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Quality Control Issues in Antibiotic Susceptibility Testing by Disc Diffusion Technique

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Abstract

Although automated susceptibility methods are widely available nowadays, antimicrobial susceptibility testing performed by the Kirby Bauer technique is still being commonly used in many laboratories especially in the developing countries. They provide a simple and inexpensive method for determining susceptibility of various Gram positive and Gram negative organisms against various drugs. The quality of results generated by the laboratory with respect to susceptibility of microorganisms to antimicrobials has an important bearing on patient management and outcomes. Any quality assurance program must include internal quality control (IQC) and external quality assessment. The important IQC measures in disc diffusion susceptibility testing include use of specific Quality Control (QC) strains, batch testing of media and antibiotics, and lot-to-lot verifications of reagents used. QC may be performed daily or weekly as per the workload of the laboratory. Some important causes of QC failure include improper storage of QC strains, media and reagents; errors in inoculums preparation and incubation temperatures; and equipment related issues like calibration errors. Accuracy and reliability of results should be regularly assessed by proficiency testing. If any failure in the QC is noted, corrective and preventive actions must be undertaken to ensure the quality of results generated.

Introduction

Quality assessment, internal quality control (IQC) and external quality assurance (EQA) are the essential elements of a laboratory's Quality Management System (QMS). These quality tools ensure the quality of end results generated by the laboratory. Antimicrobial susceptibility testing is one of the most important tests performed by the microbiology laboratory. Despite the availability of modern commercial rapid and automated systems for performing susceptibility testing for bacterial pathogens in the microbiology laboratory, the conventional disc diffusion technique [1] developed by Kirby and Bauer stills continues to be widely used especially in resource limited settings and provides a fairly simple, easy and accurate method for performing the tests. Quality is of great concern because the results have a direct bearing on patient outcomes. In the laboratory setting, analytical quality for any test is usually assessed by internal quality control (IQC) and External Quality Assurance Scheme (EQAS). The aim of conducting IQC and participating in EQAS program is to scrutinize the performance of the laboratories pre analytical, analytical and post analytical phases of testing system. It also creates awareness on changes in performance of the laboratory (especially a changing trend in the analysis including lack of reproducibility of testing results) that may lead to erroneous patient results that may be clinically significant. Proficiency Testing (PT) programs help establish the efficacy of new test methods, identify the problems related to test performance and accordingly plan corrective and preventive actions and also helps identify inter-laboratory differences [2]. These may include modalities such as 'external quality assessment programs', 'inter-laboratory comparisons', or 'split sample' testing. Most laboratory accreditation programs ensure that the laboratory's Quality Management System is compliant with the International standards of quality viz. ISO 15189 [3]. It is important to comply with the internal quality control requirements and proficiency testing to ensure total quality assurance and also meet accreditation requirements. In this mini-review we have attempted to highlight important aspects of internal quality control and organism-specific issues in antibiotic susceptibility testing by disc diffusion techniques.

Laboratory Procedures for Internal Quality Control

The use of Quality control (QC) strains is an essential component of quality assurance in antimicrobial susceptibility testing. These should be traceable to reference standards such as the American Type Culture Collection (ATCC) or the National Type Culture Collection (NTCC). Specific Quality control strains are indicated for various individual Gram positive and Gram negative pathogens. Evaluation of CLSI QC strains and methods should conform to procedures (testing methodology, temperature and incubation conditions etc.) and expected results as described in the relevant documents [4]. The QC strains must be stored as per manufacturer's recommendations at the required temperature. Stock cultures of QC strains may be stored at -20°C in a stabilizer such as 10% glycerol (CLSI M02-A11) [5]. They may also be lyophilized and stored. Weekly or daily QC strain testing may be performed as per the workload and needs of the laboratory. Primary subcultures and working subcultures must be prepared accordingly. These may be stored at 2°C-8°C or as per the temperature requirements for the individual organisms. Tests must be performed as per guidelines and procedures outlined in current reference standards such as the Clinical laboratory Standards institute (CLSI) [6] or the European committee on antimicrobial susceptibility testing (EUCAST) [7,8]. Acceptable zone diameter ranges for QC strains for each drug-bug combination are provided in these reference documents. The ranges are based on the distribution of aggregated zone values on repeated testing [9]. In cases of antibiotic –organism combination where reference limits are not available in-house ranges may be established and used after validation [10].

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Each new lot of media (e.g. Mueller Hinton agar etc.) must also be tested with appropriate QC strains (Lot QC) as per recommended methods to determine whether the zone sizes obtained with the respective antibiotics is within the expected range provided in the reference documents. Sterility testing of readymade or laboratory made media should be conducted by incubating overnight an uninoculated plate and observing for any growth. The frequency of QC testing has to be decided by each laboratory based on its workload and logistics. The CLSI M02-A11 document describes very useful and practical algorithms for daily and weekly QC testing [5]. They recommend daily performance of QC tests initially. Satisfactory performance in daily QC testing is documented when no more than one out of 20 or three out of 30 result outliers for each antimicrobial agent/organism combination are noted. The laboratory may then shift to weekly QC testing. QC must also be performed when any reagent component such as media lots, discs etc. are changed. Use of Westgard rules with warning and rejection criteria has also been proposed if lower and upper limits of zone diameter ranges are considered equivalent to two standard deviations [10,11].

Corrective and Preventive Actions

Once an outlier is noticed in the Quality control procedures it is imperative to determine the cause of the aberration. In some cases it may not be able to identify the exact cause. The important and common reasons for QC failure are as listed in Table 1. Corrective actions include analysis of these factors and the tests repeated after rectifying the errors. Most errors are corrected by this procedure. Systemic errors must be suspected, identified and corrected in cases of repeated QC failures. It is important to demonstrate and document satisfactory QC performance in order to perform patient tests and release reports. Appropriate levels of biosafety precautions (use of biosafety cabinets, wearing personal protective equipment etc.) must be strictly adhered to while performing tests on microorganisms or potentially infective material [12]. Records of all Quality control tests conducted must be kept in manual or electronic form. The ISO 15189-2012 provides international standards for management and technical requirements related to quality and competence for medical laboratories [13].

Organism Specific Issues

Some other important "organism-antibiotic" specific quality control and reporting aspects include the following [6]: First and second generation cephalosporin's, cefamycins and aminoglycosides must not be reported for salmonella and shigella organisms. Antibiotics such as fluoroquinolones, macrolides, tetracycline, clindamycin, 1st and 2nd generation cephalosporin's and "oral only" preparations should not be reported for organisms isolated form CSF. Disc diffusion results may be unreliable for certain drug-bug combinations and minimum inhibitory concentrations must be performed e.g. penicillin and cephalosporins for *Streptococcus pneumoniae*, vancomycin for *Staphylococcus aureus*, penicillin for *Streptococcus viridans*, colistin for *Acinetobacter spp*. etc. Tests for β-lactamase production, extended spectrum β-lactamases (ESBLs) and carbapenemases should be performed as indicated for relevant organisms. Enterococci do not respond to cephalosporins *in vivo* and must not be reported as susceptible. Inducible clindamycin resistance in staphylococci must be tested for by the D test. Similarly cefoxitin resistant staphylococci must not be reported susceptible to penicillins, β-lactam-β-lactamase inhibitor combinations, cephalosporins (except anti MRSA cephalosporins). A proper selection of antibiotics to be reported has to be made depending on the isolate, the site of infection and other factors described above.

1.	**Equipment related:**
	• Improper temperature for incubators • Improper incubation conditions (% of CO_2/O_2) • Calibration errors
2.	**Media and reagent related:**
	• Improper storage and transport of media, QC strains and reagents • Improper composition of media and reagents (e.g. ionic content, pH, nutritionally deficient etc.) • Contaminated media/reagents and defective plates • Wrong, contaminated, mutated or unviable QC strain • Reduced disk potency/antibiotic deterioration • Use of reagents beyond their shelf life
3.	**Testing process related:**
	• Error in inoculum preparation (including inaccurate turbidity standards) • Incubating at wrong temperature/ CO_2/O_2 concentration/ time • Media depth too thick or too thin • Improper disk placement • Measurement, transcription and interpretive errors

Table 1: Causes of QC failure [5,6].

Conclusion

Quality control measures including IQC and PT need to be followed meticulously while performing antibiotic susceptibility testing by disc diffusion techniques. The important IQC measures include testing QC strains, batch testing of media and antibiotic discs, lot-to-lot verifications of reagents used. Various organism specific issues including selection of the right antibiotic-organism combinations are also important. When errors are identified in IQC or PT, corrective and preventive actions must be undertaken to ensure quality assurance and effect better patient outcomes.

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