ANTIBIOTIC SENSITIVITY TEST

On the advent of Penicillin by Alexander Fleming in 1928, proved to be wonder drug in treating the infections. Many antimicrobial compounds were discovered and was predicted that infectious diseases would be eliminated through the use of antimicrobials. But the indiscriminate use of antimicrobials led to the development of bacterial resistance to antimicrobials, diminished the optimism and resulted in the need for physicians to request the microbiology laboratory to test a pathogen against various available and widely used array of antibiotics in medical and veterinary treatment.

Initially broth dilution method was used, later on modified to disc diffusion method in which the antimicrobials are impregnated onto the 6 mm size paper disc used for assessing the antimicrobial activity on lawn culture of pathogen on specific solid gel media incubated for specific time at 37^{O}C for 12-24 Hrs.

Antibiotic sensitivity test is carried out to find out sensitivity of organisms to particular antibiotic/antimicrobial drug. This test has great importance to direct the clinicians for employing antibiotic therapy in patients.

In 1950's many laboratories modified the disc diffusion method to suit their requirement. Changes in the media used, concentrations of drug impregnated on disc, number of bacteria in inoculums, time and temperature combination, led to mass confusion and variations in results.

The present day method, described by W. M. M. Kirby and A. W. Bauer (1966) is the result of extensive review of literature, known as **Kirby-Bauer Disc Diffusion method** and put forth one standard protocol published and accepted by all (WHO) to test the sensitivity of an organisms towards antimicrobial drugs/antibiotic.

To bring the uniformity and reproducibility, the Clinical Laboratory Standards Institute (CLSI) is authorized (USA) for updating and modifying the original procedure of Kirby and Bauer. The zone of inhibition for a particular antibiotic and pathogen should be as per the guidelines of CLSI.

Principle of AST:

When disk impregnated with antimicrobial, is placed on Mueller Hinton Agar medium, water is absorbed in to the disk immediately and the antimicrobial begins to diffuse into the surrounding agar medium. The rate of diffusion is not rapid, so the concentration of antimicrobial is highest closer to the disc and a logarithmic reduction in concentration occurs as the distance from the disc increases. The rate of diffusion depends upon the solubility property of drug in agar.

If the agar plate is seeded with pathogen prior to the placement of discs, simultaneous growth of organism and antimicrobial diffusion through agar medium occurs. The antibiotic will diffuse in agar medium and sensitive organism will not grow in the vicinity of such antibiotic while the resistant strain will show either very less or no zone of inhibition depending upon the antibiotic concentration in the agar medium near to the disc.

Zone of inhibition of any size observed in a disk diffusion test has no meaning as such. The interpretation of resistance and susceptibility to antimicrobials is determined by correlating with zone sizes resulting in the interpretive standards (CLSI).

Materials:

Muller and Hinton Agar plates, Antibiotic discs, Scale, Zone of inhibition interpretation chart, Sterile swabs, Clinical Sample/Fresh Culture, Peptone water, Inoculation loop, Forcep, Spirit lamp.

Preparation of media

MH agar plates from dehydrated media, should be prepared with a depth of 4 mm (25 ml of liquid agar for 100-mm plates). Too shallow plates will produce false susceptible results as the antimicrobial compound will diffuse further than it should, creating larger zones of inhibition. Conversely, plates poured to a depth of more than 4 mm will result in false resistant results.

Preparation of inoculums

1. Using a sterile inoculating loop or needle, touch four or five isolated colonies of the organism to be tested.
2. Suspend the organism in 2 ml of sterile saline.
3. Vortex the saline tube to create a homogenous suspension.
4. Adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more colonies if the suspension is too thin or diluting with sterile saline if the suspension is too thick. Use this suspension within 15 minutes of preparation.

Procedure:

1. With the help of sterile loop inoculate the sample in peptone water tube and incubate at 37 ^{0}C for 24 hrs.
2. Inoculate the culture on Muller and Hinton Agar plate by spread plate method using sterile swab. Allow the plate to absorb the culture.
3. With the help of sterile forcep place the antibiotic discs on agar surface keeping 2 cm distance between two adjacent discs. **Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug begins to diffuse immediately upon contact with the agar.**
4. These antibiotic discs shall be chosen depending upon the type of sample and history of symptoms provided by clinicians.
5. Incubate the plate at 37 ^{0}C for 18-24 hrs.
6. Measure the diameter of zones of inhibition of bacterial growth in mm with the help of scale.
7. The diameter is measured vertically as well as horizontally and average of both is taken as reading.
8. Record the observations and compare with their respective prescribed zones of inhibition mentioned in interpretation chart.
9. The suitable antibiotic shall be chosen for treatment amongst effective antibiotics.

Interpretation

Zone Interpretation Chart for *Staphylococcus aureus* **(Zone diameter in mm)**

Antibiotics	Susceptible (mm or more)	Intermediate (mm)	Resistant (mm or less)
Amikacin (30mcg)	17	15-16	14
Penicillin (10 Units)	29	-	28
Tetracycline (30 mcg)	19	15-18	14
Ciprofloxacin (5 mcg)	26	22-25	21
Ceftriaxone (30 mcg)	21	20-22	19
Cefotaxime (30 mcg)	23	15-22	14

Zone Interpretation Chart for *Enterobacteriaceae* **(Zone diameter in mm)**

Antibiotics	Susceptible (mm or more)	Intermediate (mm)	Resistant (mm or less)
Amikacin (30mcg)	17	15-16	14
Streptomicin (10mcg)	15	12-14	11
Gentamicin (10mcg)	15	13-14	12
Ciprofloxacin (5mcg)	21	16-20	15
Ceftriaxone (30mcg)	23	20-22	19
Cefotaxime (30 mcg)	26	23-25	22
Tetracycline (30mcg)	15	12-14	11

Exercise:

1. Write the Zone of Inhibition Chart for other commonly used antibiotics.

References:

1. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 36:493-496.

2. www.microbelibrary/Kirby-Bauer Disk Diffusion Susceptibility Test Protocol.htm

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For interpretive Zones of Inhibition log on to the links:
http://www.himedialabs.com/intl/en/products/Microbiology/Antimicrobial-Susceptibility-Sensitivty-Discs-Antibacterial-As-per-CLSI/100000449

Clinical Laboratory Standards Institute
http://www.clsi.org/blog/2012/01/13/clsi-publishes-2012-antimicrobial-susceptibility-testing-standards/