growth at 5 days"
- Anytime during the 5 days, a bottle may become positive.

Positive bottle (s)

As soon as a bottle is flagged positive, all three of the following actions are taken (most often within 1 hour of the bottle turning positive)

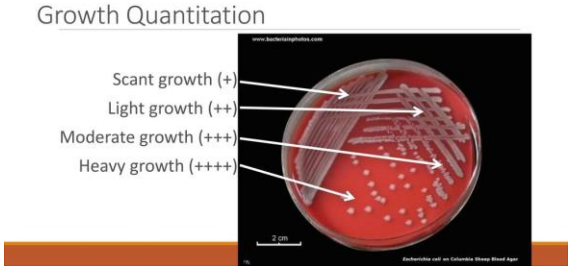
- Blood from bottle removed and Gram stained.
    - Gram stain is called to floor (i.e. Gram-negative cocci in clusters)
- Blood from bottle is removed and placed on petri dishes/plates for culture.
    - Culture plates are incubated for 3-7 days
    - If a bacteria or fungus grows on the plates the organism is identified and if appropriate (e.g., *S. aureus*), susceptibility testing is automatically performed (e.g., oxacillin S)
    - Culture results take 3-7 days to complete
- Blood from a bottle is removed and used for molecular (e.g., PCR) testing.
    - Most of these tests identify the organism and can also detect resistance markers (e.g., KPC, NDM, vanA, mecA)
    - Even if molecular testing is performed, culture-based testing results (explained above) will follow.
    - Rapid molecular blood culture assays take 1-2 hours to complete

Blood sent for sequencing

- In a very small proportion of cases, blood may be sent out for sequencing
- This may occur in difficult diagnose cases or for other reasons
- May be referred to as WGS (whole genome sequencing), NGS (next generation sequencing) or by the actual company name (e.g., Karius)

Culture Reports

- Sterile sites
  - Growth or no growth
- Normal flora
- Quantity of organisms
  - Rare, Few, Moderate, Many
  - 1+, 2+, 3+, 4+
  - Urines: colony forming units per mL of urine (CFU/mL)



Antimicrobial Susceptibility Testing and Stewardship

Antimicrobial Susceptibility Testing

- Testing to determine if an antimicrobial agent can be used to effectively treat an infection
- Phenotypic
  - Evaluating expression of a gene
  - Main method for bacteria and fungi
- Genotypic
  - Evaluating the presence of a gene
  - Can be performed for bacteria, fungi, and viruses
- Susceptibility testing for parasites rarely performed

Phenotypic Susceptibility Testing

- Purpose:
  - Predict whether the bacteria is capable of expressing resistance to the antimicrobial agents that are potential choices as therapeutic agents for managing the infection
- The tests are performed under standardized conditions
  - Ensures reproducibility
- Guide not a standard!!
  - Results should be combined with clinical information and experience when selecting the most appropriate antibiotic for a particular patient.

Choosing Antimicrobials to Test

- Number of factors play a role in this
  - Gram-negative vs. Gram-positive
  - Intrinsic resistance
  - Availability of antimicrobials
  - Site of infection

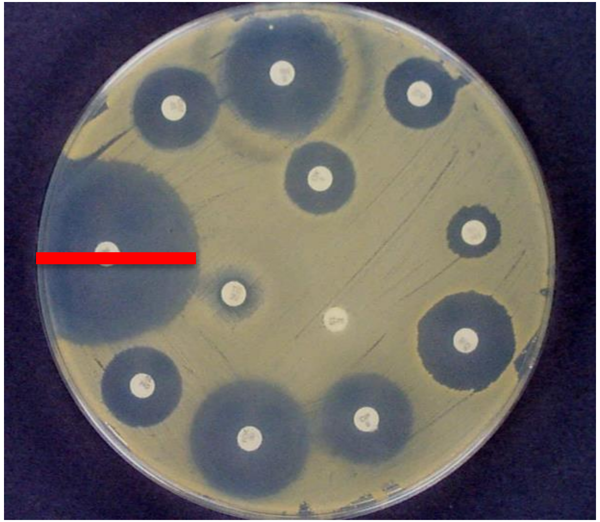
Main Methods

- Disk diffusion (Kirby-Bauer)
- Broth Dilution
- E-test

- These methods are standardized to ensure reproducibility and accuracy

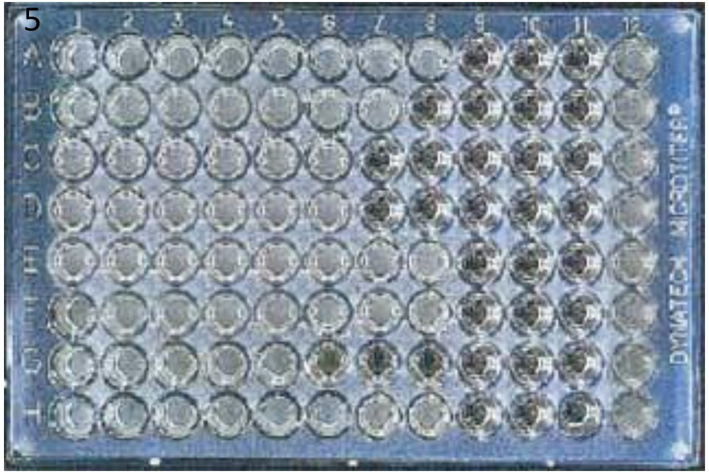
Disk Diffusion (Kirby Bauer)

- Concentrations of antimicrobial agents are incorporated into filter paper disk
- Culture plate is incubated with bacteria and discs overnight
- Measure zone of inhibition and determine if it is susceptible, intermediate, or resistant



Broth Dilution Method

- Different concentrations of antibiotics are placed in micro dilution tray
- A suspension of the bacterial organism is also added to each wells
- Incubate overnight
- Look for growth or lack of growth in well (turbidity indicates growth)
- Determine the MIC



Antimicrobial Resistance

Antibiotic Resistance Mechanisms

- Altered antibiotic transport
  - Efflux pumps
- *Enzyme inactivation*
  - *Activity of the antibiotic is hindered due to hydrolysis*
- Target by-pass
  - Prevent the drug from getting to the target site
- Target modification
  - Bacteria modify the antibiotic target rendering it inactive

- These mechanisms can be
  - inherited (passed on generation to generation)
  - acquired

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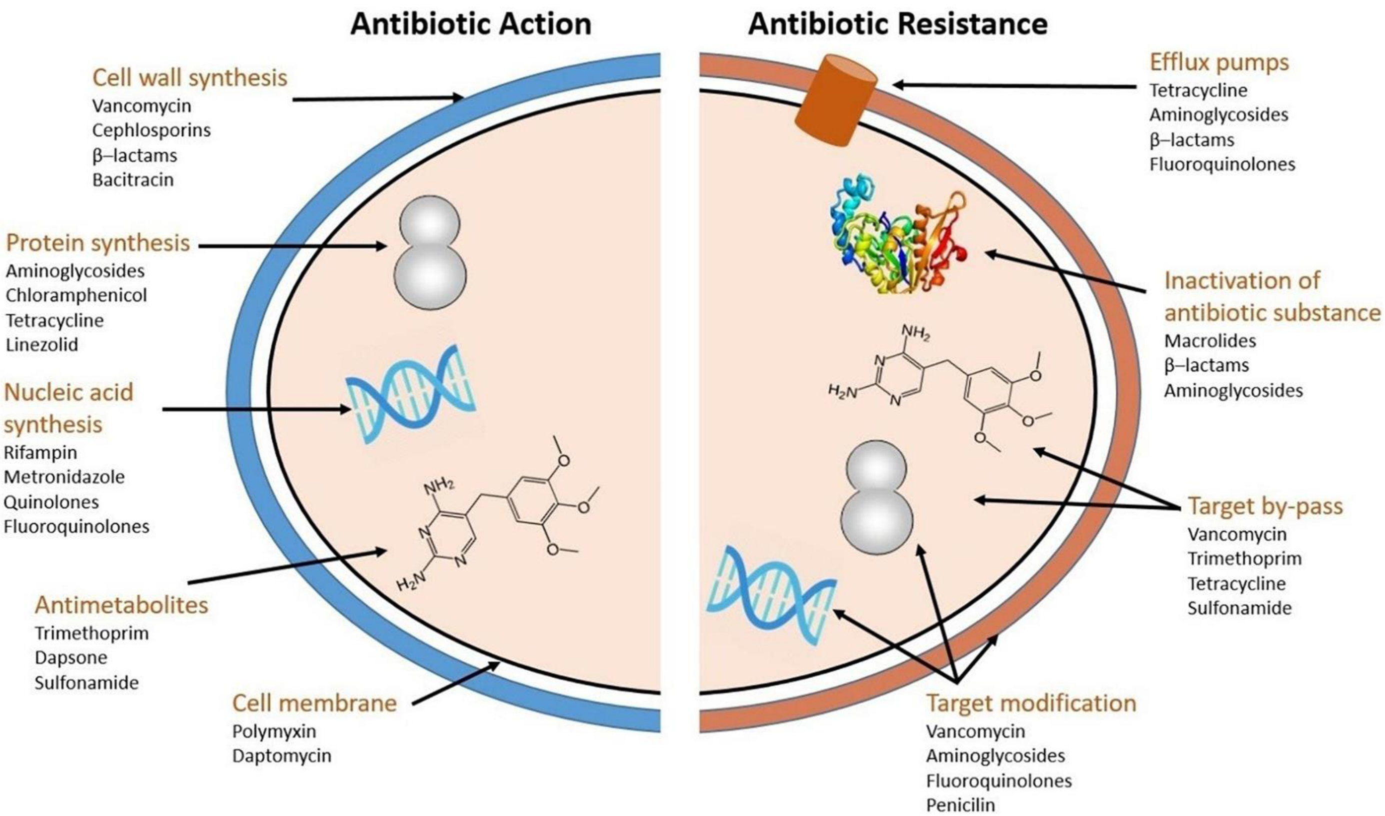

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Bacterial Genome

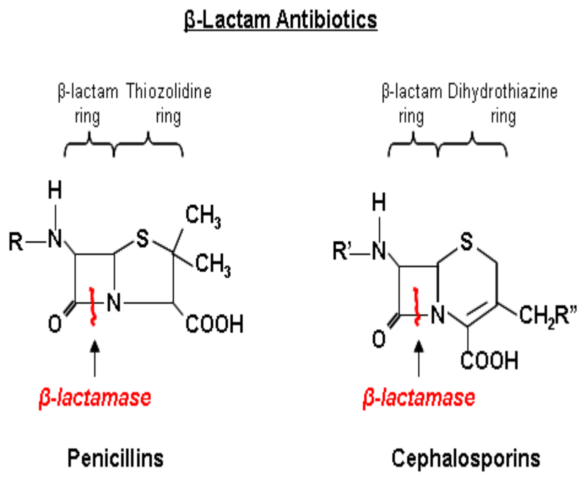
- Chromosomal DNA

- Extrachromosomal DNA (Plasmid)
  - Physically separated from chromosome
  - Contains few genes, not essential for the organism to function
  - Replicates independently of the genome
  - Type of mobile genetic element
  - Can be horizontally transferred

Production of Beta-Lactamase Enzymes

- Bacteria destroy the beta-lactam ring
  - The antibiotic is no longer active

- Examples:
  – Extended spectrum beta-lactamase (ESBL)
  – AmpC beta-lactamase
  – Carbapenemase



Rocha, Antônio & Barsottini, Mario & Rocha, Renan & Laurindo, Maria & Leandro, Francisco & Moraes, Francisco & Rocha, Soraya. (2019). Pseudomonas Aeruginosa: Virulence Factors and Antibiotic Resistance Genes. Brazilian Archives of Biology and Technology. 62. 19180503. 10.1590/1678-4324-2019180503.

Carbapenemase Genes

The "Big-5"

- *Klebsiella pneumoniae* carbapenemase (KPC)
- Imipenemase Metallo-β-lactamase (IMP)
- New Delhi Metallo-β-lactamase (NDM)
- Oxacillinase-48-type carbapenemase (OXA-48)
- Verona integron-encoded Metallo-β-lactamase (VIM)

Summary

- Identification of microorganisms from patient specimen begins in specimen collection
  - Most important step
- Types of tests include culture, molecular and immunological
- Antimicrobial susceptibility testing is performed to help determine which antimicrobial can be used for treatment
  - Testing can be phenotypic or genotypic
- Beta-lactamases are enzymes produced by some bacteria that inactivate antibiotics resulting in resistance

Questions?

Melphine.Harriott@tn.gov