



**SNS COLLEGE OF ALLIED HEALTH SCIENCES**

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Affiliated to Dr MGR Medical University, Chennai



**DEPARTMENT OF PHYSICIAN ASSISTANT**

**COURSE NAME : CLINICAL MICROBIOLOGY**

**TOPIC : ANTIMICROBIAL METHOD**



# ANTIBIOTICS



- Antibiotics are biochemicals produced by microorganisms that inhibit the growth of, or kill, other microorganisms - Major class of antimicrobial agents.
- Antibiotics must exhibit selective toxicity because they are produced by one microorganism and exert varying degrees of toxicity against others.

There are different types of antibiotics.

- Penicillins
- Cephalosporins
- Tetracyclines
- Macrolides
- Fluoroquinolones
- Sulfonamides
- Glycopeptides



# Antimicrobial method

- It is a laboratory procedure to identify which antimicrobial regimen is specifically effective for individual patients.
- **Other names:** Antibiotic susceptibility test, Sensitivity testing, Antimicrobial susceptibility test
- Helps to find out which antibiotic will be most effective in treating your infection.
- Also be helpful in finding a treatment for antibiotic-resistant infections.
- [Antibiotic resistance](#) happens when standard antibiotics become less effective or ineffective against certain bacteria.



# METHODS OF ANTIMICROBIAL



- There are several different procedures to determine the sensitivity of microorganisms to antibiotics.
- 1. Kirby-Bauer Disc Method** - Used to determine which antibiotic is the most effective against a certain pathogen.
  - 2. MIC** - Used to determine the lowest concentration that is needed to kill the pathogen at the site of infection.



# The Kirby-Bauer Disc Method



- Also called the agar diffusion method or the disk diffusion method.
- A filter disk impregnated with an antibiotic is applied to the surface of an agar plate containing the organism to be tested
- Then, the plate is incubated at 37°C for 24-48 hours.
- As the substance diffuses from the filter paper into the agar, the concentration decreases as a function of the square of the distance of diffusion.
- At some particular distance from each disk, the antibiotic is diluted to the point that it no longer inhibits microbial growth.



- The effectiveness of a particular antibiotic is shown by the presence of growth-inhibition zones.
- **These zones of inhibition (ZOIs)** appear as clear areas surrounding the disk from which the substances with antimicrobial activity diffused.
- The diameter of the ZOI can be measured with a ruler.
- The agar diffusion method uses commercially available filter paper disks, each containing a defined concentration of a specific antibiotic.



# PROCEDURE

## The Kirby-Bauer Disc Method

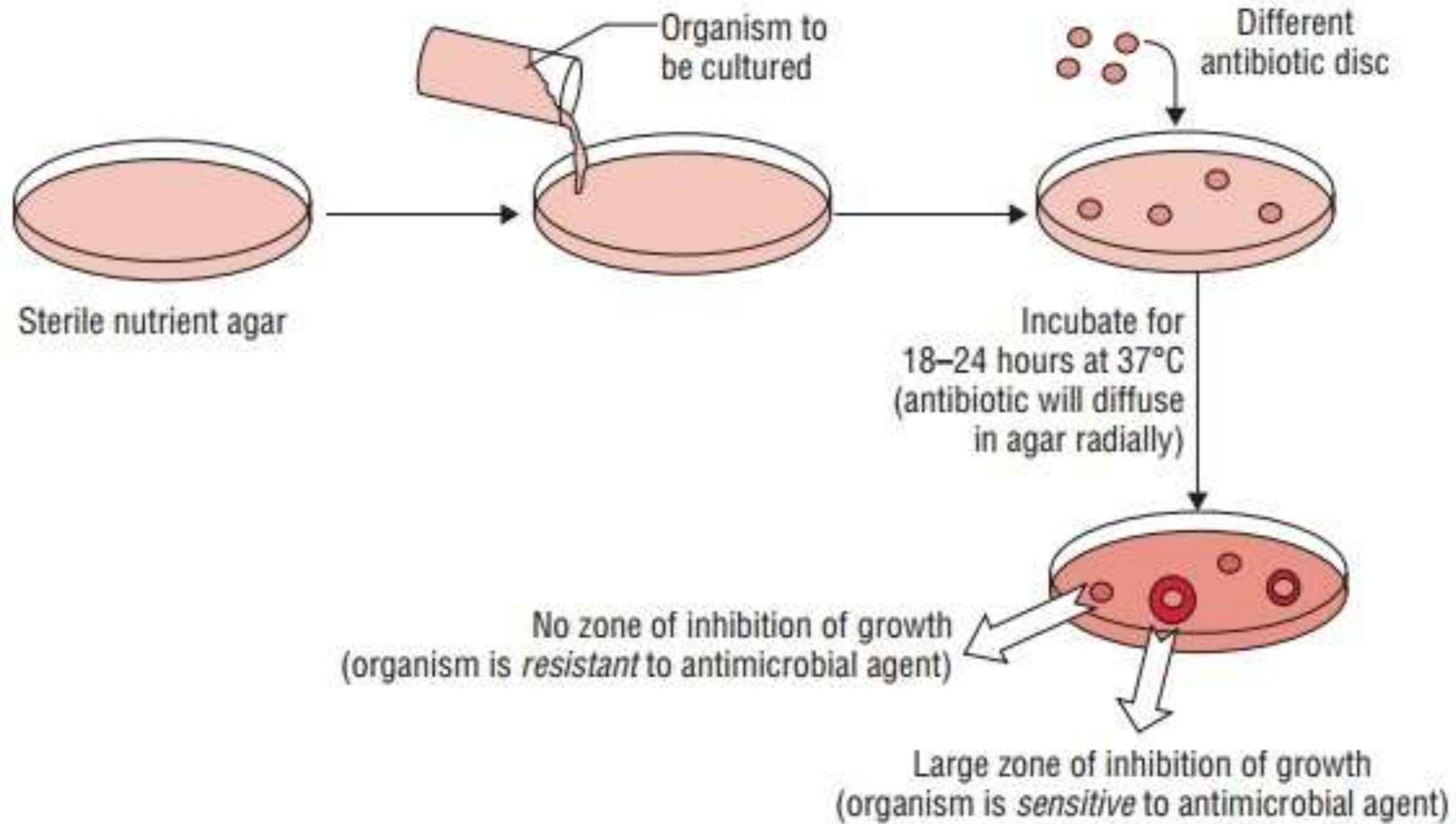


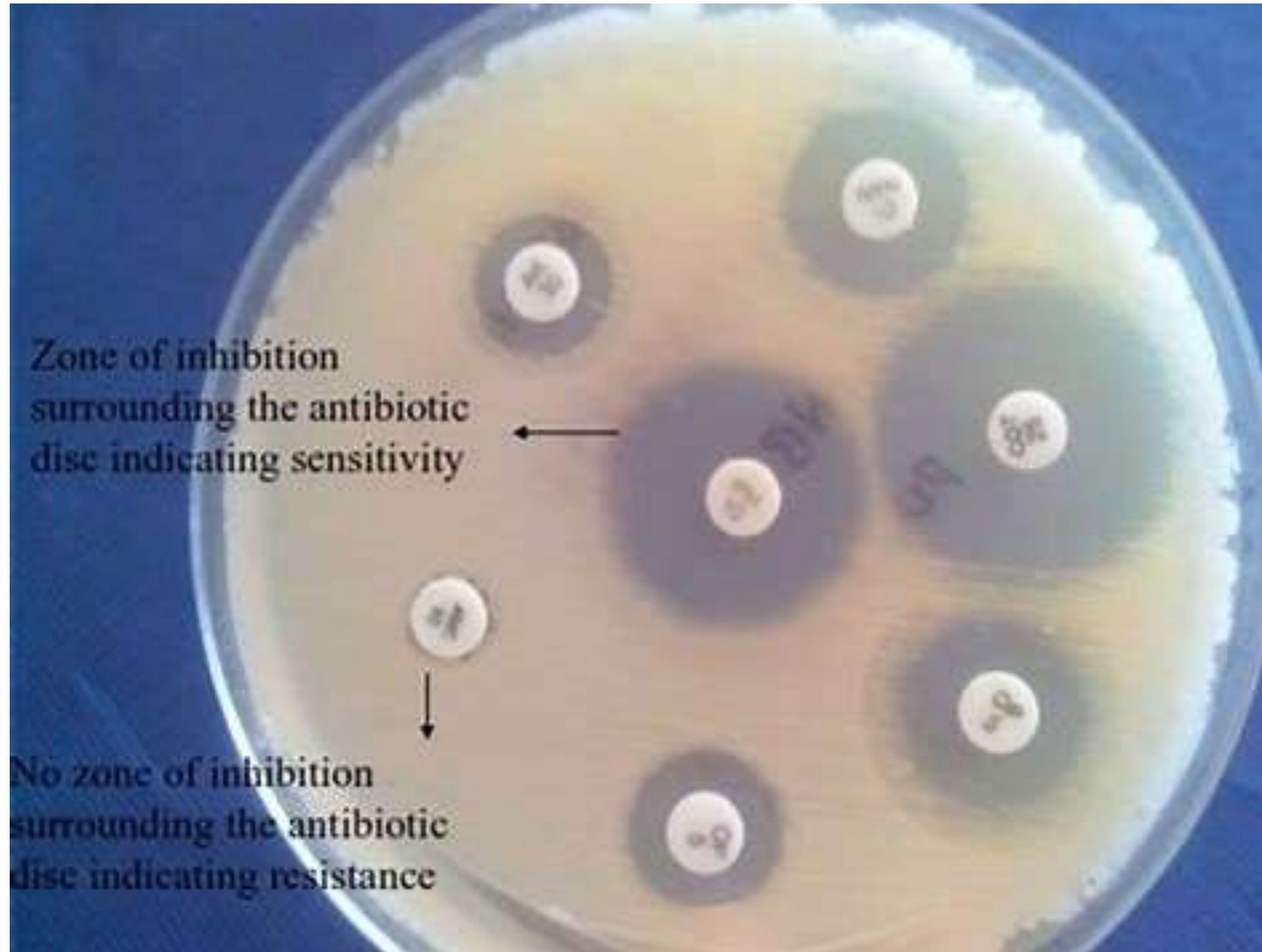
1. Obtain 2 plates and the cultures of E. coli and Staphylococcus aureus.
2. Obtain a swab and dip it into the E. coli broth culture. Roll the swab against the inside of the tube to remove excess liquid.
3. Streak one of the plates with the swab in even strokes to obtain a uniform growth pattern across the entire surface of the plate.
4. Rotate the plate 90 degrees and using the same swab, streak the plate again.
5. Rotate the plate 45 degrees and reswab.
6. Repeat the above procedure for Staphylococcus aureus with a new plate.
7. Allow the plates to dry for 2-5 minutes.



8. Use the sterile forceps to aseptically remove one of each antibiotic disc from the dispenser and place it on each plate.
9. The antibiotic discs used are: gentamicin, tetracycline, penicillin G, chloramphenicol, ampicillin and erythromycin.
10. Incubate at 37°C for 2 days.
11. Record the results by measuring the diameters of the zone of inhibition (ZOI).







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# Assessment



1. Name some of the commonly used Antibiotics?
2. 2 types of antimicrobial methods?
3. Explain the procedure of Kirby-Bauer Disc Method?
4. What is Zone of Inhibition?



# Minimum inhibitory concentration [MIC]



**MIC - The smallest concentration of antibiotic that inhibits the growth of organism.**

1. Liquid media (dilution) allows MIC estimation
2. Solid media (diffusion)
  - Disk diffusion (Kirby-Bauer)
  - E-tests



- The lowest concentration of an antibiotic that will inhibit the growth of the organism being tested is known as the minimal inhibitory concentration (MIC).
- The MIC may assist a physician in deciding the concentration of the antibiotic needed to inhibit the pathogen.

MIC = The minimum concentration leads to inhibition of bacteria under testing .

MBC = The minimum concentration that kill the bacteria under testing.



# MIC Testing

## Dilution method

It is a quantitative method, depend on preparation of series of gradually duplicate concentration of antibiotic in a suitable medium for growth, then adding limited number of bacteria and checking the ability of antibiotic to inhibit or kill the bacteria under testing.



# Tube dilution Method

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

# Methods of MIC determination

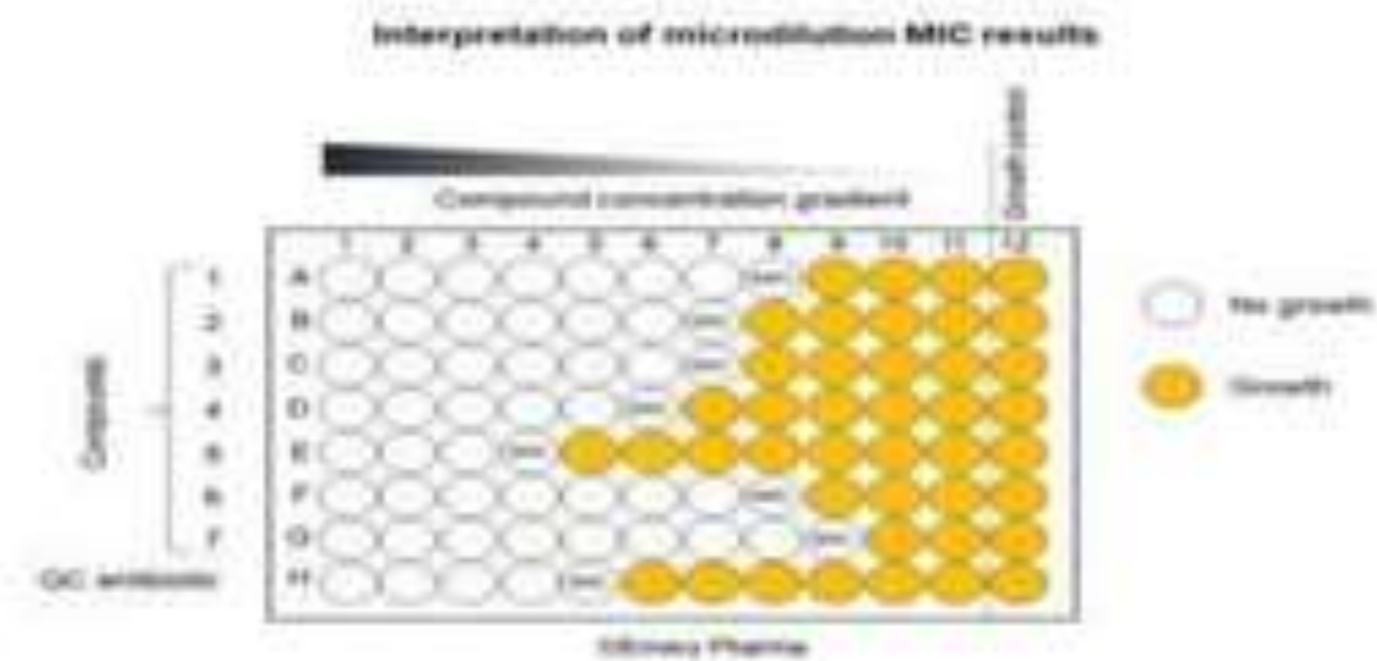
## 1. Agar based dilution

- i. Agar dilution method
- ii. Well diffusion method



## 2. Broth based dilution

- i. Macrodilution method
- ii. Microdilution method







## **Macrodilution Method**

### **Preparation of antibiotic stock solution**

- A stock solution is a concentrated solution that will be diluted to some lower concentration for actual use.
- Stock solutions are used to save preparation time, conserve materials, reduce storage space, and improve the accuracy with which working lower concentration solutions are prepared.



# Macrodilution Method

## Preparation of antibiotic stock solution

- The antibiotic stock solutions should be made to a final concentration of **10 mg/mL** or 10 times the highest concentration to be tested and then diluted to an appropriate concentration in broth.
- Sterile water is generally used in the preparation of the antibiotic solutions as solvent and diluent.
- Antibiotic powder obtained from the manufacturer is not always 100% pure. Therefore, prior to making a stock solution, it is important to ensure the potency of each antibiotic.



## Macrodilution Method

- Small volumes of the stock solution can be stored at  $-70^{\circ}\text{C}$  in freezer vials, without the loss of any activity.
- Solutions may be stored for up to 6 months unless otherwise indicated in the manufacturer's package insert.
- Frozen stocks should be thawed the day of use, and any left over is generally discarded.



## Procedure

1. Label sterile capped test tubes 1 through 11
2. Pipette 0.5 mL of Mueller-Hinton broth into tubes 1–11.
3. Pipette 0.5 mL of antibiotic solution into tubes 1.
4. Transfer 0.5 mL from tube 1 to tube 2 and continue through tube 9. Be certain to change pipettes between tubes to prevent carryover of antibiotic.
5. Discard 0.5 mL from tube 9. The tenth tube, which serves as a control, receives no antibiotic.
6. add 0.5 mL of bacterial broth suspension to each tube except the eleventh (last) tube, which is the broth control tube.
7. incubated overnight at 35–37°C.



## Macrodilution Method

### Result interpretation

- The lowest concentration of the antimicrobial agent that will inhibit the growth of the microorganism being tested as detected by lack of visual turbidity, matching with a negative control included with the test, is known as MIC.
- **To determine MIC :** select the 1st clear tube (no bacterial growth) ranked at serial turbidity tubes, this tube contains MIC of antibiotic.
- **To determine MBC :** take 0.1 ml from clear tubes and transfer to petri dishes containing Mueller Hinton agar and spread on the surface of agar then incubate at 37°C for 24-48 hrs and then check the growth of colonies in each plate ;the 1st plate that dose not show any colony represents the concentration of antibiotic for clear tube and is considered MBC.

# Minimum Inhibitory Concentration Test

### 1. Preparation of test inoculum

### 2. Preparation of different dilutions of antimicrobial agent

### 3. Inoculation on 96 well plate

(Antimicrobial agents are transferred into a 96-well microtiter plate and inoculated with bacterial suspension)

### 4. Results - Determination of Minimum Inhibitory Concentration (MIC)

(Turbidity of the sample is determined)

The lowest concentration of antimicrobial agent that is capable of inhibiting bacterial growth is called MIC.



# Assessment



1. Define MIC?
2. Methods of MIC determination?
3. What is Macrodilution method?
4. Procedure for MIC?



# THANK YOU