



ANTIBIOTIC SUSCEPTIBILITY TESTING

Bacteria demonstrate two kinds of resistance to antibiotics, namely intrinsic resistance and acquired resistance. Intrinsic resistance means that the species was resistant to an antibiotic even before its introduction. Acquired resistance means that the species was originally susceptible to an antibiotic, but later became resistant. Bacteria can acquire antibiotic resistance either by mutation or through exchange of genetic material among same or closely related species. The sudden acquisition of resistance to antibiotics poses difficulties in treating infections. Resistance to several different antibiotics at the same time is even more significant problem. It is because of the acquired resistance that bacterial isolates must be subjected to antibiotic susceptibility testing. Bacteria showing reduced susceptibility or resistance to an antibiotic implies that it should not be used on the patient.

Various methods of antibiotic susceptibility testing are:

1. Quantitative Methods
2. Qualitative Methods
3. Automated Susceptibility Tests
4. Newer Non-Automated Susceptibility Tests
5. Molecular Techniques

Quantitative Methods:

In these tests, the minimum amount of antibiotic that inhibits the visible growth of an isolate or minimum inhibitory concentration (MIC) is determined. Bacterial isolate is subjected to various dilutions of antibiotics. The highest dilution of antibiotic that has inhibited the growth of bacteria is considered as MIC. These tests can be performed on broth or agar.

1. Broth dilution methods
 - a. Macrobrotth dilution MIC tests
 - b. Microbroth dilution MIC tests
2. Agar dilution methods

Macrobrotth dilution tests:

A serial two-fold dilution of antibiotic are made in test tubes from 0 to maximum concentration that is achieved in vivo without toxic effect on patient. The inoculum density of bacterial isolate to be tested is standardized with 0.5 McFarland turbidity standard. The suspension should have a final inoculum of 5×10^5 cfu/ml. 1ml of bacterial suspension is added to rows of antibiotic solution and incubated at 37°C overnight. The lowest concentration of antibiotic that completely inhibits visual growth of bacteria (no turbidity) is recorded as MIC.

Microbroth dilution tests:

A polystyrene tray containing 80 wells is filled with small volumes of serial two-fold dilutions of different antibiotics. The inoculum suspension and standardization is done according to McFarland standard. The bacterial inoculum is then inoculated into the wells and incubated at 37°C overnight. MIC is determined as in macrobroth dilution test.

Agar dilution method:

A serial two-fold dilution of the antibiotic is prepared in Mueller-Hinton agar. The bacterial inoculum is standardized according to McFarland standard. Using calibrated loops a volume of 0.001-0.002 ml is inoculated on the surface of agar and incubated at 37°C overnight. The lowest concentration of antibiotic that inhibits visible growth on surface of agar is taken as MIC.

Qualitative Methods:

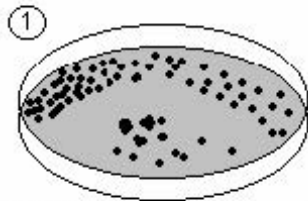
These tests categorize a bacterial isolate as sensitive, intermediate or resistant to a particular antibiotic.

Disk diffusion test:

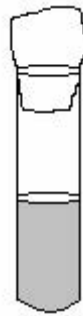
In this method the standardized bacterial isolate is spread on an agar plate and then paper disc containing specific concentration of antibiotics are placed and incubated at 37°C overnight. If the isolate is susceptible to the antibiotic,

it does not grow around the disk thus forming a zone of inhibition. Strains resistant to an antibiotic grow up to the margin of disk. The diameter of zone of inhibition must be measured and result read from the Kirby Bauer chart as sensitive, intermediate or resistant.

Procedure for disk diffusion testing



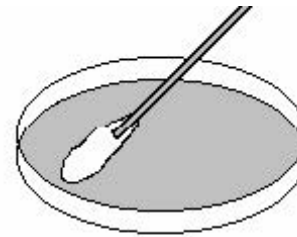
①
2-3 identical colonies are picked from the plate and transferred to the broth



②

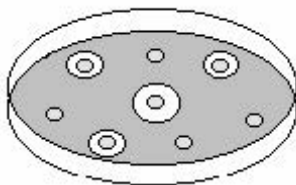
The tube is incubated for the bacteria to grow.

The inoculum density is standardized using McFarland standard



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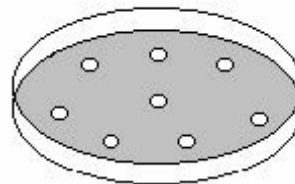
A cotton swab dipped in the inoculum suspension is swabbed over the entire surface of agar to give a lawn culture.



⑤

Zone diameter around the disk are measured and result read from Kirby Bauer chart

Plate incubated at 37°C overnight



④

Filter paper disks containing known antibiotic in known concentration is placed on the surface of inoculated agar.

Automated Susceptibility Methods:

Determination of bacterial growth in wells containing antimicrobial agent are performed in short period of time using computer-assisted instruments. Various techniques include:

Turbidimetric detection: VITEK (report ready in 4-15 hours)

Fluorimetric detection: AutoSCAN Walkaway (report ready in 3.5-7 hours)

Video Image processing: ALADIN (report ready in 18-24 hours)

Newer Non Automated Susceptibility Tests:

Alamar system, E-test and Spiral gradient endpoint system

Molecular Techniques:

Detection of gene coding for resistance to one or several drugs by techniques such as PCR and DNA hybridization.