# Antibiotic Sensitivity Test

**Antimicrobial Susceptibility Tests :-** are very essential step for the proper treatment of infectious diseases.

**Antimicrobial susceptibility** tests measure the ability of an antibiotic or other antimicrobial agent to **inhibit** bacterial growth.

**Terms Defined :-**

**1-Prophylaxis:-** use of drugs to prevent infection (**before development of a disease** )**.**

**2- Selective Toxicity :-** selective inhibition of the growth of the microorganisms without damage to the host .

**3- Broad Spectrum :-** affect variety of bacteria both Gram positive and Gram negative ( e.g. **tetracycline** ).

**4- Narrow Spectrum :-** antibiotic are active  against one or very few types ( e.g. **vancomycin** is primarily used against certain Gram positive cocci ( e.g. *Staphylococci* ).

**5- Bacteriostatic Antibiotic :-** drugs inhibits their growth but does not kills bacteria.

**6- Bactericidal Antibiotic :-** drugs kills bacteria .

**7-Minimum Inhibitory Concentration (MIC):-** The lowest concentration of antimicrobial agents required to **inhibit** the growth of microorganism. Unit of MIC : micrograms per milliliter (μg/ml) or milligrams per liter (mg/L).  . **Importance :-**

1- Clinically , MIC used to determine the amount of antibiotic that the patient will receive.

2- Determine the types of antibiotic used.

3- This will lower the opportunity for **microbial resistance** to specific antimicrobial agents.

4-Determine the degree of **sensitivity** or **resistance** of the pathogen to a specific range of antibiotic concentration.

**8-Minimum Bactericidal Concentration (MBC):-** the lowest concentration of the antimicrobial agent to **kill** microorganism. Unit of MBC : μg/ ml .

**Mechanisms of Action of Antimicrobial Agents:-**

**1- Inhibition of Cell Wall Synthesis:-** They are **bactericidal** . They block the synthesis of the peptidoglycan. They do not affect Eukaryotic cells ( e.g. human ). **Major groups of antibacterial agents are :-**

➢ **Beta-lactams :-** e.g. penicillin's , cephalosporin's.

➢ **Glycopeptide :-** e.g. vancomycin.

➢ **Polypeptide :-** e.g. Bacitracin.

**2- Inhibition of Protein Synthesis:-** several drugs inhibit protein synthesis in bacteria without significantly interfering with protein synthesis in human cells, because they selective is due to the differences between bacteria and human ribosomal protein. Bacteria have 70S ribosomes with 50S and 30S subunit , whereas human cells have 80S ribosomes with 60S and 40S subunits.

<span style="color:red">**Mode of Action of Antibiotic That inhibit protein synthesis:-**</span>

| Antibiotic | Ribosomal subunit | Bactericidal or bacteriostatic |
|---|---|---|
| Aminoglycosides | 30S | Bactericidal |
| Tetracycline's | 30S | bacteriostatic |
| Chloramphenicol | 50S | Both |
| Clindamycin | 50S | bacteriostatic |
| Macrolides | 50S | bacteriostatic |
| Telthromycin | 50S | Both |
| Streptogramines | 50S | Both |

**3- Inhibition of Nucleic Acid Synthesis:-**

The mode of action and clinically useful activity of the important drugs that act by inhibiting nucleic acid synthesis , they are generally **<u>bactericidal</u>**.

**Several classes of antimicrobial agent act  microbial nucleic acid including :-**

> ➢ **Fluoroquinolones :-** block DNA gyrase , lead inhibit DNA synthesis.

> ➢ **Rifampicin :-** block RNA polymerase, lead inhibit RNA synthesis.

**4- Inhibition of Cell Membrane :-**

There are few antimicrobial compounds that act on the cell membrane because the structure and chemical similarities of bacteria and human cell membrane make it difficult to provide sufficient selective toxicity include **polymyxins** & **daptomycins** .
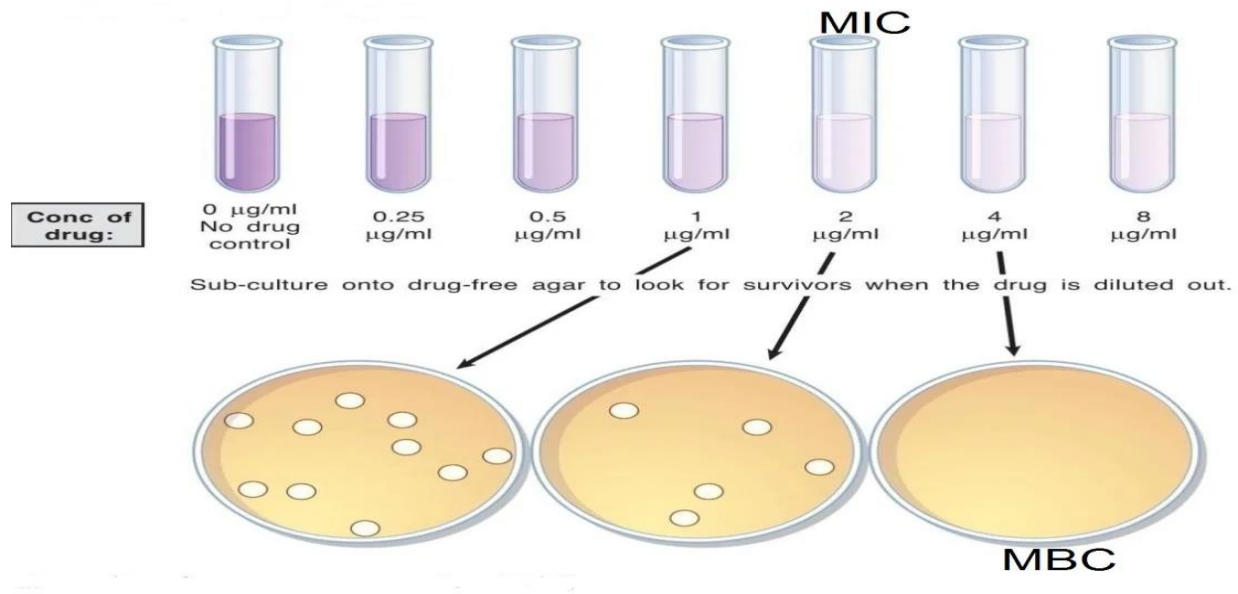
 **Methods of Antibiotic Susceptibility Testing:-**

The methods of antimicrobial susceptibility testing are divided into types based on the principle of testing involved: -

**1) Dilution:** Broth dilution.

**2) Diffusion:** Kirby-Bauer method (antibiotics disks) .

**3) Diffusion and Dilution**: E-test method.

**1-Dilution Method :-**

The **<u>Broth dilution</u>** method involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment. Each **antimicrobial agent** is tested using a range of concentrations commonly expressed as (μg) of active drug/ ml of broth (**i.e., μg/ ml**). Typically, the range of the concentrations tested for each antibiotic are series of **<u>doubling dilution</u>** (e.g., 16, 8, 4, 2, 1, 0.5 μg/ ml). For broth dilution methods determine minimal inhibitory concentration ( MIC ). The MIC is the minimum concentration of the antibiotic that will inhibit this particular isolate. The microbes inoculate in the tubes and are incubated for 16–20 hours. The MIC is generally determined by **turbidity**.

## 2) Diffusion: Kirby-Bauer method (antibiotics disks):-

➢ **The Kirby-Bauer** are generally used for antibiotics susceptibility testing. The Kirby-Bauer method makes use of **antibiotics disks** which are placed on the plates which are inoculated with the test organism. After incubation for the required time, the **zone of inhibition** is measured for the test antibiotics. Depending on the diameter of the zone of inhibition, There are three ways in which the microorganism is affected :-

❑ **Sensitive (susceptible).**

❑ **Intermediate.**

❑ **Resistant.**

➢ **Mueller – Hinton Agar** it is a special media containing beef infusion, peptone and starch, used primarily for the disk diffusion method.
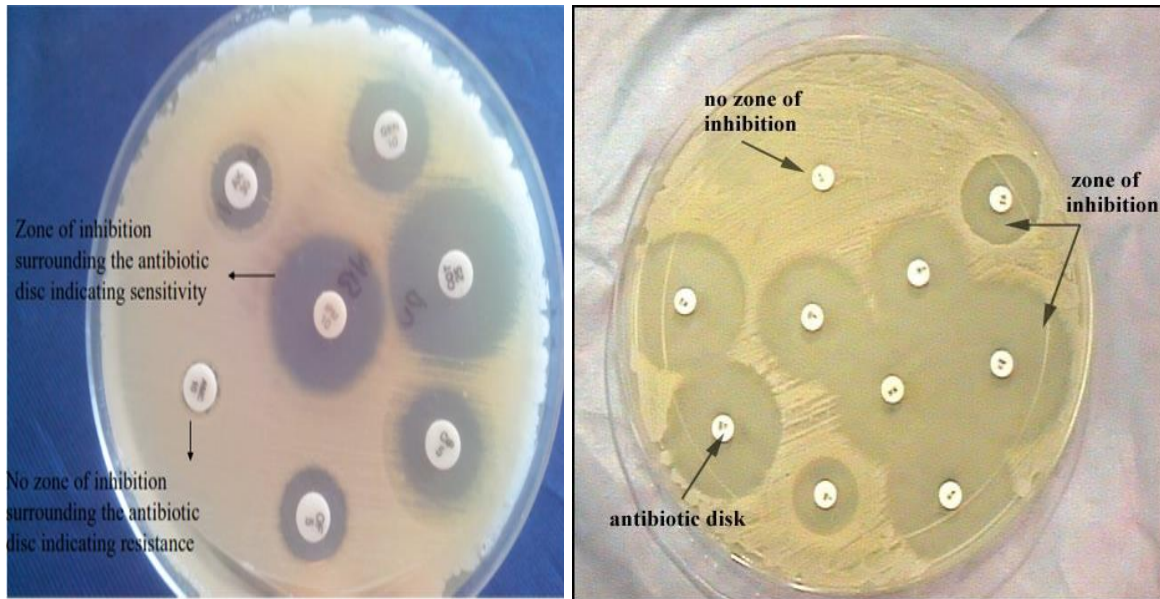
**Procedure of The Kirby-Bauer Methods :-**

**1- Preparation of Plates:** only **Mueller – Hinton medium** can be used. The medium should be allowed to cool to **45-50°C** before **5 %** percent of blood is added when susceptibility testing is done on *Streptococcus* and Campylobacter species.

**2- Preparation of Inoculums:** A **small** colonies form an agar medium are touched with a wire loop and the growth is transferred to a test tube containing sterile **normal saline**. Comparison should be made against a white background with a contrasting black line. Before use the standard should be **shaken vigorously** (**0.5 McFarland standard**). Comparison between test tube culture and McFarland standard depending on **turbidity**.

**3- Inoculation: Lawn culture** are prepared by flooding the surface of the plate with a liquid culture . A sterile cotton – **wool swab** is dipped in to the suspension and surplus removed by rotation of the swab against the side of the tube above the fluid level. The medium is inoculated by even **streaking** of swab over the **entire surface** of the plate in **three direction**.
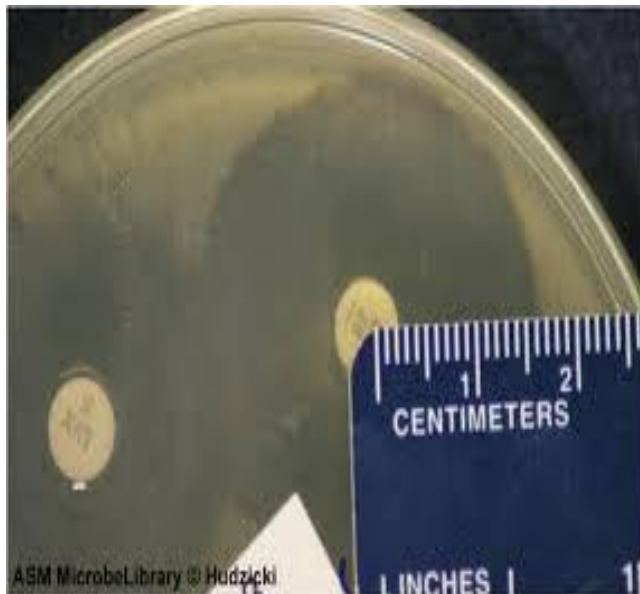
 **4- Antibiotic Disc:** After the inoculum has dried, **single discs** are applied with **forceps**, or a **sharp needle** and pressed gently to ensure even contact with the medium. Not more than **six discs** can be accommodated on an 8.5cm circular plate.
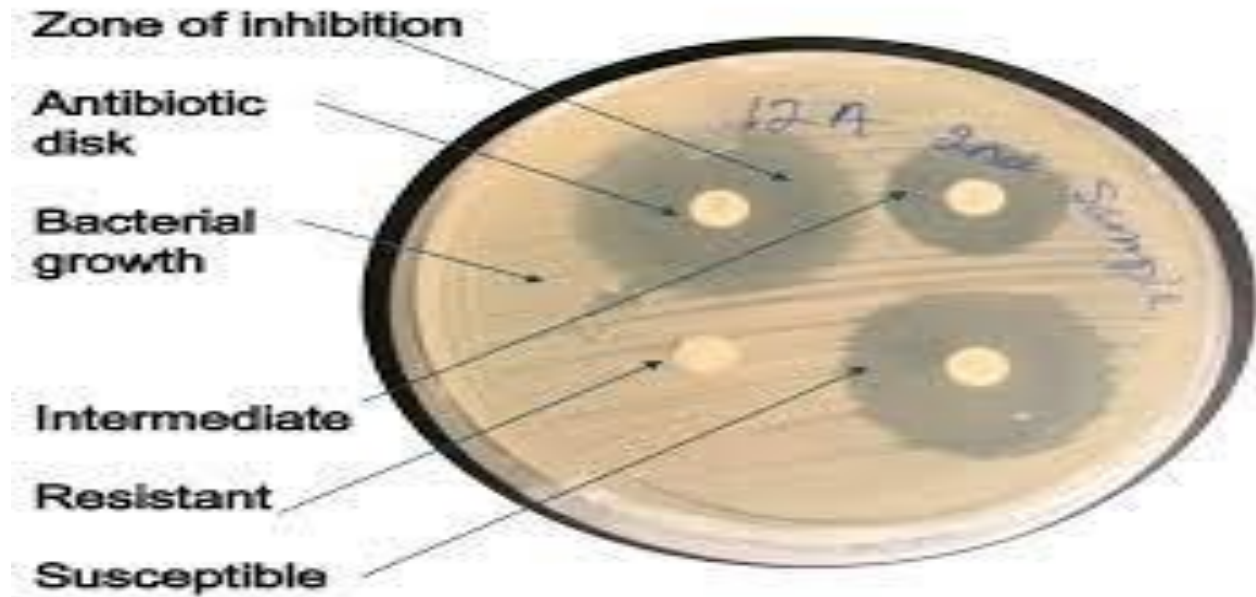
**5-Incubation:** plates are incubated inverted for (16-18) hr. at(35 – 37) °C.

**6- Reading of Zones of Inhibition :**the diameters of the zones are measured to the nearest (mm) with **calipers** or millimeter **rule**.
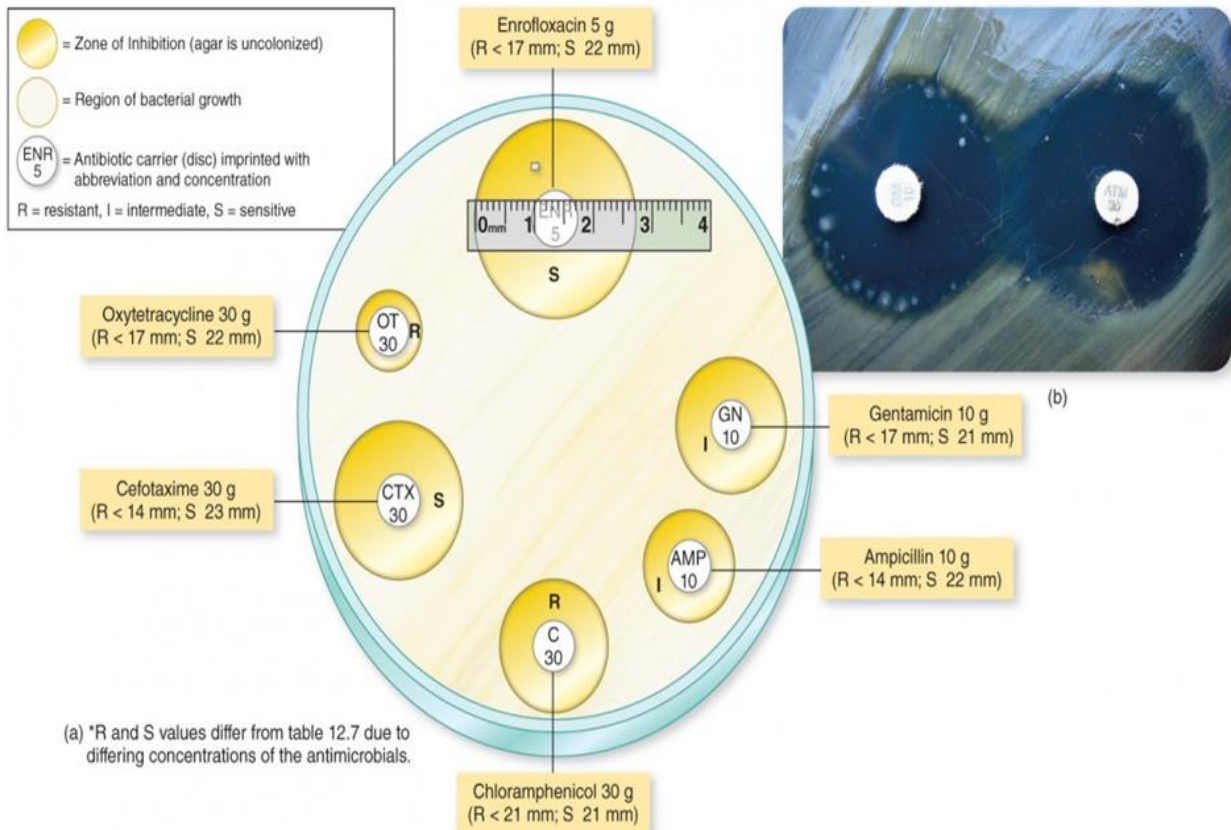
**Interpretation:**

> **Sensitive:** an organism is **susceptible** to an antibiotic when infection occurs within its normal dose.
> **Resistant:** an organism is **resistant** to antibiotics when infection occurs.

Zone of inhibition
Antibiotic disk
Bacterial growth
Intermediate
Resistant
Susceptible

= Zone of Inhibition (agar is uncolonized)

= Region of bacterial growth

ENR 5 = Antibiotic carrier (disc) imprinted with abbreviation and concentration

R = resistant, I = intermediate, S = sensitive

Enrofloxacin 5 g
(R < 17 mm; S  22 mm)

Oxytetracycline 30 g
(R < 17 mm; S  22 mm)

Gentamicin 10 g
(R < 17 mm; S  21 mm)

Cefotaxime 30 g
(R < 14 mm; S  23 mm)

Ampicillin 10 g
(R < 14 mm; S  22 mm)

(a) *R and S values differ from table 12.7 due to differing concentrations of the antimicrobials.

Chloramphenicol 30 g
(R < 21 mm; S  21 mm)

(b): © Kathy Park Talaro

### *E. coli* and other enteric Gram Negative Rods
### (Zone Diameter, nearest whole mm)

|  | Resistant | Intermediate | Susceptible |
|---|---|---|---|
| Amikacin (30 μg) | ≤14 | 15-16 | ≥17 |
| Ampicillin(10 μg) | ≤13 | 14-16 | ≥17 |
| Cefazolin (30 μg) | ≤14 | 15-17 | ≥18 |
| Gentamicin (10 μg) | ≤12 | 13-14 | ≥15 |
| Tetracycline (30 μg) | ≤14 | 15-18 | ≥19 |
| Ticarcillin (75 μg) | ≤14 | 15-19 | ≥20 |
| Trimethoprim (5 μg) | ≤10 | 11-15 | ≥16 |
| Tobramycin (10 μg) | ≤12 | 13-14 | ≥15 |

Activate Windows

**Table 31.2** Zones of Inhibition in the Kirby-Bauer Method of Antimicrobic Sensitivity Testing

| ANTIBIOTIC | CODE | POTENCY | Zone of Inhibition (mm) | | |
|---|---|---|---|---|---|
|  |  |  | RESISTANT | INTERMEDIATE | SENSITIVE |
| Amikacin | AN-30 | 30 μg |  |  |  |
|   Enterobacteriaceae |  |  |  |  |  |
|   P. aeruginosa, Acinetobactor |  |  |  |  |  |
|   staphylococci |  |  | ≤14 | 15–16 | ≥17 |
| Amoxicillin/Clavulinic acid | AmC-30 | 20/10 μg |  |  |  |
|   Enterobacteriaceae |  |  | ≤13 | 14–17 | ≥18 |
|   Staphylococcus spp. |  |  | ≤19 | — | ≥20 |
|   Haemophilus spp. |  |  | ≤19 | — | ≥20 |
| Ampicillin | AM-10 | 10 μg |  |  |  |
|   Enterobacteriaceae |  |  | ≤13 | 14–16 | ≥17 |
|   Staphylococcus spp. |  |  | ≤28 | — | ≥29 |
|   Enterococcus spp. |  |  | ≤16 | — | ≥17 |
|   Listeria monocytogenes |  |  | ≤19 | — | ≥20 |
|   Haemophilus spp. |  |  | ≤18 | 19–21 | ≥22 |
|   β-hemolytic streptococci |  |  | — | — | ≥24 |
| Aziocillin | AZ-75 | 75 μg |  |  |  |
|   P. aeruginosa |  |  | ≤17 | — | ≥18 |
| Bacitracin | B-10 | 10 units | ≤8 | 9–12 | ≥13 |

**3) Diffusion and Dilution Method:** E test also known as **Epsilometer** test is a quantitative method for antimicrobial susceptibility testing applies both **dilution** of antibiotics as well as **diffusion** of antibiotic into the medium. Is a manual in vitro diagnostic device used by laboratories to determine the **MIC**(Minimum Inhibitory Concentration). This type of test is most commonly used in **healthcare** settings to help guiding **physicians** in treatment of patients by indicating what concentration of antimicrobial would successfully treat an infection.
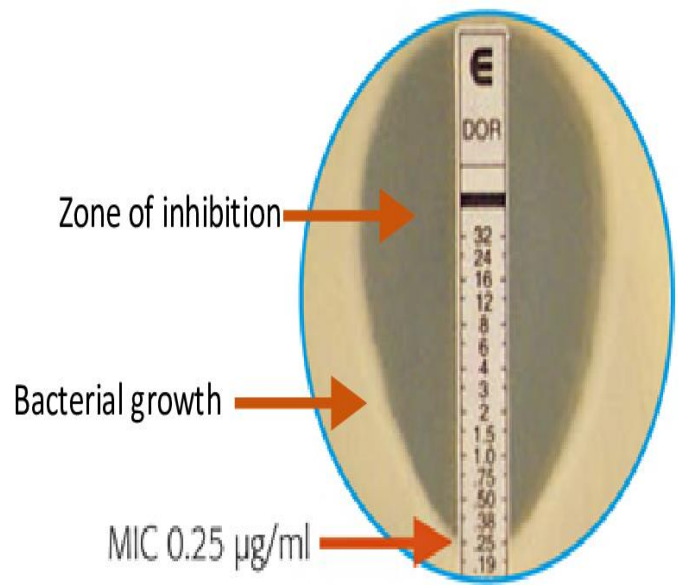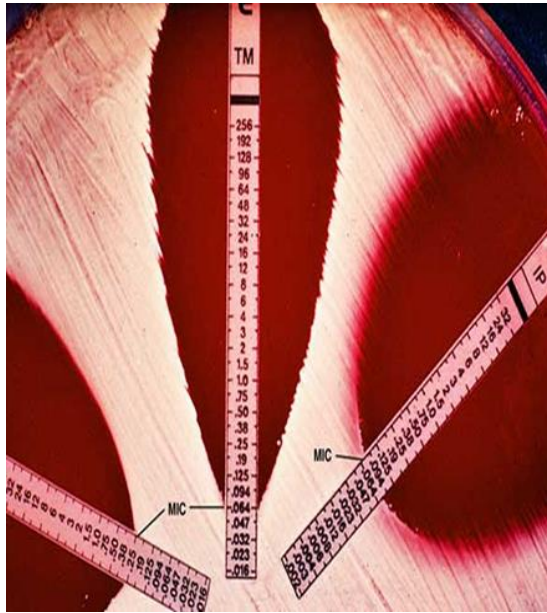
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