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Research Article

Antimicrobial susceptible test

Anti-microbial susceptibility test is useful to guide the clinician in the selection of antimicrobial agents to which clinical condition being treated will respond. There are three principal methods of antimicrobial susceptibility testing like disc diffusion, broth dilution and agar dilution¹.

Disc diffusion test

It is performed by impregnating of antimicrobial agents onto an agar plate seeded with the bacterium to be stored. The antimicrobial agents diffuse into agar creating a zone saturated with the agent, in which organisms susceptible to that agent will not growth. The edges of zone are the point of minimum inhibition concentration [1].

Reagents for the disk diffusion test: Mueller Hinton agar medium is considered to be the best for routine susceptibility testing of non fastidious bacteria for the following reasons:

- ✓ It shows acceptable batch-to-batch reproducibility for susceptibility testing.
- ✓ It is low in sulphonamide, trimethoprim, and tetracycline inhibitors.
- ✓ It gives satisfactory growth of most nonfastidious pathogens.
- ✓ A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Müeller-Hinton agar preparation includes the following steps.

- * Müeller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer's instructions.

- * Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath.
- * Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.
- * The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C).
- * Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.
- * A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

Procedure for performing the disc diffusion test

The growth method is performed as follows:

- * At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.
- * The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours)
- * The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standards. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

- * **Direct Colony Suspension Method:** As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used).
- * The suspension is adjusted to match the 0.5 McFarland turbidity standards, using saline and a vortex mixer. This approach is the recommended method for testing the fastidious organisms, *Haemophilus* spp., *N. gonorrhoeae*, and streptococci, and for testing staphylococci for potential methicillin or oxacillin resistance.

Inoculation of Test Plates

- * **Adjust Turbidity Of Inoculum:** Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, 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.
- * **Inoculate the swab over dried mellerian hintan agar by streaking:** 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.
- * **Impregnated disks:** The lid may be left a jar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

Reading plates and interpreting results

The interpretation is listed as following [1,2]:

Susceptibility is high probability of success following treatment, whereas resistance is low probability of success following resinsatce. After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc.

Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate (Figure 1). The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light. If blood was added to the agar base (as with streptococci), the zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed. If the test organism is a *Staphylococcus*

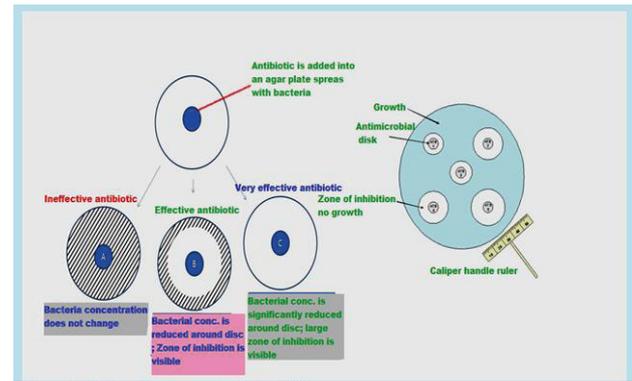


Figure 1: Agar diffusion and antimicrobial test.

or *Enterococcus* spp., 24 hours of incubation are required for vancomycin and oxacillin, but other agents can be read at 16 to 18 hours. Transmitted light (plate held up to light) is used to examine the oxacillin and vancomycin zones for light growth of methicillin- or vancomycin- resistant colonies, respectively, within apparent zones of inhibition. Any discernable growth within zone of inhibition is indicative of methicillin or vancomycin resistance.

The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored. When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter.

Dilution methods

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are tested in log² serial dilutions (two fold). Minimum Inhibitory Concentration (MIC) Diffusion tests widely used to determine the susceptibility of organisms isolated from clinical specimens have their limitations; when equivocal results are obtained or in prolonged serious infection e.g. bacterial endocarditis, the quantitation of antibiotic action vis-a-vis the pathogen needs to be more precise (Figure 2). Also the terms 'Susceptible' and 'Resistant' can have a realistic interpretation. Thus when in doubt, the way to a precise assessment is to determine the MIC of the antibiotic to the organisms concerned. There are two methods of testing for MIC are Broth dilution method and Agar dilution method.

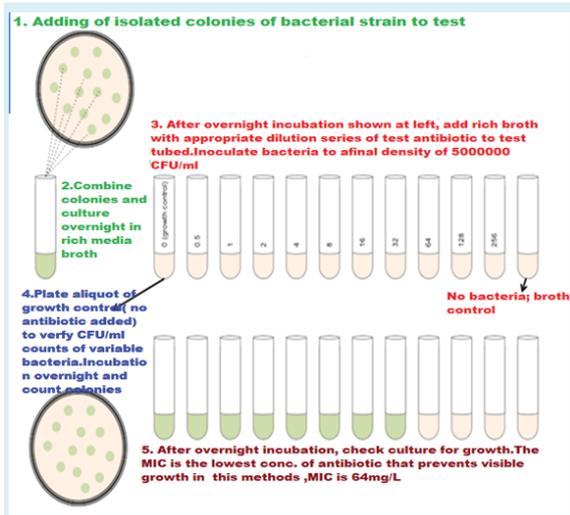


Figure 2: Broth dilution methods. The MIC is the lowest concentration of antibiotic that lacks turbidity.

Broth dilution method

The Broth Dilution method is a simple procedure for testing a small number of isolates, even single isolate. The broth dilution method is based on serial dilutions of an antimicrobial agent in tubes, or microtitre plates, to which a standard concentration of the test bacterium is added [3]. Results are read as a MIC in mg/mL, that is, the lowest concentration of antibiotic that completely inhibits the growth of the bacterium; this is the first tube showing no discernible growth. If the contents of the tubes are cultured onto agar plates then the plate with no growth represents the tube with the minimal bactericidal concentration (MBC) of antimicrobial and may

be different to the tube indicating the MIC. The tubes are incubated and examined for turbidity. A turbid sample is an indication of bacterial growth, whereas a clear sample is an indication of inhibition of bacterial growth. The MIC is the lowest concentration of the antibiotic being tested that inhibits the growth of the bacteria, resulting in a sample that lacks turbidity [4].

The agar dilution method

The agar dilution method is similar to the broth dilution method in that the antimicrobial agent is serially diluted and the dilutions added to the agar medium. The test bacterium is spot-inoculated at a standardized concentration on to the agar surface. Agar plates are prepared with antimicrobial concentrations that encompass the quality control range and the breakpoints. Agar plates containing antimicrobial concentrations must be used within a week of preparation, and therefore, the method requires more media preparation than the disk diffusion method and is more likely to be used by laboratories with a high sample throughput [4].

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