Quality Control of Antimicrobial Susceptibility Tests

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Abstract :

Background: Susceptibility test may be performed in the clinical laboratory for two main purposes. 1)To guide the clinician in selecting the best antimicrobial agent for an individual patient. 2) To accumulate epidemiological information on the resistance of microorganisms of public health importance within the community. **Aims & Objectives:** To define variables affecting antimicrobial susceptibility testing in disc diffusion method in microbiology laboratory, B.J. Medical College, Ahmedabad, Gujarat. **Materials & Methods:** This is an observational study which was done by using disc diffusion method . Total 600 Antimicrobial Susceptibility tests of weekly Quality Control(QC) were observed from 1st September 2014 to 30th April 2015. Out of total 600 Antimicrobial Susceptibility tests of weekly QC,200 tests were of E. coli ATCC 25922 , 200 tests were of S. aureus ATCC 25923 and 200 were of P. aeruginosa ATCC 27853. **Results:** Out of 600 Antimicrobial Susceptibility Tests of weekly QC , abnormal results were found with 22 (3.7 %) tests and unaffected tests were 578 (96.4 %). Variables affecting Antimicrobial Susceptibility Testing (AST) were in 22 tests. Out of them, factors contributed by media were 5 (0.8 %), antimicrobial disc were 3(0.5 %), inoculums were 5(0.8%), environmental factors were 3 (0.5%), incubation were 2(0.33%), observational factors were 2 (0.33%) & equipment problems in 2(0.33%). **Conclusion:** Internal quality assessment is a useful complementary approach to external quality assessment and may detect problem areas not highlighted by other control methods. Education, accuracy and training are an important part of the quality assurance process.

Key words: Antimicrobial Susceptibility Test, Quality Control

Introduction :

Most of the clinically important bacteria causing infections in humans are capable of exhibiting resistance to antimicrobial agents commonly used for the treatment. Thus, the report produced by clinical microbiology laboratory for the physician, also includes organism's susceptibility profile to different antimicrobials along with its identification.[1] Antimicrobial susceptibility testing (AST) is performed on bacteria which are isolated from clinical specimens to determine if they are killed, inhibited or unaffected by the antimicrobial drugs that may be potential choices for therapy. Although the importance of antimicrobial susceptibility testing is well established, the procedure itself is very sensitive to changes in the environment and test conditions. Therefore, it is crucial that each variable in the procedure should be standardized and carefully controlled. Owing to the numerous variables that may affect the results,[2] rigorous quality control is of utmost importance for susceptibility testing. Properly performed quality control would aid in providing accurate, reproducible and timely results.

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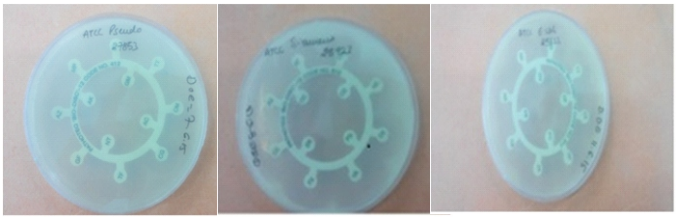
Materials and Methods:

This is an observational study which was done by using disc diffusion method.

Total 600 Antimicrobial Susceptibility tests of weekly QC were observed from 1st September 2014 to 30th April 2015. Out of the total 600 Antimicrobial susceptibility tests, 200 tests were of E. coli ATCC 25922, 200 tests were of S. aureus ATCC 25923 and 200 were of P. aeruginosa ATCC 27853.

Figure 1: Antimicrobial Sensitivity Testing of the QC strains.



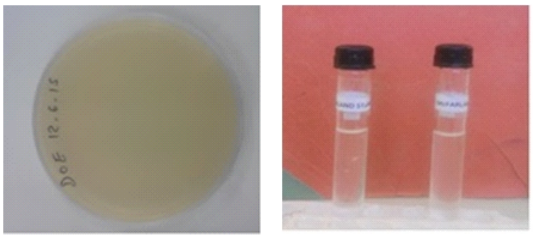
ATCC P.aeruginosa 27853 | ATCC S.aureus 25923 | ATCC E.Coli 25922

We have used Agar disc diffusion method taking into account the following particulars:

Table 1 : Specifications for performing AST

Medium	Mueller Hinton Agar	4 mm thickness pH : 7.2 to 7.4
Antibiotic discs	Storage temperature	-20 °C minimum
Inoculum	McFarland 0.5	10^8 bacteria/mL
Incubator	Temperature	37° C
	Atmosphere	Ambient air

Figure 2(a) &2(b): Mueller Hinton Agar medium & McFarland Standard

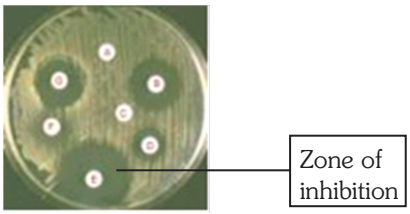


(a) Mueller Hinton agar (b) McFarland Standard 0.5

Procedure:

We used well-isolated, 18-24 hour old colonies of the organism which was transferred to a sterile saline tube with a turbidity of 0.5 McFarland. Then Mueller Hinton agar was inoculated by swabbing in three different directions forming a "Lawn of growth" and filter paper discs impregnated with antimicrobial agents were placed on the agar. And finally it was inverted and incubated for 16-18 hours at 37°C. During incubation, drug diffuses into agar. Depending on the susceptibility of organisms, areas of no growth form a zone of inhibition. Zones are measured to determine whether the organism is susceptible, intermediate, or resistant to the drug.

Figure 3: AST of test organism showing zones of inhibition.



Amongst the tests indicating unacceptable performances, the sources of the errors were investigated with help of daily records of:

- Refrigerator temperature, deep freeze temperature and incubator temperature.
- Thickness and pH of media.
- Antimicrobial disc expiry date records.
- Zone diameter of weekly QC strain.
- Over growths , scanty growths and mix growths.
- Sterility testing of petridish ,MH agar ,swab , bio safety cabinet & incubator.
- Humidity & environment temperature.
- QC of MH agar plate.

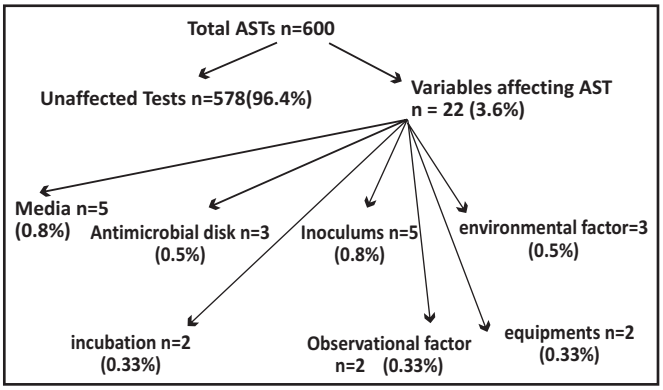
Errors in media, antimicrobial discs, inoculum, incubation ,equipments, observation and environmental factors were noted.

Result:

In the study ,we have done a total of 600 Antimicrobial Susceptibility Tests of weekly QC of E. coli ATCC 25922 , S. aureus ATCC 25923 and P.aeruginosa ATCC 27853.

Out of 600 tests, 578 (96.4%) tests were unaffected while 22(3.6%) tests were affected due to various variable factors. In them, media was the contributing factor in 5 (0.8%) tests, in 3 (0.5%) tests problems were found in antimicrobial discs, inoculums were contributing factors in 5 (0.8%) tests, 3 (0.5 %)tests were affected by environmental factors, observational error was affecting 2 (0.33%) tests and problems in equipment and incubation were found in 2 (0.33%)tests each.

Figure 4 : Antimicrobial Sensitivity Testing of Quality Control Strains & The Effect of Different Variables.



Discussion:

Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agent to inhibit bacterial growth in vitro. This ability may be estimated by either the dilution method or the diffusion method.[3]

The dilution method:

For quantitative estimates of antibiotic activity, dilutions of the antibiotic may be incorporated into broth or agar medium, which is then inoculated with the test organism. The lowest concentration that prevents growth after overnight incubation is known as the minimum inhibitory concentration (MIC) of the agent. The MIC value is then compared with known concentrations of the drug obtainable in the serum, and in other body fluids to assess the likely clinical response.

The Kirby-Bauer disc diffusion method:

Paper discs impregnated with a defined quantity of antimicrobial agent are placed on agar medium uniformly seeded with the test organism. A concentration gradient of the antibiotic occurs due to diffusion from the disc and the growth of the test organism is inhibited at a particular distance from the disc, that is related among other factors to the susceptibility of the organism.

Agar:

Mueller Hinton Agar is considered as the best medium for the routine susceptibility testing,[4] since it has batch-to-batch reproducibility, low concentration of inhibitors of sulphonamide, trimethoprim and tetracyclines and produces satisfactory results for most of the non-fastidious pathogens.

Mueller-Hinton agar should be prepared from a dehydrated base according to the manufacturer's recommendations. It is important not to overheat the medium. Cool the medium to 45-50°C and pour into the plates. Allow to set on a level surface, to a depth of approximately 4 mm. A 9 cm diameter plate requires approximately 25 mL of the medium. When the agar has solidified, dry the plates for immediate use for 10-30 minutes at 36°C by placing them in an upright position in the incubator with the lids tilted. The medium should be such that control zone sizes within the standard limits are produced.

Any unused plates may be stored in a plastic bag, which should be sealed and placed in the refrigerator. Plates stored in this way can be kept for 2 weeks.

To ensure that the zone diameters are sufficiently reliable for testing susceptibility to sulfonamides and co-trimoxazole, Mueller-Hinton agar must have low concentrations of the inhibitors thymidine and thymine. Each new lot of Mueller-Hinton agar should therefore be tested with a control strain of Enterococcus faecalis (ATCC 29212 or 33l86) and a disc of co-trimoxazole. A satisfactory lot of medium will give a distinct inhibition zone of 20 mm or more, that is essentially free of hazy growth or fine colonies.

Table 2 : Factors affecting AST & their effect on it (variables).

Factor	Variables
pH	If pH is low:→ *Aminoglycosides, Quinolones & Macrolides lose potency. * Tetracyclines have excess action.
Moisture	Affects accuracy of susceptibility testing.
Medium component	Excess of thymine reversibly inhibits action of antibiotics like trimethoprim group.
Thickness	If thickness of medium is more, growth of organism is less and vice versa.

Antibiotic discs:

To minimize error we should

- Use commercially available discs with the proper diameter (6 mm).
- Keep appropriate distance between discs.
- Use disc with proper potency.

Stocks of antibiotic discs should preferably be kept at -20°C; the freezer compartment of a home refrigerator is convenient. A small working supply of discs can be kept in the refrigerator for up to 1 month. On removal from the refrigerator, the containers should be left at room temperature for about l hour to allow the temperature to equilibrate. This procedure reduces the amount of

condensation that occurs when warm air reaches the cold container. Antibiotic discs should be placed within 15 minutes of swabbing.

Inoculum:

Prepare the turbidity standard by pouring 0.6 mL of a 1% (10 g/L) solution of barium chloride into a l00-mL graduated cylinder, and filling to l00 mL with 1% (10 mL/L) sulfuric acid. The turbidity standard solution should be placed in a tube identical to the one used for the broth sample. It can be stored in the dark at room temperature for 6 months, provided it is sealed to prevent evaporation.

Stocks of sterile cotton wool swabs on wooden applicator sticks should be prepared. These can be sterilized in tins, culture tubes, or on paper in the autoclave.

To prepare the inoculum from a primary culture plate, touch the tops of each of the 3-5 colonies of similar appearance of the organism to be tested, with a loop. When the inoculum has to be made from a pure culture, a loopful of the confluent growth is similarly suspended in saline, or peptone water.

Compare the tube with the turbidity standard and adjust the density of the test suspension to that of the standard by either adding more bacteria or more sterile saline. Proper adjustment of the turbidity of the inoculum is essential to ensure that the resulting lawn of growth is confluent or almost confluent.

Inoculate the plates by dipping a sterile swab into the inoculum. Remove excess inoculum by pressing and rotating the swabs firmly against the side of the tube above the level of the liquid.

- The mean MIC of the isolates increases as the inoculum size progressively increases from 10^3 to 10^7 Colony Forming Units(CFUs).
- Identical results were noted when isolates were maintained for two or four days prior to testing.
- Inoculum size should be carefully controlled when assessing the in vitro susceptibility .
- Size of zone inhibition increases if inoculum density is less.
- Size of zone inhibition decreases if inoculums is heavy.

Incubation:

Knowledge of requirements for incubation is also needed as different organisms have specific requirements, e.g., non fastidious aerobic and facultative anaerobic bacteria require ambient air at 35^0C for generally 16-18 hours; while 24 hours are needed for S.aureus and Enterococcus spp; and 5% CO_2 is needed for Haemophilus spp.

Equipment factors:

If there is problem with petridish or measuring calibre, then also, the result of antimicrobial susceptibility test may be affected. Observational error and other environmental factors also affect outcome of Antimicrobial susceptibility test.

The final result of a disc diffusion test is influenced by a large number of variables. Some of the factors, such as the inoculum density and the incubation temperature, are easy to control; but a laboratory rarely knows the exact composition of a commercial medium, the antimicrobial content of the discs or the batch-to-batch variations in their quality, and it cannot be taken for granted . The results of the test must, therefore, be monitored constantly by a quality control program which should be considered part of the procedure itself.

The precision and accuracy of the test are controlled by the parallel use of a set of control strains, with known susceptibility to the antimicrobial agents. These quality control strains are tested using exactly the same procedure as for the test organisms. The zone sizes shown by the control organisms should fall within the range of diameters standardized. When results regularly fall outside this range, they should be regarded as evidence that a technical error has been introduced into the test, or that the reagents are at fault. Each reagent and each step in the test should then be investigated until the cause of the error has been found and eliminated. The quality control program should use standard reference strains of bacteria that are tested in parallel with the clinical culture. They should preferably be run every week or with every fifth batch of tests, and in addition, every time that a new batch of Mueller Hinton agar or a new batch of discs is used.

To avoid errors:

- Use antibiotic discs of 6 mm diameter, with proper space between the discs kept in AST.
- Use correct content of antimicrobial agent per disc.
- Store supply of antimicrobial discs at -20˚C.
- Use Mueller-Hinton medium for antibiotic sensitivity determination.
- Use appropriate control cultures.
- Use standard methodology for the test.
- Use coded strains from time to time for internal quality control.
- Everyone should be aware of the importance of QC.
- Training of personnel with the correct technique.
- Checking of expiry date of discs before use.
- Maintaining of proper temperature of all the equipments required in AST.
- Periodic service and calibration of instruments required in AST.

Conclusion:

Internal quality assessment is a useful complementary approach to external quality assessment and may detect problem areas not highlighted by other control methods. Education, accuracy and training is an important part of the quality assurance process. Knowledge of atypical results for different organism–agent combinations may provide warning of possibly erroneous results, and an understanding of the limitations and sources of error in disc diffusion methods contributes significantly to the recognition, resolution and avoidance of errors.

References:

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