

Q

1 B.Sc 2nd Sem

STUDENT'S NAME	
CLASS	SUBJECT
ROLL NO.	DATE

Antimicrobial agents

- Antimicrobial agents are the chemicals which act against microorganisms and they prevent infection due to microorganisms.

Terms of Antimicrobials

- 1) Antiseptics : — • These are the chemical substances which are able to kill and to prevent the growth of microorganisms (bacteria, fungi, protozoa etc) and they are generally applied to living tissues

- An ideal antiseptic should destroy bacteria, fungi, viruses without causing any harm to the tissue.

ex: Dettol

- 2) Disinfectant : — • A chemical agent which destroys microorganism on inanimate objects (eg. Instruments, equipment's etc)

- They prevent the infection by destruction of pathogenic micro organisms.
- Disinfectants are used for home and hospital sanitation and disinfection can be achieved by heat, chemicals or radiation (UV rays).
- They do not kill microorganism but reduces them to a level which is not harmful to health

ex: Sulphur dioxide (Lysol, Domex)

3) Germicides:— These are substances which kill microorganisms.
ex- bactericide, fungicide.

=> Bactericide:

A chemical agent which is capable of killing bacteria, but not its spores.

=> Fungicide:

A chemical agent which is capable of killing fungi including their spores.

• Bacteriostatic:

These are the chemical substances which act by inhibiting the growth of microorganisms.

4) Sterilization:— It is a process by which an object is rendered completely free from microorganisms. It involves the use of chemical or mechanical process (heat, radiation, filtration or chemical treatment).

5) Sanitation:— Disinfectants that are used to maintain general public health standards are termed as sanitizers.

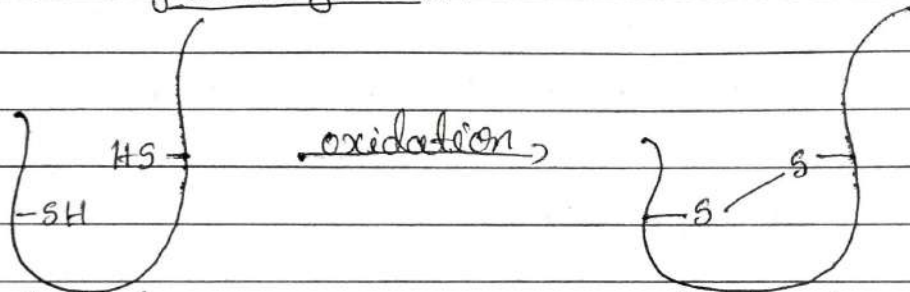
6) Preservative:— A chemical agent used to prevent microbial spoilage of preparations. It reduces microbial population to acceptable levels.

* Mechanism of anti-microbial action

- The mechanism of antimicrobial action ranges from mild astringent to powerful oxidative process.
- Inorganic compounds generally exhibit antimicrobial action by following 3 mechanisms.
 - 1) Oxidation
 - 2) Halogenation
 - 3) Protein binding or precipitation.

Oxidation

- Examples of compounds acting by this mechanism are peroxides of peroxy acids, oxygen liberating compounds like potassium permanganate.



Sulfhydryl group
in protein
(Normal protein
structure)

Disulfide bond in
protein (Protein
structure after
oxidation)

Halogenation

- Examples of compounds acting by this mechanism are the compounds which

⑧

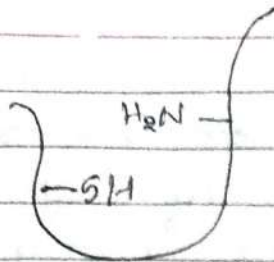
STUDENT'S NAME

CLASS

SUBJECT

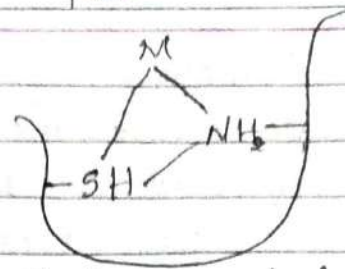
ROLL NO.

DATE



(Normal protein structure)

Protein precipitant



(Protein metal complex)

⇒ Characteristics of Anti microbial agent

- It should possess antiseptic / germicide activity & not bacteriostatic activity.
- It should have rapid onset of action and sustained activity.
- It must have good therapeutic index in the concentrations used.
- It should not cause local cell damage.
- It should not interfere with body defence.
- It should show no systemic toxicity from topical applications.
- It should have broad spectrum of activity against bacteria, fungi, protozoa, virus etc.
- It should have favourable lipid water distribution coefficient.

Antibiotic Sensitivity Test

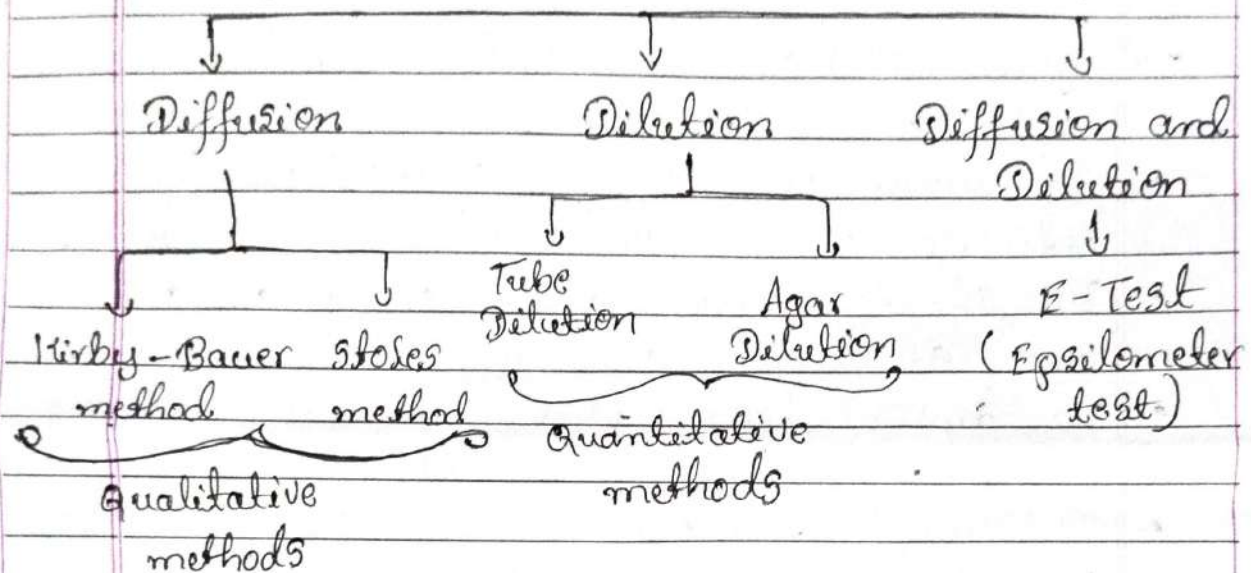
⇒ Definition :- This is a test done to check the effectiveness of a drug against a bacterium and to select the best drug with the right dosage.

- This procedure is done *in vitro*.

→ Purpose :-

- To guide the doctor to select best antibiotic.
- To control the use of wrong drugs.
- To give advance treatment for drug resistant bacteria.

Types of antibiotic sensitivity test



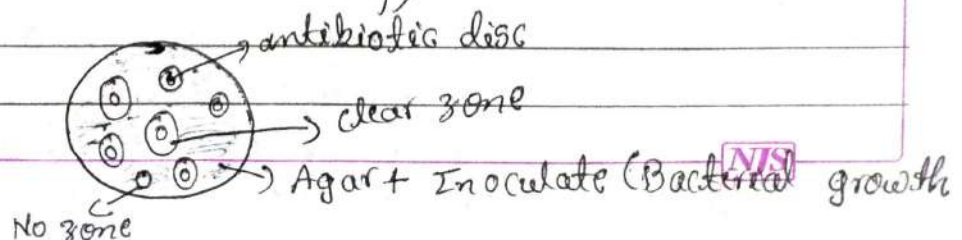
→ Diffusion method is a qualitative method and is done in case of less complicated cases

→ Dilution method is a quantitative test which are done in more complicated cases like transplantation.

★ ★

★ Diffusion method

① Kirby-Bauer / Disk diffusion method



- Principle

A paper disc with a definite amount of antibiotic is used to generate a transparent zone in the agar close to the disc.

- Materials

Mueller-Hinton Agar, Antibiotic Discs, Turbidity standard & Scrabs.

- Media - Preparation

a) Mediums containing beef infusion, peptone, and starch.

b) This media helps in the growth of non-fastidious organisms.

c) Cool the medium to $45-50^{\circ}\text{C}$ and pour into the plates. Allow to set on a level surface, to a depth of approximately 4 mm.

d) When the agar has solidified, dry the plates for 10-30 min at 35°C by placing them in the upright position in the incubator with the lids tilted.

e) If it is not to be used immediately, the agar medium can be stored in a refrigerator (2 to 8°C) for 2 weeks.

- Antibiotic Discs

a) Any commercially available disc with the proper diameter & potency can be used.

b) They have to be brought to room temp. at least 1 hour before use.

- Turbidity Standard

- a) Prepared by pouring 0.6 ml of a 1% (10 g/l) solution of barium chloride dihydrate into a 100-ml graduated cylinder, and filling to 100 ml with 1% (10 ml/l) sulfuric acid

- Procedure

- 1) To prepare the inoculum from the primary culture plate, touch with a loop the tops of each of 3-5 colonies, of similar appearance, of the organism to be tested.
- 2) Transfer this growth to a tube of saline.
- 3) Compare the tube with the turbidity standard and adjust the density of the test suspension to that of the standard by adding more bacteria or more sterile saline
- 4) Inoculate the plates by dipping a sterile swab into the inoculum.
- 5) Streak the swab all over the surface of the medium 3 times, rotating the plate through an angle of 60° after each application
- 6) Leave the inoculum to dry for a few minutes (but no more than 15 min) at room temp; with the lid closed
- 7) Place the appropriate antimicrobial - discs on the surface of the agar by using a sterile forceps, and Discs should be placed at a distance of 24 mm

STUDENT'S NAME

CLASS

SUBJECT

ROLL NO.

DATE

- 8) Each disc should be gently pressed down to ensure complete contact with the agar surface.
- 9) Incubate the plate upside down for 24 hours at 37°C.
- 10) After 24 hours of incubation, use a metric ruler to measure the zone of inhibition & include the diameter of the disc in the measurement.
- 11) Compare the result with CLSI (Clinical and Laboratory Standards Institute) guidelines to report the result.
- 12) The results are reported as Susceptible(S), Intermediate(I) or resistant.

Advantages

- This test is used in determining the antibiotics of choice to treat an infection.
- It can be useful in monitoring antimicrobials and for the selection of proper antibacterial agents.
- It doesn't require special equipment to perform and can be interpreted by all medical personnel.
- It costs less to perform this test.

★
★
★
⇒

Agar well Diffusion method

- 1) Agar well diffusion method mainly helps in the research purpose to check the antimicrobial activity of some plant, leaf or the seed extract.
- 2) The well diffusion assay is performing in Mueller Hinton agar.
- 3) Similarly to the procedure used in disk-diffusion method, the agar surface plate is inoculated by spreading a volume of the microbial inoculum over the entire agar surface (lawn culture) & make the wells in the MHA with around 6-8mm in diameter.
- 4) The wells are puncture with a sterile cork borer or with the backside of a sterile blue micropipette tip.
 - Then seal the underside of the well with melted ~~MHA~~ Mueller-Hinton agar.
 - Then the extracts with different conc. were added in different wells, also you can add a negative control and a positive control disc to compare the test result. The plates were then incubated in an upright position at 35-37°C for 18-24 hrs.
 - The antimicrobial activity was assayed by measuring the diameter of zone of inhibition formed around the wells.