ANTIBIOTIC SUSCEPTIBILITY

1-Diffusion method:

The Kirby Bauer method(K-B) used for antibiotic susceptibility test, it's common method used for years.

This test is useful to find or chose the most effective drug to kill the infective microorganism

The basics are : the filter paper discs containing measured concentration of drug is placed on the agar, the antibiotic diffuses from the disc into the surrounding area .after incubation, if the organism is killed or inhibited by the concentration of the antibiotic, there will be clear zone around the disc (no growth) ;this is called the inhibition zone . the zone diameter are looked up on the standardized chart to give result as sensitive, resistance or intermediate.

The method:

1-lable the agar plate with information.
2-dip the sterile swab into the broth .
3-swab the surface of agar completely (you do not leave any un swabbed agar area at all) when the plate is not swabbed correctly with even coverage of bacterium over the entire agar.
4-after completely swabbing the plate, turn it 90 degrees and repeated the swabbing process (it is not necessary re moisten the swab) run the swab around the circumference of plate before discharge .
5-alow the surface to dray for 2-5 minute .
6-placing (disrupting) the antibiotic disc on the surface of inoculum agar using sterile forceps then touch each disc with sterile forceps to make sure that it is good contact with agar surface.
7-incubate the plate at 37c over night.
8-record the result by place the metric ruler across the zone of inhibition and measured the diameter from edge to edge of the zone.
9-zone diameter is reported in millimeter, looked up on the chart and result reported as sensitive, resistance or intermediate.

#sensitive(S): the bacterial strain is susceptible to a given antibiotic when it is inhibited in vitro by a concentration of this drug that is associated with a high likelihood of therapeutic success.

Intermediate(I) : the susceptibility of bacterial strain to a given antibiotic is intermediate when it is inhibited in vitro by a concentration for this drug that is associated with uncertain therapeutic effect.

#Resistant(R):the bacterial strain is resistant to a given antibiotic in vitro by a concentration of this drug that is associated with high likelihood of therapeutic failure.

#the factors affect on antimicrobial activity :

1-sensivit y of microbe to the antibiotic.
2-the rate of antibiotic diffusion through the Muller Hinton agar(M-H-A).
3-PH of environment , some drugs are active in acidic while other active in alkaline .
4-components of medium, there are some compounds interact with drug activity.
5-stability of drug, at incubator temperature several antibiotics lose their activity.
6-size of inoculum , in general the larger bacterial inoculum, the lower sensitivity (lower drug activity).
7-length of incubation, in many instance the microorganisms are not killed but only inhibited , the longer incubation continues, the greater the chance for resistance.

#Muller -Hinton agar(M-H-A) is most frequently used for routine susceptibility test for the following reasons:

1-M-H-A is non selective or deferential medium this mean that all non fastidious microorganisms grow on it.
2-it contains starch, starch is known to absorb toxins released from bacteria ,so that they cannot interfere with the antibiotics.
3-it is loss agar, this allows for better diffusion of the antibiotic than other agar.

4-it is low in sulfonamide, trimethoprim and tetracycline inhibitor.

2-Dillution method:

The basics is: graded amount of drug are add into liquid or solid media. in the other word the tested antibiotic diluted to produced a series of concentration. The media inoculated with test microorganisms and incubated, the end point is taken as that amount of antibiotic concentration required to inhibit the growth or kill test the bacteria.

There are two type of this method: agar dilution & broth dilution, both of them are used to determine minimum inhibitory concentration(MIC) and minimum bactericidal concentration (MBC).

MIC: is the lowest concentration of antimicrobial agent which prevent bacterial growth.

MBC: is the lowest concentration of antimicrobial agent required to kill the bacteria.

Some antibiotic actually kill the bacteria it's called **Bactericidal**, whereas others merely prevent bacterial growth and multiply it's called **Bacteriostatic.**

Antimicrobial action generally falls within one of four mechanisms inhibition of cell wall biosynthesis ,protein synthesis, nucleic acid metabolism and repair, or disruption of cell membrane .

Lecturer : Rawaa M.O. Hraishawi