**Antimicrobial Susceptibility Testing**

**Disk Diffusion**

**Objective**:-

To determine the effectiveness of antibiotics against bacteria.

**Factors affect Antibiotic Susceptibility Testing**:-

1. pH**.**
2. Moisture.
3. Effects of Thymine (Müeller-Hinton agar is low thymidine content)
4. Effects of Variation in Divalent Cations like magnesium, calcium and zinc (Müeller-Hinton agar must conform to the control limits)

**Methods** :-

* Müeller-Hinton Agar media were prepared.
* Inoculum Preparation,,, a) Growth Method: from an agar plate culture. Inoculating a tube containing 4 to 5 ml of a suitable broth medium was done.( The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard)
* B) Direct Colony Suspension Method: prepared by making a direct broth or saline suspension of isolated colonies selected from a 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used).
* Inoculation of Test Plates: a) Sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. b) The dried surface of a Müeller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60 each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.
* **NOTE**: Extremes in inoculum density must be avoided.
* Antimicrobial discs are dispensed onto the surface of the inoculated agar plate (Not more than: 12 discs one 150 mm plate or 5 discs on a 100 mm plate). Each disc must be pressed down to ensure complete contact with the agar surface.
* The plates are inverted and placed in an incubator set to 37◦ C within 15 minutes after the discs are applied.

**Reading Plates and Interpreting Results**:-

* After 16 to 18 hours of incubation, each plate is examined (Staphylococcus or Enterococcus spp., 24 hours of incubation are required for vancomycin and oxacillin). the resulting zones of inhibition will be uniformly circular.
* The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. using a ruler, which is held on the back of the inverted petri plate.
* Oxacillin >0.5 Ug /ml-(S.aureus) >13 mm (Susceptible) --11-12 mm (Intermediate)--<10 mm (Resistant).