

Antibacterial susceptibility testing

Antibiotics: are natural chemical substances produced by certain groups of microorganisms (fungi, bacteria) that inhibit the growth of or kill the other bacteria that cause infection. Several hundreds of compounds with antibiotic activity have been isolated from microorganisms over the years, but only a few of them are clinically-useful. The reason for this is that only compounds with selective toxicity can be used clinically. Antibiotics may have a cidal (killing) effect or a static (inhibitory) effect on a range of microbes.

The **selective toxicity** of antibiotics means that they must be highly effective against the microbe but have minimal or no toxicity to humans

Types of antibiotic:- **Narrow spectrum antibiotic** active against either Gram +ve bacteria only or gram –ve bacteria only

Broad spectrum antibiotic active against both Gram's +ve and -ve bacteria

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Mechanism of action of antibiotics

Classification of antibiotics based on mode of action include:

■ Inhibit Cell synthesis

Penicillin (P) Bacitracin (Ba) –Vancomycin (Va)

■ Inhibit Protein synthesis

Erythromycin (E) - Gentamycin (GN)

■ Inhibit nucleic acids

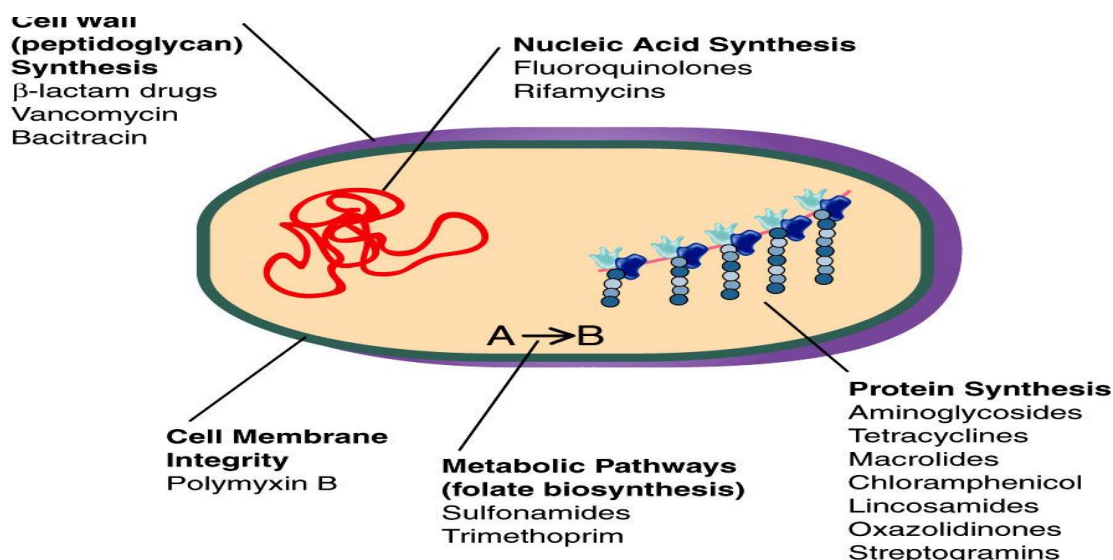
DNA/RNA Rifampicin (RF) –Ciprofloxacin (CIP)

■ Inhibit of selective permeability of cell membrane

Polymyxins

■ Inhibit bacterial metabolism

(SXT) sulphamethoxazole/trimethoprim



Antimicrobial Susceptibility testing can be done by the ways:

1. Disk diffusion

(Kirby Bauer)

(Modified Stock Technique)

2- Broth dilution method

Minimum Inhibitory Concentration (MIC)

Minimum Bactericidal Concentration (MBC)

Etest

Disc Diffusion Method

The effectiveness of an antibiotic in this technique is based on the size of the zone of inhibition that surrounds a disc that has been impregnated with a specific concentration of the agent.

Advantages: Rapid

Accurate

Inexpensive

The disk-diffusion method (Kirby-Bauer) is more suitable for routine testing in a clinical laboratory where a large number of isolates are tested for susceptibility to numerous antibiotics.

An agar plate is uniformly inoculated with the test organism

A paper disk impregnated with a fixed concentration of an antibiotic is placed on the agar surface.

Growth of the organism and diffusion of the antibiotic commence simultaneously resulting in a circular zone of inhibition in which the amount of antibiotic exceeds inhibitory concentrations.

The diameter of the inhibition zone is a function of the amount of drug in the disk and susceptibility of the microorganism.

This test must be rigorously standardized since zone size is also dependent on:

- inoculum size,
- medium composition,
- temperature of incubation,
- excess moisture and
- thickness of the agar.

Zone diameter can be correlated with susceptibility as measured by the dilution method.

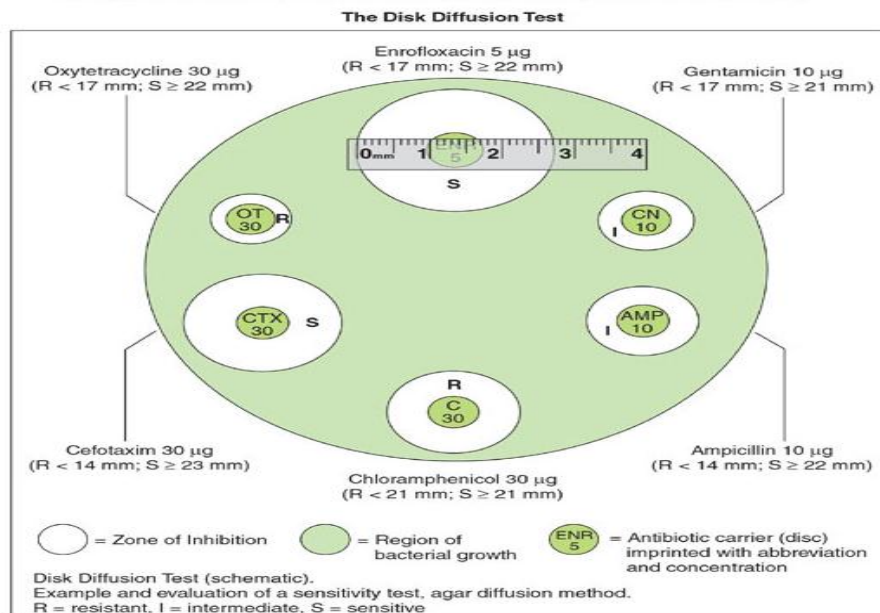
Further correlations using zone diameter allow the designation of an organism as "**susceptible**", "intermediate", or "**resistant**" to concentrations of an antibiotic which can be attained in the blood



or other body fluids of patients requiring chemotherapy.

Using a dispenser, antibiotic-impregnated disks are placed onto the agar surface.

As the bacteria on the lawn grow, they are inhibited to varying degrees by the antibiotic diffusing from the disk.



Method

Preparation of inoculum

1. Using a sterile inoculating loop, touch four or five isolated colonies of the organism to be tested.
2. Suspend the organism in 5 ml of sterile saline.
3. Vortex the saline tube to create a smooth suspension.
4. Adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy.
5. Use this suspension within 15 minutes of preparation.

FIG. 1. McFarland standards (left to right) 0.5, 1.0, 2.0, 3.0, positioned in front of a Wickerham card.

McFarland standards are used to prepare bacterial suspensions to a specified turbidity. In the Kirby-Bauer disk diffusion susceptibility test protocol, the bacterial suspension of the organism to be tested should be equivalent to the 0.5 McFarland standard.

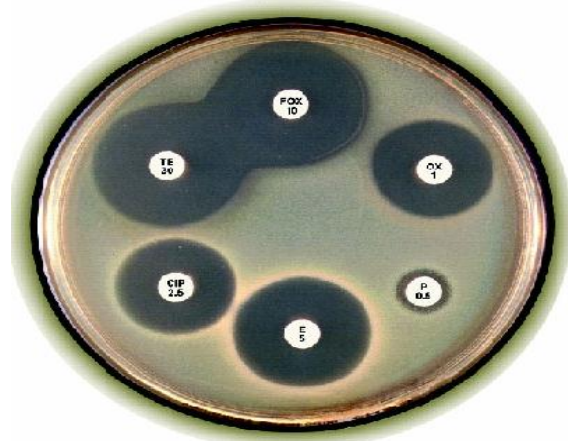
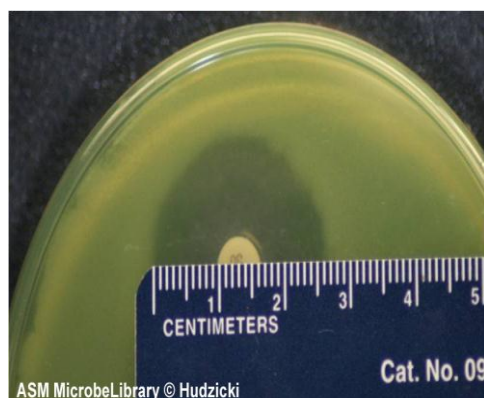


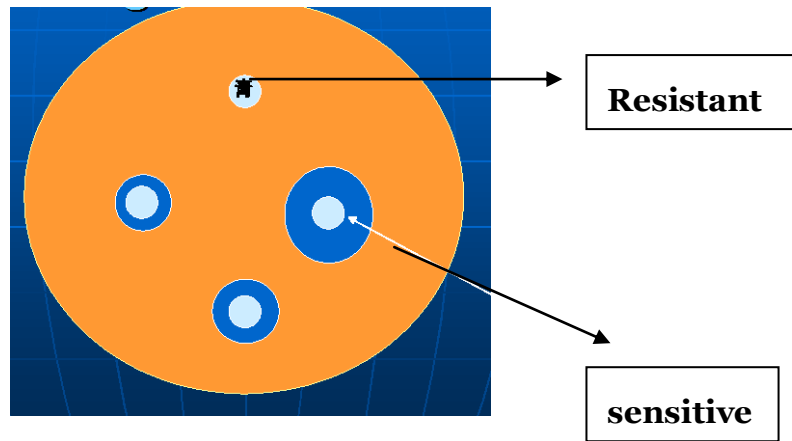
Inoculation of the Mueller-Hinton plate (MH) plate

1. Dip a sterile swab into the inoculum tube.
2. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet
3. Inoculate the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface
4. Discard the swab into an appropriate container.
5. Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes

Placement of the antibiotic disks

1. Place the appropriate antimicrobial-impregnated disks on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time, or a multidisk dispenser to dispense multiple disks at one time.
2. Once all disks are in place, replace the lid, invert the plates, and place them in a 35°C air incubator for 16 to 18 hours.





Minimum Inhibitory Concentration (MIC) :

Principle:

- The tube dilution test is the standard method for determining levels of resistance to an antibiotic.
- Serial dilutions of the antibiotic are made in a liquid medium which is inoculated with a standardized number of organisms and incubated for a prescribed time.
- The lowest concentration of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC).

MIC: It is the lowest concentration of the antimicrobial agent that inhibits the growth of the test organism but not necessarily kills it.

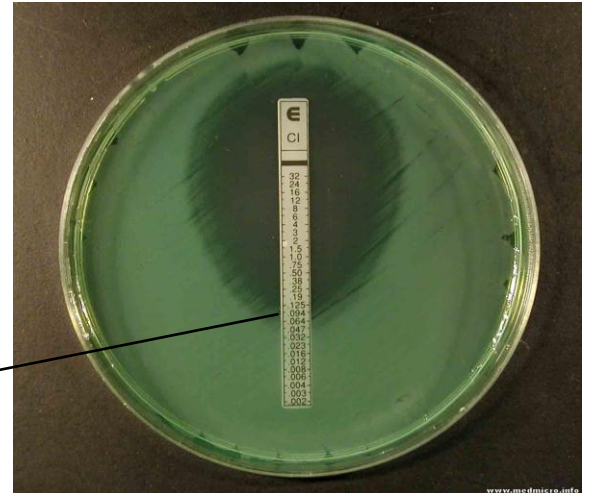
MBC (minimum bactericidal conc.): It is the lowest concentration of the antimicrobial agent that kills the test organism.

E-test: A plastic coated strip contains a gradient of antibiotic concentrations and the minimal inhibitory concentration is read from a scale printed on the strip

E-test for *P.aeruginosa*



MIC



Chemical class	Examples	Biological source	Spectrum (effective against)	Mode of action
Beta-lactams (penicillins and cephalosporins)	Penicillin G, Cephalothin	<i>Penicillium notatum</i> and <i>Cephalosporium</i> species	Gram-positive bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Semisynthetic beta-lactams	Ampicillin, Amoxicillin		Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Clavulanic Acid	Augmentin is clavulanic acid plus Amoxicillin	<i>Streptomyces clavuligerus</i>	Gram-positive and Gram-negative bacteria	Inhibitor of bacterial beta-lactamases
Monobactams	Aztreonam	<i>Chromobacterium violaceum</i>	Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Carboxypenems	Imipenem	<i>Streptomyces cattleya</i>	Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Aminoglycosides	Streptomycin	<i>Streptomyces griseus</i>	Gram-positive and Gram-negative bacteria	Inhibits translation (protein synthesis)
	Gentamicin	<i>Micromonospora</i> species	Gram-positive and Gram-negative bacteria esp. <i>Pseudomonas</i>	Inhibits translation (protein synthesis)
Glycopeptides	Vancomycin	<i>Amycolatopsis orientalis</i> <i>Nocardia orientalis</i> (formerly designated)	Gram-positive bacteria, esp. <i>Staphylococcus aureus</i>	Inhibits steps in murein (peptidoglycan) biosynthesis and assembly
Lincomycins	Clindamycin	<i>Streptomyces lincolnensis</i>	Gram-positive and Gram-negative bacteria esp. anaerobic <i>Bacteroides</i>	Inhibits translation (protein synthesis)
Macrolides	Erythromycin, Azithromycin	<i>Streptomyces erythreus</i>	Gram-positive bacteria, Gram-negative bacteria not enterics, <i>Neisseria</i> , <i>Legionella</i> , <i>Mycoplasma</i>	Inhibit translation (protein synthesis)
Polypeptides	Polymyxin	<i>Bacillus polymyxa</i>	Gram-negative bacteria	Damages cytoplasmic membranes
	Bacitracin	<i>Bacillus subtilis</i>	Gram-positive bacteria	Inhibits steps in murein (peptidoglycan) biosynthesis and assembly
Polyenes	Amphotericin	<i>Streptomyces nodosus</i>	Fungi (<i>Histoplasma</i>)	Inactivate membranes containing sterols
	Nystatin	<i>Streptomyces noursei</i>	Fungi (<i>Candida</i>)	Inactivate membranes containing sterols
Rifamycins	Rifampicin	<i>Streptomyces mediterranei</i>	Gram-positive and Gram-negative bacteria, <i>Mycobacterium tuberculosis</i>	Inhibits transcription (bacterial RNA polymerase)

Tetracyclines	Tetracycline	<i>Streptomyces</i> species	Gram-positive and Gram-negative bacteria, Rickettsias	Inhibit translation (protein synthesis)
Semisynthetic tetracycline	Doxycycline		Gram-positive and Gram-negative bacteria, Rickettsias <i>Ehrlichia</i> , <i>Borrelia</i>	Inhibit translation (protein synthesis)
Chloramphenicol	Chloramphenicol	<i>Streptomyces venezuelae</i>	Gram-positive and Gram-negative bacteria	Inhibits translation (protein synthesis)
Quinolones	Nalidixic acid	synthetic	Mainly Gram-negative bacteria	Inhibits DNA replication
Fluoroquinolones	Ciprofloxacin	synthetic	Gram-negative and some Gram-positive bacteria (<i>Bacillus anthracis</i>)	Inhibits DNA replication
Growth factor analogs	Sulfanilamide, Gantrisin, Trimethoprim	synthetic	Gram-positive and Gram-negative bacteria	Inhibits folic acid metabolism (anti-folate)
	Isoniazid (INH)	synthetic	<i>Mycobacterium tuberculosis</i>	Inhibits mycolic acid synthesis; analog of pyridoxine (Vit B6)
	para-aminosalicylic acid (PAS)	synthetic	<i>Mycobacterium tuberculosis</i>	Anti-folate