

MEDICAL MICROBIOLOGY

LAB 5

Antimicrobial Susceptibility Test



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Antimicrobial chemotherapy

Many diseases are treated with chemotherapeutic agents , such as antibiotics , that inhibit or kill the pathogen while harming the host as little as possible .

Some drugs kill the organism and are said to be **bactericidal** . Other drugs are **bacteriostatic** ; they stop growth but don't kill the microbe. The effectiveness of chemotherapeutic agents on microorganisms depends on many factors:

- 1-The route of administration.
- 2-Location of the infection.
- 3-The presence of interfering substances.
- 4-The concentration of the drug in the body.
- 5-The presence of drug allergies .
- 6-The nature of the pathogen.
- 7-The resistance of microorganisms to the drug.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing is a standardized method that is used to measure the effectiveness of antibiotics and other chemotherapeutic agents on pathogenic microorganisms .

Minimum Inhibitory Concentration (MIC) : is the lowest concentration of drug that prevents growth of a particular pathogen .

Minimal Lethal Concentration (MLC) : is the lowest drug concentration that kills the pathogen .

There are four methods to perform the antibiotic sensitivity

test : 1-**Disk diffusion (Kirby- Bauer test)**

2- **Dilution Susceptibility Test**

3- E-Test(Epsilometer)

4- Vitek 2 test

The Kirby –Bauer test , also called the **disk diffusion test** , is widely used for testing the sensitivity of micro-organisms to antibiotic. A disk diffusion technique may be used to save time ,media and is fairly simply

, in this method Filter paper disks used which contain a specified amount of the microbial agent (printed on the disk).

Procedure

1- Broth culture of bacteria diluted to match a 0.5 McFarland turbidity standard.

2- A sterile cotton swab is dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of a Mueller -
-Hinton agar plate .

2-Allow the agar surface to dry for about 5 minutes .

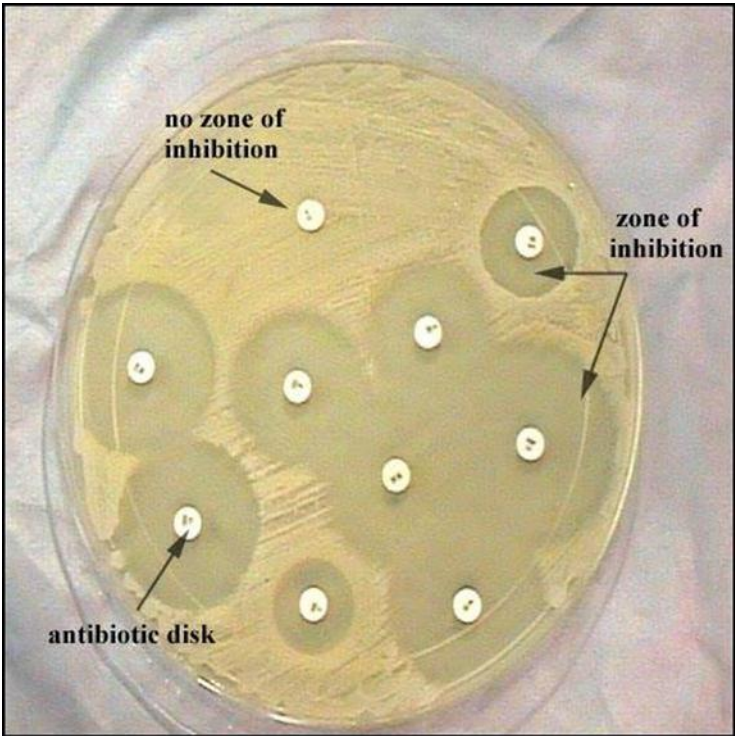
3-Using sterile forceps (flamed with alcohol 70% and cooled) or a multiple applicator device to placed antibiotic disk on a plate.

4- The plate is incubate at 37 °C for 16-18 hours .

5-After incubation ,examine the plate (without opening) if the organisms is susceptible to it , a clear zone will appear around the disk where growth has been inhibited . The diameters of the zones of inhibition are measured to the nearest millimeter (mm) . Results : S=Sensitive , R=Resistant , I=Intermediate



Multiple applicator device



Dilution Susceptibility Test

Dilution susceptibility test can be used to determine MIC and MLC values. In the **broth dilution test**, a series of broth tubes (usually Mueller-Hinton broth) containing antibiotic concentrations in the range of 0.1 to 128 µg/ml is prepared and inoculated with standard numbers of the test organism. The lowest concentration of the antibiotic resulting in no growth after 16-20 hours of incubation is the MIC. The MLC can be ascertained if the tubes showing no growth are subcultured into fresh medium lacking antibiotic.

The **agar dilution test** is very similar to the broth dilution test. Plates containing Mueller-Hinton agar and various amounts of antibiotic are inoculated and examined for growth.

E-Test(Epsilometer)

The E-test system for determining antibiotic sensitivity, is an alternative to the Kirby – Bauer method and has the added advantage of allowing the MIC to be determined. It consists of a paper strip with a gradient of antibiotic concentrations on one surface and a printed scale on the other. After an agar plate is inoculated with a lawn of bacteria, the strip is placed, antibiotic side down, on the agar surface. During incubation, the antibiotic will diffuse into the agar. The point at which the inhibition zone intersects the scale printed on the strip is the MIC.

