Antibiotic Sensitivity Test

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In Clinical laboratories antibiotic sensitivity testing is carried out as a guide to antibiotic therapy. This test is important in the case of organisms responsible for an infectious disease whose susceptibility cannot be predicted because of their ability to acquire resistance against commonly used antimicrobial agents. There are two types of sensitivity tests which are commonly used-dilution tests and diffusion tests.

The disc diffusion method is the most convenient one under field conditions. In this test, a solid culture medium is evenly inoculated with the organism to be tested and standard filter paper discs containing given amounts of antibiotics are put on the surface. During incubation, antibiotic diffuses radially from the disc into the medium. After incubation, the degree of sensitivity is measured by measuring the zone of inhibition of growth.

Isolation of the causative agent in pure culture is a pre requisite for this test. For that, appropriate clinical material is inoculated on a suitable medium and incubated. After obtaining the growth of the organism and staining it by Gram's method, disc diffusion method of sensitivity is performed.

For easy description the entire procedure is mentioned under different headings.

1. Medium used.

Mueller-Hinton agar is used and it is prepared and sterilized as per manufacture's instructions. For growing fastidious organisms the agar is supplemented with 5 percent defibrinated blood.

2. Preparation of the plates

The sterilized medium is poured in to 150 mm or 100 mm diameter petridishes when the temperature is about 45°C. Agar depth should be between 4 to 6mm (20-25 ml is required for 100 mm petridish and 70-80 ml for 150 mm petridish). After sterility checking (by keeping the plates at 37°C overnight), the plates without any visible growth are stored at 4°C and preferably used within two weeks of preparation. Immediately prior to use, the plates should be dried in an incubator for about 30 minutes in order to avoid droplets of moisture on the agar surface.

3. Preparation of inoculum

Inoculum is prepared from a pure primary culture of the organism. The isolated colonies should be stained by Gram's method before preparing the inoculum. This will help us to select the suitable antibiotics for the causative agent. Three to four similar colonies are selected and used as inoculum in 5 ml of Trypticase soya broth to obtain moderate turbidity. If turbidity from inoculation is sufficient, incubation is not necessary. Otherwise incubate the broth culture at 35-37°C for 2-5 hours to get suitable turbidity. It is then diluted with sterile normal saline to obtain a density equivalent to barium sulphate standard prepared by adding 0.5 ml of 1 percent barium chloride to 99.5 ml of 0.36N sulphuric acid

4. Inoculation

A sterile swab is immersed in to the diluted inoculum suspension. Excess broth is expressed by pressing and rotating firmly against the upper inside wall of the tube. The entire agar surface is streaked with the swab in three different directions by turning the plate 60°C between each streaking. A final circular rotation is made around the agar rim. The plates are dried at room temperature for 5 minutes.

5. Application of discs

Antibiotic impregnated discs are applied on the surface of the medium by means of a sterile forceps using aseptic technique. Various antimicrobial discs are being marketed by different firms like Hi Media, Pasteur, Span diagnostics etc. Discs are deposited with centres at least 24mm apart. so the number of discs that can be placed in a 150mm plate is 12 to 13 or 4 to 5 in a 100mm plate. The discs should not be

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Zone-diameter interpretive standards				
Antimicrobial agent	Disc content	Inhibition zone diameter		
		Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)
Amikacin	30/ug	14	15-16	17
Ampicillin				
a) Enterobacteriaceae and Enterococci	10/ug	11	12-13	14
b) Staphylococcus and penicillin sensitive organism.	10/ug	20	21-28	29
c) Haemophilus	10/ug	19	---	20
Carbenicillin				
a) Proteus & E. coli	100/ug	17	18-22	23
b) P. aeruginosa	100/ug	11	12-14	15
Cephalothin	30/ug	14	15-17	18
Chloramphenicol	30/ug	12	13-17	18
Clindamycin	2/ug	14	15-16	17
Erythromycin	15/ug	13	14-17	18
Gentamycin	10/ug	12	13-14	15
Kanamycin	30/ug	13	14-17	18
Methicillin	5/ug	9	10-13	14
Nalidixic acid	30/ug	13	14-18	19
Neomycin	30/ug	12	13-16	17
Nitrofurantoin	300/ug	14	15-16	17
Penicillin G				
a) Staphylococci	10iu	20	21-28	29
b) Other organisms	10iu	11	12-21	22
Polymyxin B	300 iu	8	9-11	12
Streptomycin	10/ug	11	12-14	15
Sulphonamides				
a) N. meningitidis only	250/ug	--	---	40
b) Other organisms	300/ug "	12	13-16	17
Tetracycline	30/ug	14	15-18	19
Tobramycin	10/ug	11	12-13	14
Co-trimoxazole	25/ug	10	11-15	16
Vibramycin	30/ug	14	15-18	19
Bacitracin	10iu	8	9-12	13

removed once it has come in contact with the agar surface because some diffusion of the drug is almost instantaneous.

6. Incubation of plates

The plates are inverted and immediately placed at 37°C and incubated for 16 to 18 hours or later if necessary.

7. Reading of the results and interpretation

After the incubation period, plates are examined and the diameter of the zone of complete inhibition is measured to the nearest whole mm with a ruler.The tiny colonies or faint growth in the inhibition zone are ignored. Large colonies growing

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