Lecture-11: Antimicrobial Susceptibility Testing (AST)

Antimicrobial agents are nontoxic antimicrobial therapeutic agents, which include antiseptics, antibiotics, preservatives, sterilants, and disinfectants; all have the capacity to kill or suppress the growth of microorganisms. **Antimicrobial agents are used to treat, prevent, and control the distribution of bacterial pathogens**. The term **antibiotic** is a compound that is naturally produced by **living microorganisms**, **such as bacteria and fungi**. The antibiotic either **natural**, **synthetic or semisynthetic** are used to treat or prevent disease. **The primary goal of antimicrobial susceptibility testing is** to determine whether the bacterial isolate is resistant to antimicrobial agents selected for treatment. The procedures used to detect resistance to therapeutic agents are referred to as *antimicrobial susceptibility testing (AST).*

Antibiotics Mode of Action:

1- Cell wall synthesis. **2- Cell membrane synthesis.** **3- DNA replication.**
4- RNA transcription. **5- Protein synthesis.** **6- Cell metabolism**

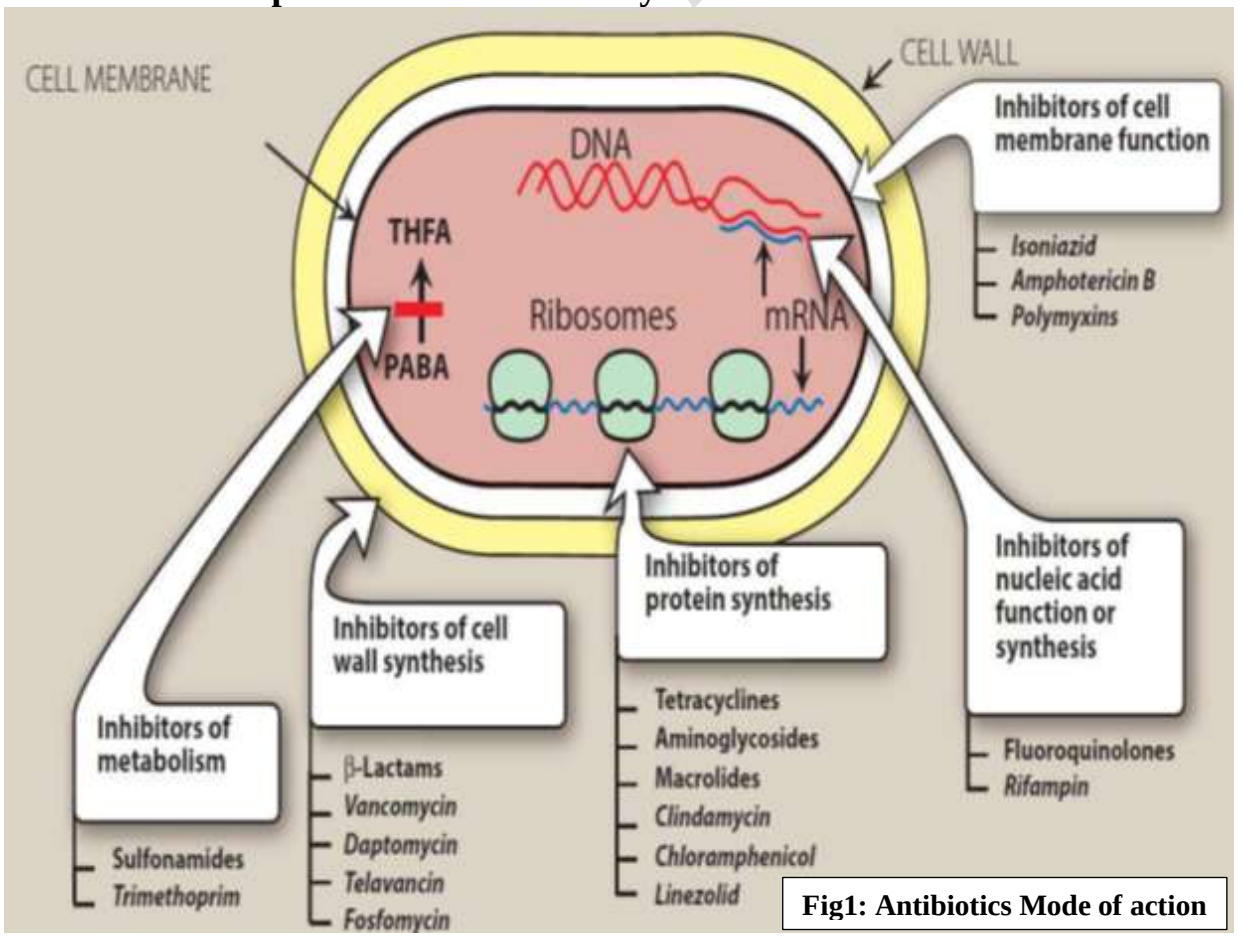

Fig1: Antibiotics Mode of action

The procedures and guidelines of AST are published by the **Clinical and Laboratory Standards Institute (CLSI)** which describe various methods of antimicrobial susceptibility testing which are continuously updated.

The standardized conditions of antimicrobial susceptibility teste:

- Bacterial inoculum size.
- Growth medium (most frequently a Mueller-Hinton base)
- pH
- Cation concentration
- Incubation atmosphere
- Incubation temperature
- Incubation duration
- Antimicrobial concentrations

Standard inoculum size is used by comparing the turbidity of the organism suspension with a turbidity standard of McFarland turbidity standards (which is prepared by mixing **1% sulfuric acid** and **1.175% barium chloride** to obtain a solution with a specific optical density), are commonly used. **The 0.5 McFarland standard,** which provides a bacterial suspension of **1.5×10^8 colony forming units (CFU)/ ml.**

Procedure: Susceptibility testing is usually performed by **two** main methods; **the disk diffusion (Kirby-Bauer) method or/and the minimal inhibitory concentration (MIC)** according to the standards reports published and frequently updated by (CLSI).

1- Minimal inhibitory concentration (MIC):

A- Broth Dilution: Broth dilution testing is divided into two general categories: **microdilution** and **macrodilution**. **The principle of each test is the same; the only difference is the volume of broth in which the test is performed.** For microdilution testing, the total broth volume is 0.05 to 0.1 mL; for macrodilution testing, the broth volumes are usually 1 mL or greater.

B-Tube dilution: In this method, tubes containing serial dilutions of an antibiotic are inoculated with the tested organism. The tubes are incubated and later observed to determine the minimal inhibitory concentration (MIC) of the antibiotic necessary to prevent bacterial growth **(Figure-3).** Quantitative susceptibility testing is necessary **for patients who either fail to respond to antimicrobial therapy or who relapse during therapy.** In some clinical

cases, the minimal bactericidal concentration (MBC) may need to be determined. **This is the lowest concentration of antibiotic that kills 100% of the bacteria, rather than simply inhibiting growth.**

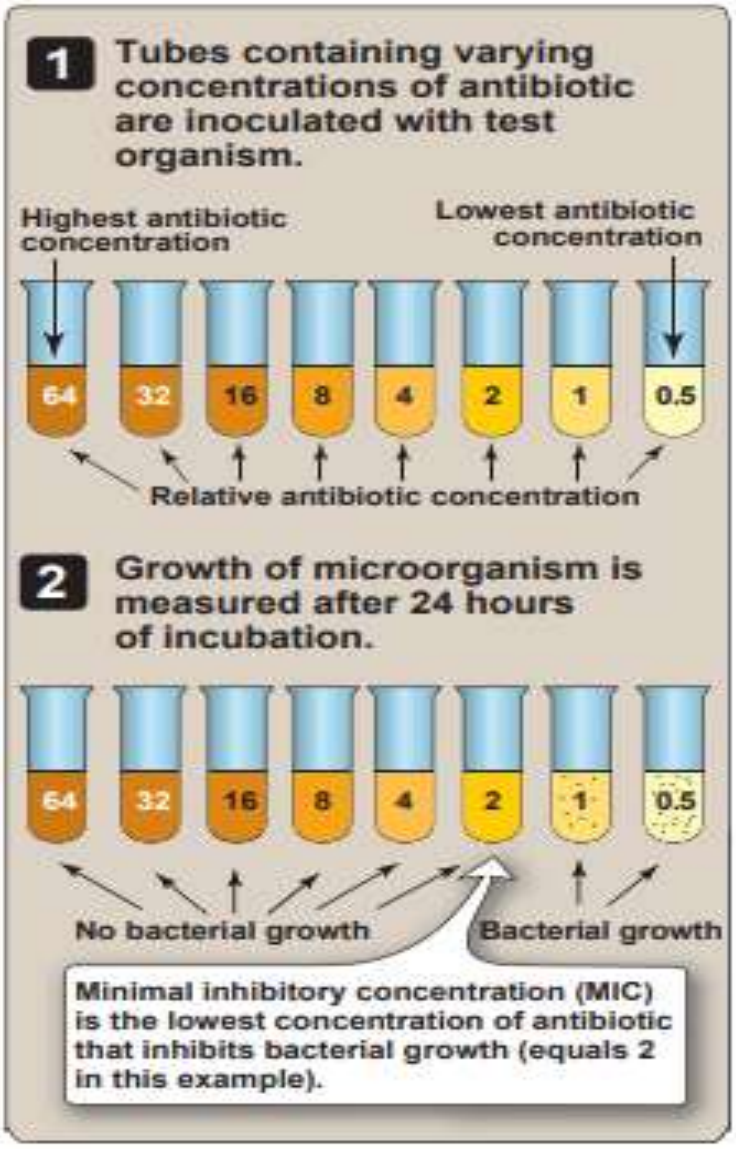

Figure-3

Determination of minimal inhibitory concentration (MIC) of an antibiotic.

2- **Disk Diffusion:** Disk diffusion test **(Kirby-Bauer method)** was developed from the study by **Bauer et al.1 in 1966, who used the antibiotic filter paper disks** (antibiotic disks) with MICs using many bacterial strains. With the disk diffusion susceptibility test, antimicrobial resistance is detected by bacterial isolates with antibiotic disks placed on the surface of an agar plate that has been seeded with a lawn of bacteria **(Figure-4).**

Figure-4: A, Disk diffusion method: antibiotic disks are placed on the agar surface just after inoculation of the surface with the test organism.

B, Zones of growth inhibition around various disks are apparent after 16 to 18 hours of incubation.

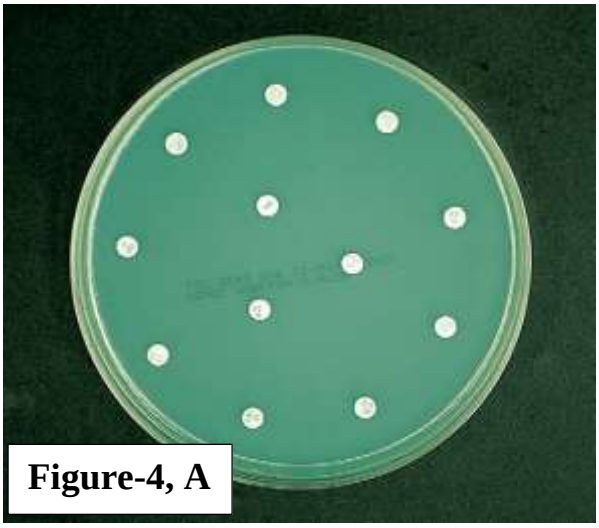

Figure-4, A

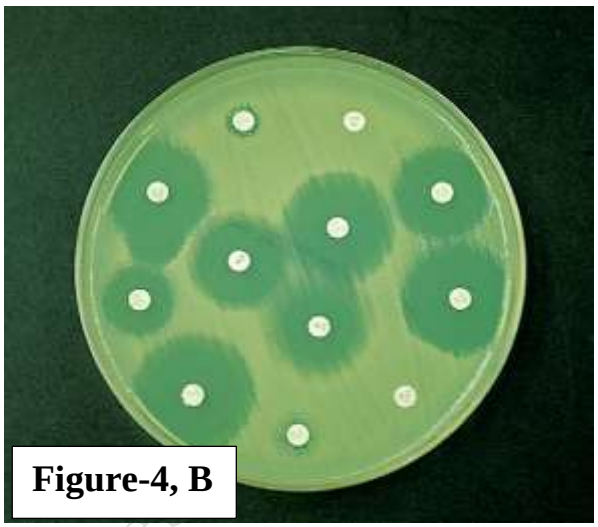

Figure-4, B

When disks containing a known concentration of antimicrobial agents are placed on the surface of a freshly inoculated plate, the agent immediately begins to diffuse into the agar and establish a concentration gradient around the paper disk. **The highest concentration is closest to the disk.** During incubation, the bacteria grow on the surface of the plate except where the antibiotic concentration in the gradient around each disk **is sufficiently high to inhibit growth.** After incubation, the **diameter of the zone of inhibition around each disk is measured in millimeters** (see Figure -3). The inhibition zone sizes obtained are then compared with MICs obtained by broth **(Figure -5).** As the **MICs of the bacterial strains** tested increase (i.e., the more resistant bacterial strains), the corresponding inhibition zone sizes (diameters) decrease.

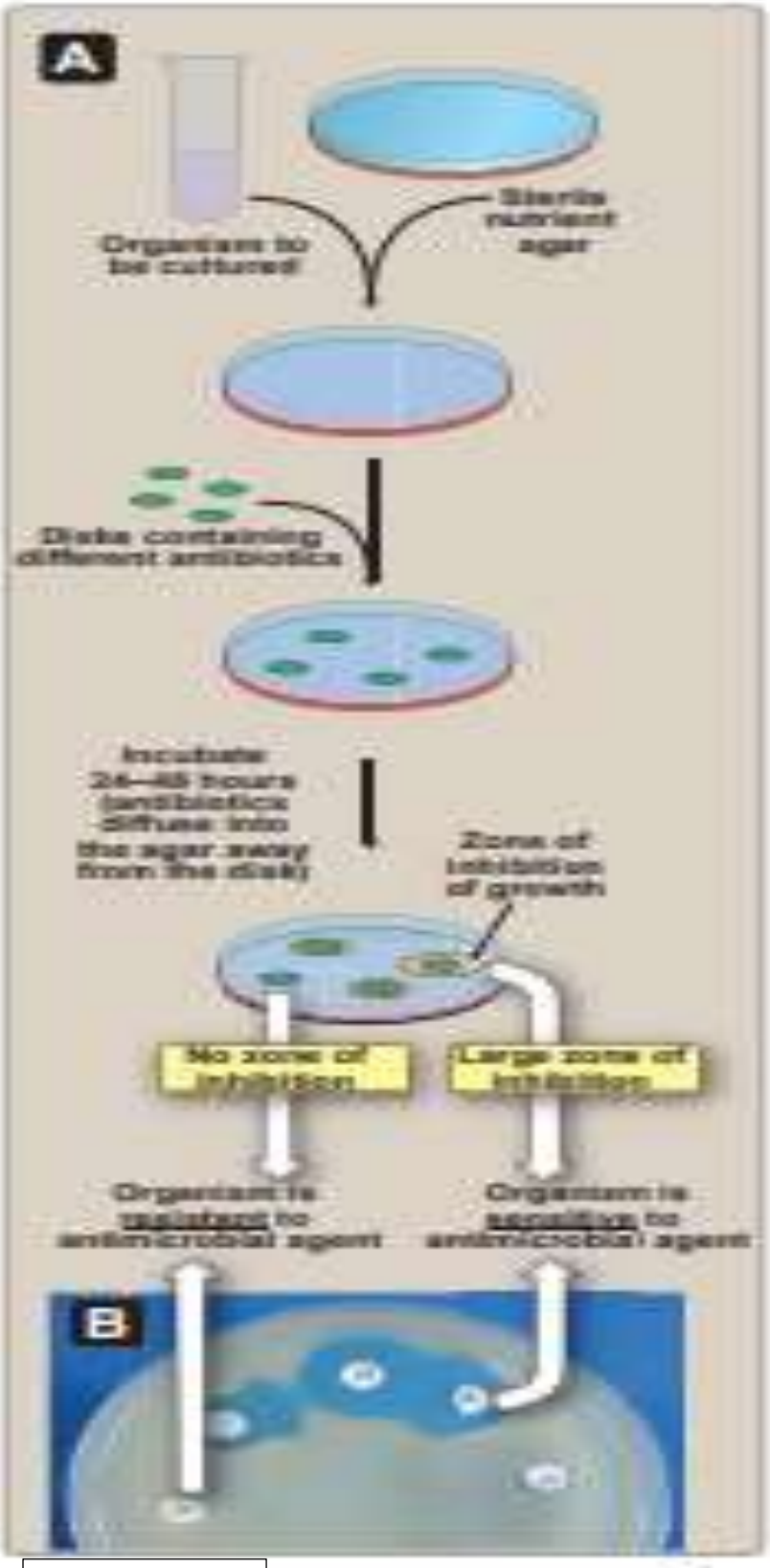

Figure-5

A. Outline of disk-diffusion method for determining the sensitivity of bacteria to antimicrobial agents. B. Photograph of culture plate with antibiotic-impregnated disks.

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