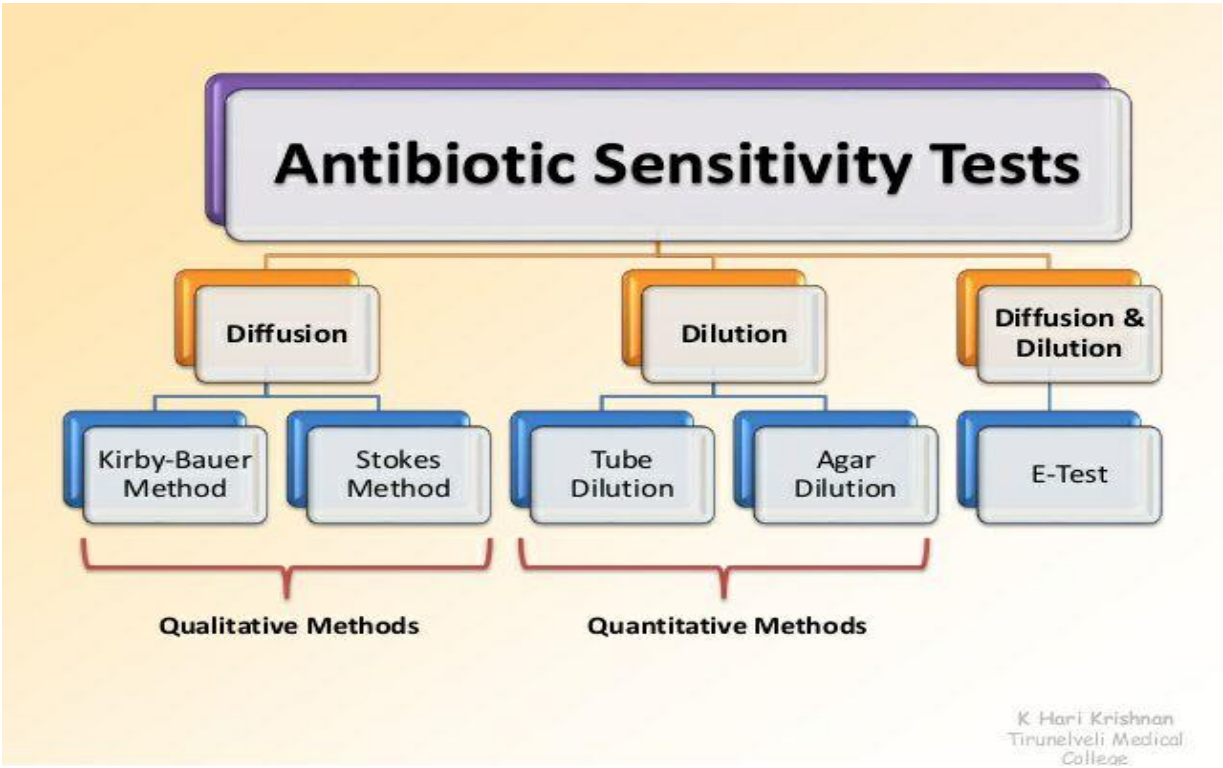
Lab : 8 Technique of antibiotic sensitivity

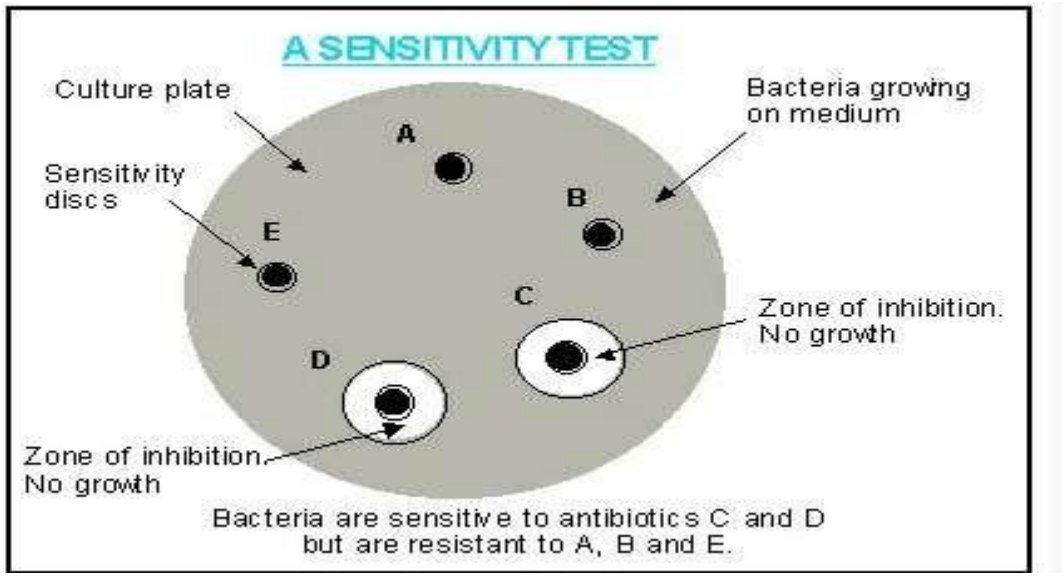
Antibiotic is a chemical substance produced by a *microorganism* that inhibits the growth or kills other microorganisms.



1- Diffusion method

Kirby-Bauer test

In Kirby–Bauer testing, discs containing antibiotics are placed on agar where bacteria are growing, and the antibiotics diffuse out into the agar. If an antibiotic stops the bacteria from growing, one can see circular areas around the wafers where bacteria have not grown.



KB tests are performed under standardized conditions and standard-sized zones of inhibition have been established for each antibiotic. KB test results are usually reported as sensitive, intermediate, or resistant, based on the size of the zone of inhibition. If the observed zone of inhibition is greater than or equal to the size of the standard zone, the microorganism is considered to be sensitive to the antibiotic. Conversely, if the observed zone of inhibition is smaller than the standard size, the microorganism is considered to be resistant. The size of a zone of inhibition in a KB test is inversely related to the minimum inhibitory concentration (MIC), which is the amount of antibiotic required to prevent bacterial growth in an overnight culture. The MIC (in µg/ml) can be calculated from known standard-curve (linear regression) . graphs based on the diameter of the observed inhibition zone diameter (in millimeters).

Clinicians can use KB test results to choose appropriate antibiotics to combat a particular infection in a patient. Administering antibiotics that specifically target the particular bacteria that are causing the infection can avoid using broad-spectrum antibiotics, which target many types of bacteria. Thus, clinical

application of KB testing results can decrease the frequency with which antibiotic-resistant bacteria evolve.

Materials Required

Petriplate containing microbial culture(For example, Escherichia coli) Inoculation loop , Bunsen burner, Saline solution, McFarland solution, MHA plate, Cotton swab, Antibiotic disks(Ciprofloxacin (CIP), Penicillin G (P), Gentamycin (G), Tooth pick, Incubator and Ruler

Procedure

1. Select a pure culture plate of one of the organisms to be tested.

2. Aseptically emulsify a colony from the plate in the sterile saline solution. Mix it thoroughly to ensure that no solid material from the colony is visible in the saline solution.

3. Repeat until the turbidity of the saline solution visually match that of the standard turbidity.

4. Take a sterile swab and dip it into the broth culture of organism.

5. Gently squeeze the swab against the inside of the tube in order to remove excess fluid in the swab.

6. Take a sterile Mueller-Hinton agar (MHA) plate or a nutrient agar (NA) plate.

7. Use the swab with the test organism to streak a MHA plate or a NA plate for a lawn of growth.

8. After the streaking is complete, allow the plate to dry for 5 minutes.

9. Antibiotic discs can be placed on the surface of the agar using sterilized

forceps.

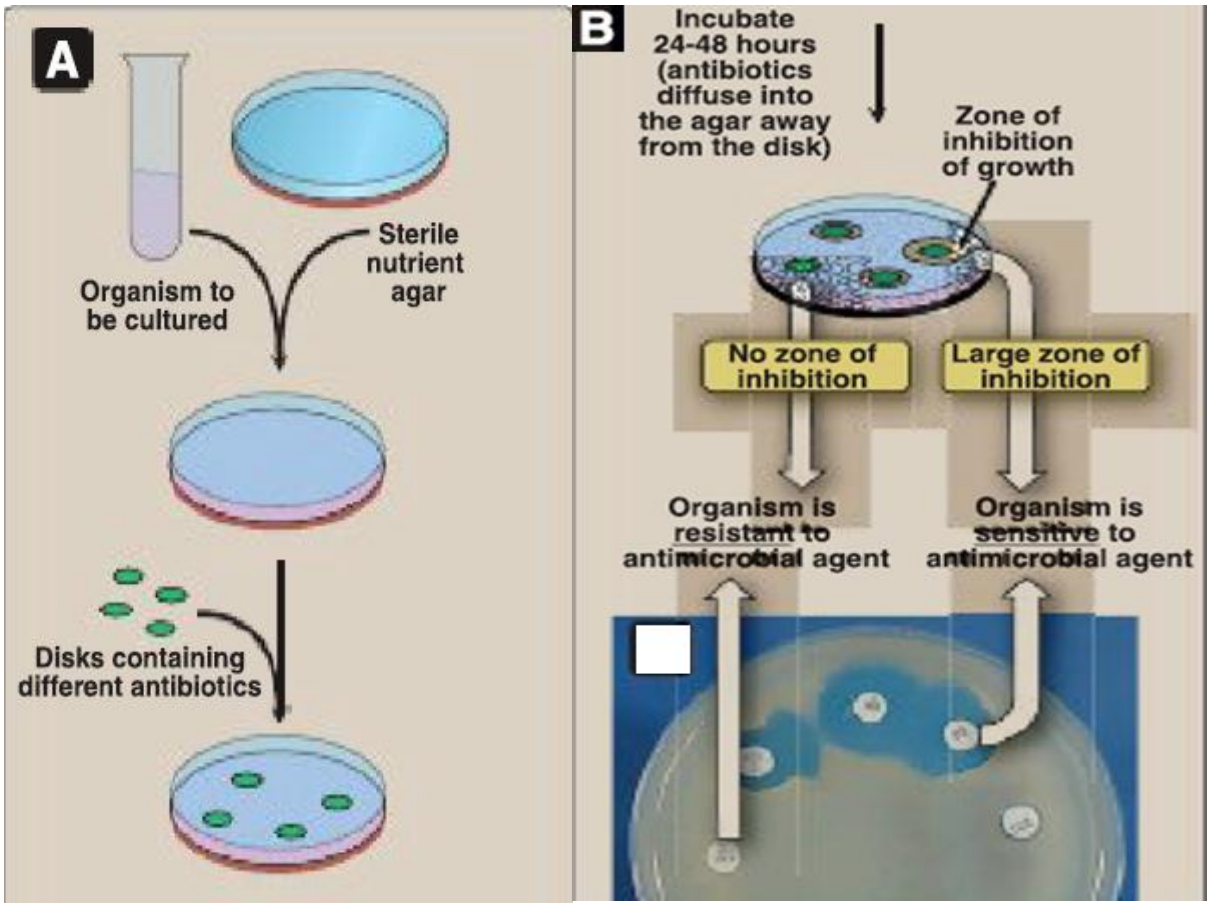
10.Gently press the discs onto the surface of the agar using flame sterilized forceps or inoculation loop.

11.Carefully invert the inoculated plates and incubate for 24 hours at 37° C.

12.After incubation, use a metric ruler to measure the diameter of the zone of inhibition for each antibiotic used.

13.Compare the measurement obtained from the individual antibiotics with the standard table to determine the sensitivity zone.

14.Compare the measurement obtained from the individual antibiotics to the standard table to determine whether the tested bacterial species is sensitive or resistant to the tested antibiotic.



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