Ministery of Higher education and scientific research

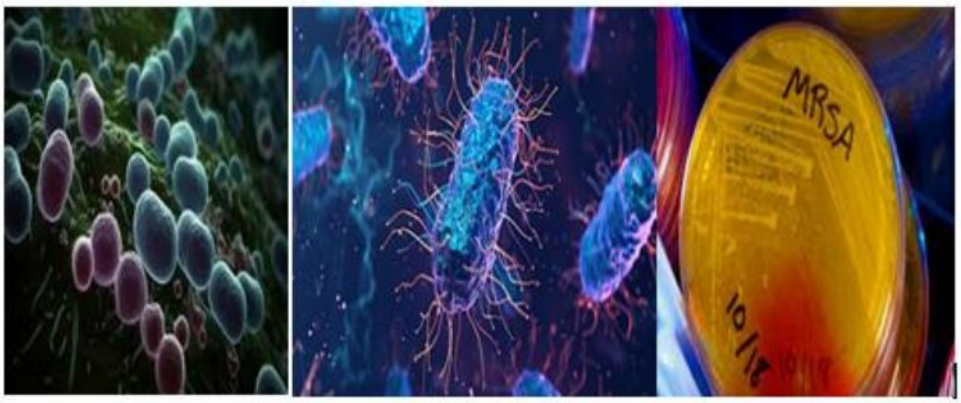
University of Tikrit

College of science

Department of Biology

Lab. of Practical Microbiology (1)

For second stage - 2025-2026



مدرس المادة .: بشرى علي كاظم

Determination of bacterial sensitivity to antibiotics

Antibiotics:

Substances that are produced by some microorganisms or synthesized chemically (partially or completely) ; this substances Possessed an affect of killing or inhibiting growth of different microorganisms, when these antibiotics are used at low concentration.

Bactericidal: Antibiotics or any chemical substance which kill the bacteria.

Bacteriostatic: Chemicals or antibiotics inhibit the bacterial multiplication.

Mechanisms of antibiotic action

1. Inhibits formation of bacterial cell wall: ex. Pencillins, cephalosporines

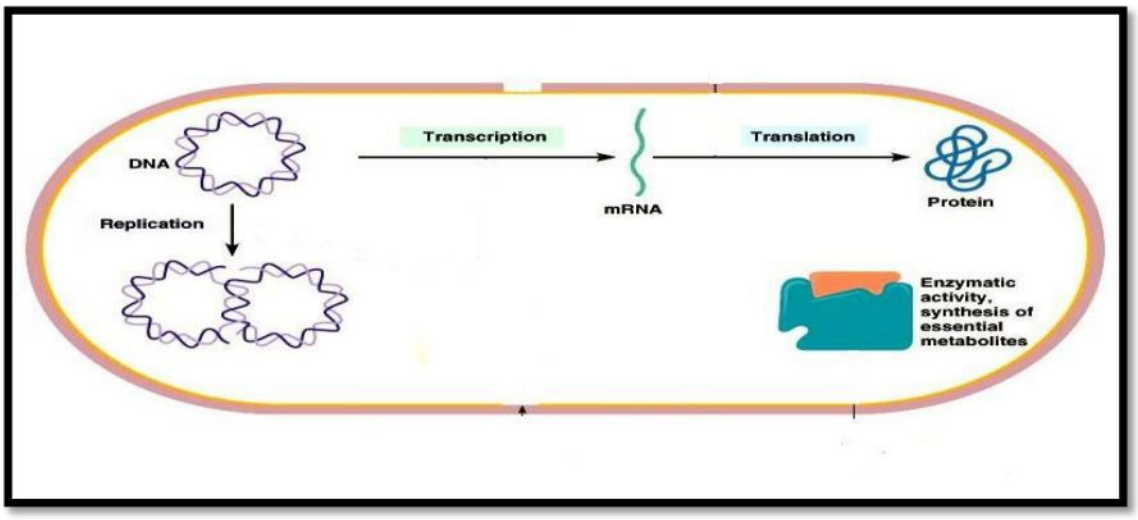
2. Injury of plasma membrane: ex. Polymyxin B

3. Blocks DNA synthesis: ex. Quinolones

4. Inhibits RNA synthesis: ex. Rifampin

5. Inhibit protein synthesis: ex. Aminoglycosides, chloramphenicole.

6. Blocks cell metabolism by inhibiting enzymes: ex. Trimethoprim and Sulfonamides



Classification of antibiotics according to their affect on bacterial cell:

1. Narrow spectrum antibiotics: Kill only a specific group of microbes like, penicillin (usually Gram+) and Erythromycin.

2. Broad spectrum antibiotics: have the ability to kill all clinically important bacteria like, Gentamicin and Ciprofloxacin.

Methods of obtaining antibiotics:

1.Micro-organisms: from bacteria like: Streptomyces or from fungi like *Penicillium notatum* .

2.Chemosynthetic: Using of chemical processes to produce antibiotics like producingof chloramphenicol.

3.Semi- chemosynthetics: This processes depend on producing antibiotics by fermentation and changing the product by chemical methods like producing of Methicillin and Ampiclox.

Antibiotic sensitivity testing

There are two methods to detect the antibiogram of each bacterial strain:

1.Disk diffusion method: It is the most of effective practical means of clinical use. A sterile filter paper disc usually used of two types:

a. Uni- disk

b. Multi- disks (with projecting arm)

- **Medium**

Muller- Hinton agar was proper for antibiotic sensitivity testing . In all types of media which were used for such test , should permit free diffusion of high molecular weight antibiotics with no inhibitory properties.

- **The inoculum:**

The organism should be selected from pure culture, and evenly spread on the surface of agar plate.

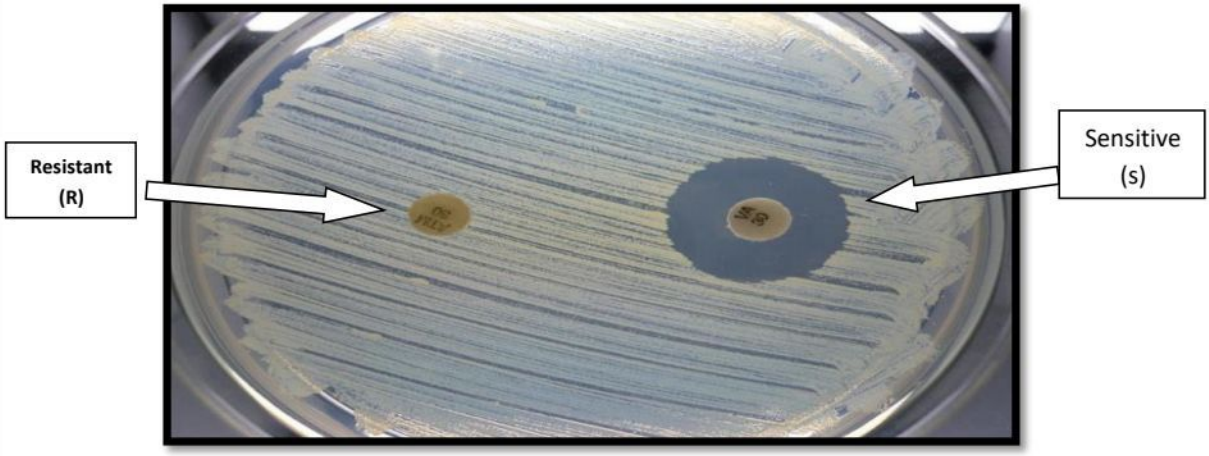
A sterile cotton swab (or loop) may be used for this purpose.

Application of discs

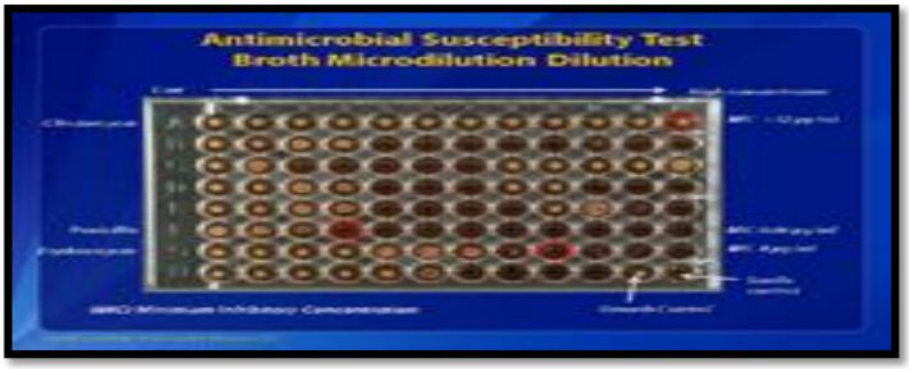
The discs should be removed from container (with flamed forceps in order to avoid contamination) and placed on the inoculated medium, one by one. It may be gently pressed down with flamed forceps. The dish is then inverted and incubated usually at 37c for (12-24) hrs.

Interpretation of the test:

- **<u>Sensitive (S)</u>: Clear zone around the disc**
- **Resistant (R): No inhibition zone or less than sensitive diameter.**



<u>2. Dilution method:</u> Serial dilution of antibiotics is inoculated with bacterial isolates in test tubes to detect the minimum inhibitory concentration (MIC).



<u>MIC</u>

Is the minimum inhibitory concentration (MIC) which the lowest concentration of antibiotic or disinfectant / antiseptic that inhibit the bacteria by using dilution method (Serial dilution method) .