

Practical Medical Bacteriology

Lab 2

Antibiotic Sensitivity Test



2023/2024

Antibiotic sensitivity test

Antibiotic sensitivity test : An *in vitro* test used to check the effectiveness of antibiotics against bacteria and to select the best antibiotics that act against the bacteria.

Targets of antimicrobial agents:

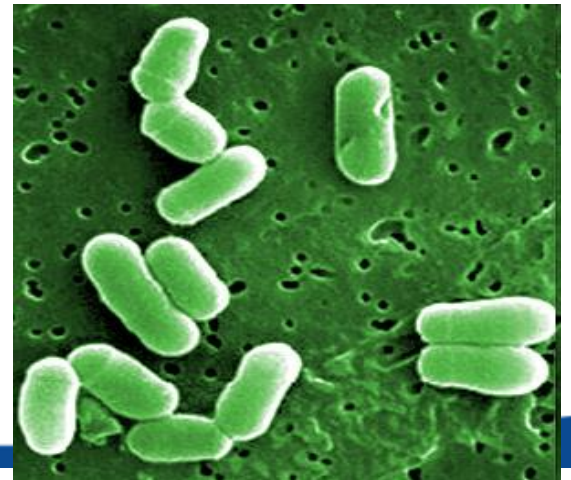
1. Inhibition of cell wall synthesis
2. Inhibition of nucleic acid synthesis
3. Inhibition of protein synthesis
4. Disruption of cell membrane structure or function
5. Inhibition of metabolic pathways



Purpose of Antibiotic sensitivity test

Because

1. To choose the most effective drug against particular pathogen especially in those infections caused by more than one pathogen with different antibiogram like urinary tract infection (UTI).
2. To detect drug resistant pathogen like MRSA.
3. To find out the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of drugs which are required for certain serious bacterial infections such subacute bacterial endocarditis.



Antibiotic Sensitivity Testing

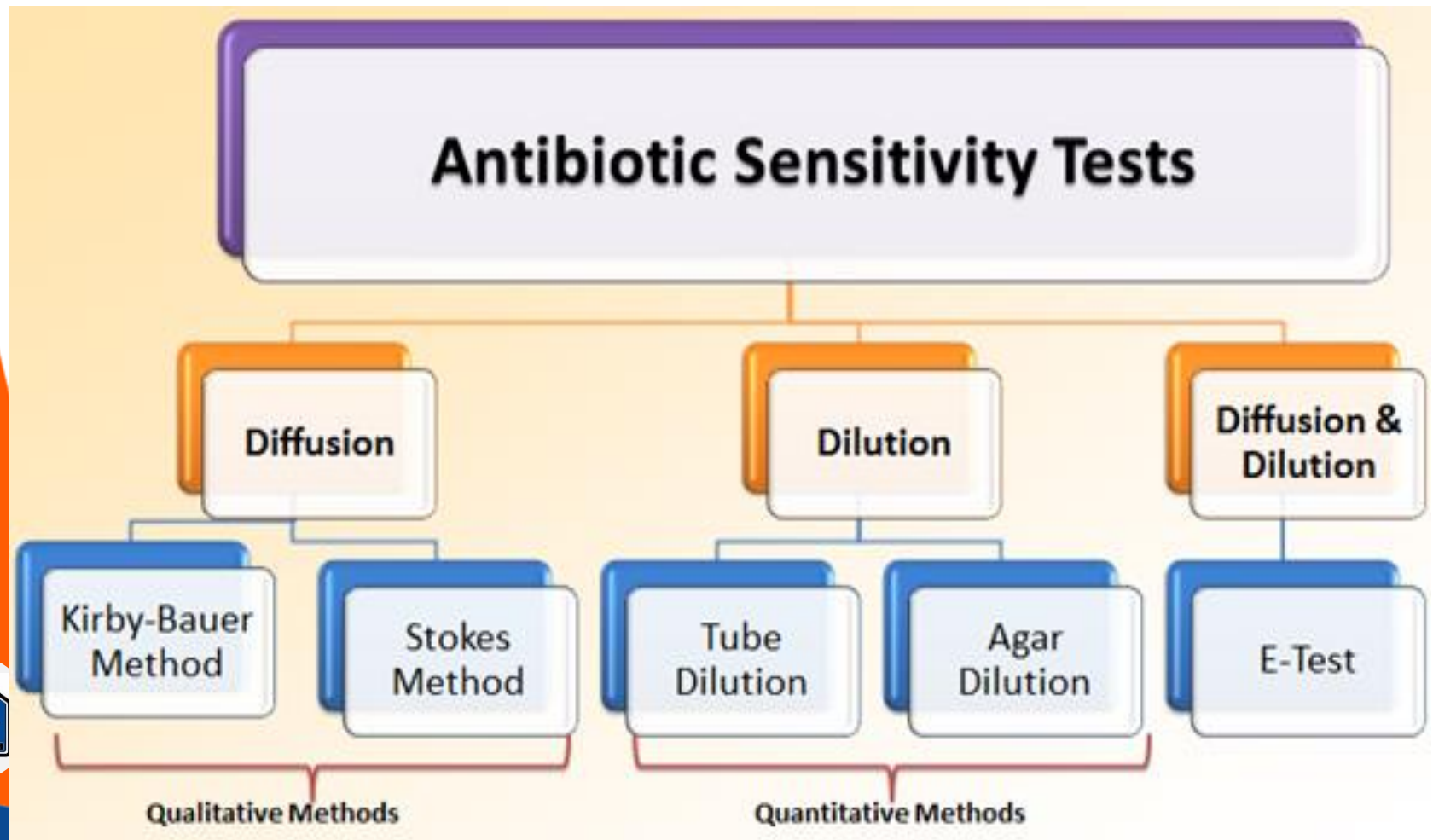
• Right Antibiotic

• Right Microbe

• Right Cure



Antibiotic sensitivity test



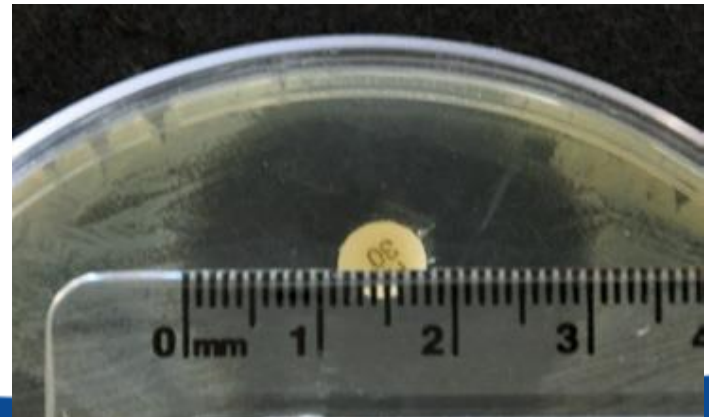


Kirby-Bauer Method

Qualitative method

Disk diffusion (Kirby-Bauer) method (1966).

- It is the most commonly used method in clinical laboratories
- In this method, antibiotics are impregnated onto paper disks and then placed on a seeded **Mueller-Hinton agar** plate. The plate is then incubated for 16 to 18 hours, and the diameter of the **Zone of Inhibition** around the disk is measured to the nearest millimeter.



Disk diffusion method or Kirby-Bauer method.

- The inhibition zone diameter indicates the susceptibility or resistance of a bacterium to the antibiotic.
- Antibiotic susceptibility patterns of a particular pathogen are called **antibiograms**.
- Antibiograms can be determined by comparing the zone diameter obtained with the known zone diameter size for susceptibility (Reference chart like **C**linical and **L**aboratory **S**tandard **I**nstitute = **CLSI**).
- For example, a zone of a certain size indicates susceptibility, zones of a smaller diameter or no zone at all show that the bacterium is resistant to the antibiotic.



Clinical and Laboratory Standard Institute

| Disk Symbol | Antibiotic | Disk Content | Diameter of Zones of Inhibition (mm) | | |
|-------------|--|--------------|--------------------------------------|--------------|-------------|
| | | | Resistant | Intermediate | Susceptible |
| AM | Ampicillin ^a when testing gram-negative microorganisms and enterococci | 10 µg | 16 or less | | 17 or more |
| AM | Ampicillin ^a when testing staphylococci and penicillin G-susceptible microorganisms | 10 µg | 28 or less | | 29 or more |
| B | Bacitracin | 10 units | 8 or less | 9–12 | 13 or more |
| CB | Carbenicillin when testing <i>Proteus</i> species and <i>E. coli</i> | 50 µg | 19 or less | 18–22 | 23 or more |
| CB | Carbenicillin when testing <i>P. aeruginosa</i> | 50 µg | 13 or less | 14–16 | 17 or more |
| C | Chloramphenicol (Chloromycetic®) | 30 µg | 12 or less | 13–17 | 18 or more |
| CC | Clindamycin ^c when reporting susceptibility to clindamycin | 2 µg | 14 or less | 15–20 | 21 or more |
| CC | Clindamycin ^c when reporting susceptibility to lincomycin | 2 µg | 16 or less | 17–20 | 21 or more |
| CL | Colistin ^d (Coly-mycin®) | 10 µg | 8 or less | 9–10 | 11 or more |
| E | Erythromycin | 15 µg | 13 or less | 14–22 | 23 or more |
| GM | Gentamicin | 10 µg | 12 or less | 13–14 | 15 or more |
| K | Kanamycin | 30 µg | 13 or less | 14–17 | 18 or more |
| ME | Methicillin ^e | 5 µg | 9 or less | 10–13 | 14 or more |
| N | Neomycin | 30 µg | 12 or less | 13–16 | 17 or more |
| NB | Novobiocin ^f | 30 µg | 17 or less | 18–21 | 22 or more |
| OL | Oleandomycin ^g | 15 µg | 11 or less | 12–16 | 17 or more |
| P | Penicillin G. when testing staphylococci ^h | 10 units | 28 or less | | 29 or more |
| P | Penicillin G. when testing other microorganisms ^{h,i} | 10 units | 14 or less | | 22 or more |
| PB | Polymyxin B ^d | 300 units | 8 or less | 9–11 | 15 or more |
| R | Rifampin when testing <i>N. meningitidis</i> susceptibility only | 5 µg | 16 or less | 17–19 | 20 or more |
| S | Streptomycin | 10 µg | 6 or less | 7–9 | 10 or more |
| S | Sulfonamides | 300 µg | 12 or less | 13–16 | 17 or more |
| T (TE) | Tetracycline ^j | 30 µg | 14 or less | 15–18 | 19 or more |
| VA | Vancomycin | 30 µg | 14 or less | 15–16 | 17 or more |

Factors affecting susceptibility of antibiotics

- Many factors are involved in sensitivity disk testing and must be carefully controlled, these include
 - **Size of the inoculums**
 - **Distribution of the inoculums**
 - **Incubation period**
 - **Depth of the agar**
 - **Diffusion rate of the antibiotic**
 - **Concentration of antibiotic in the disk**
 - **Growth rate of the bacterium.**
- If all of these factors are carefully controlled, this type of testing is highly satisfactory for determining the degree of susceptibility of a bacterium to a certain antibiotic.



Procedure

Preparation of a bacterial inoculum



Application of Antibiotic Disks



Incubation
At 35° C for 16-18 hours



Measurement of inhibition zone diameter



Procedure

1. Select 4-5 well separated colonies from *Staph. aureus* or *E. coli* and suspend in 4-5 ml of sterile broth such as brain heart infusion or sterile normal saline.
2. Use a separate, sterile cotton swab for each bacterium. The swab is immersed in the culture tube, and the excess culture is squeezed on the inner side of the test tube.
3. The swab is then taken and swabbing on the surface of the Mueller-Hinton plate three times, rotating the plate 60° after each swab.
4. Finally, run the swab around the edge of the agar. this procedure ensures that the whole surface has been seeded.
5. Allow the culture to dry on the plate for 5 to 10 minutes at room temperature with the top in place.



Procedure

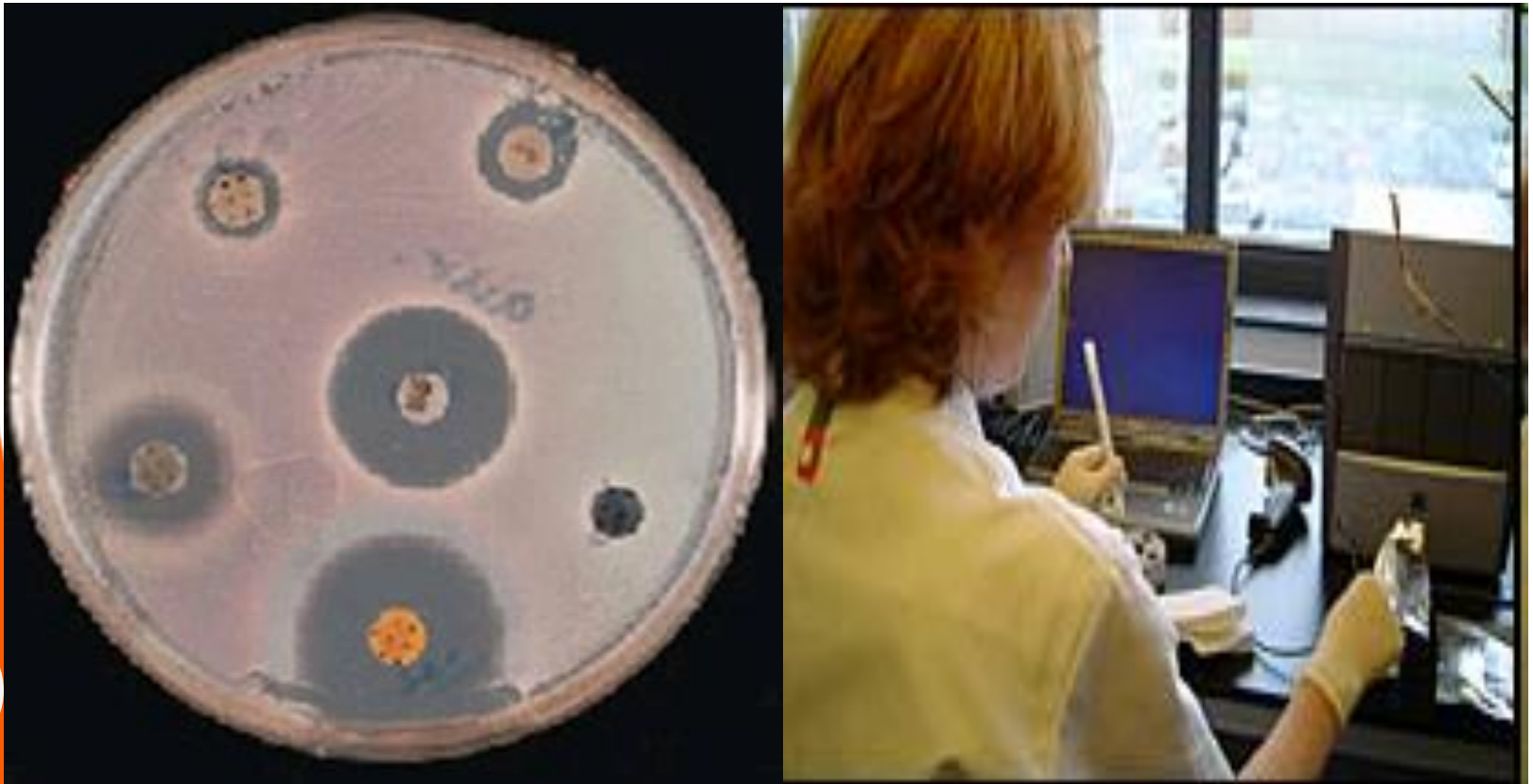
6. Sterilize the forceps by flaming before picking up the antibiotic disks (choose according to the type of pathogen and age and gender) onto the plate.
7. Make sure that contact is made between the antibiotic disk and the culture by gently pressing the disk with alcohol-flamed forceps. do not press the disk into the agar, and do not move the disk once it is placed on the agar. The disks should be placed 15 mm away from the edges and 20 mm away from each other.
8. Incubate the plates for 16 to 18 hours at 35 °C.
9. Measure the zones of inhibition to the nearest mm for each of the antibiotics tested.
10. Record the results in the report, Use table. For each antibiotic, determine whether the bacteria are resistant, susceptible or moderate or intermediate susceptible.



Read the plates in transmitted light



The zone of inhibition guides the right choice of Antibiotic



Challenge your understanding

- ✓ Why there are few colonies in the zone of inhibition?
- ✓ Why there are two zones of inhibition?

