EDUCATIONAL COMMENTARY – ANTIMICROBIAL SUSCEPTIBILITY TESTING

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Learning Outcomes
Upon completion of this exercise, the participant will be able to:

- Explain why laboratorians should follow standards developed by the Clinical and Laboratory Standards Institute when performing antimicrobial susceptibility testing.
- Discuss deficiencies in susceptibility testing practices that have been identified by researchers.

To perform antimicrobial susceptibility testing, most laboratories today use either disk diffusion or a broth microdilution method. In addition, many laboratories use gradient diffusion strips to perform minimum inhibitory concentration (MIC) tests on certain drug-organism combinations. All of these methods have been thoroughly researched and standardized, and all are reliable when they are performed correctly. However, studies have shown that many laboratories often fail to correctly perform susceptibility testing, a finding that has raised concerns that susceptibility test results may often be unreliable. To ensure that their test results are reliable, laboratories should adhere to the standards that have been developed by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]).

Goals of Standardization
The goals of standardized susceptibility testing procedures are threefold:

1. To optimize growth conditions so that test results cannot be attributed to limitations of nutrients, temperature, or atmosphere;
2. To optimize antimicrobial integrity and activity so that test results cannot be attributed to environmental drug deactivation; and
3. To maintain interlaboratory reproducibility and consistency of results.

Standardized susceptibility testing procedures accomplish these goals by controlling variables that can cause erroneous test results. For example, factors such as inoculum size, growth medium, incubation conditions, and antimicrobial concentrations can significantly impact test results. Also, some organisms have unique characteristics that require special primary or confirmatory testing procedures. Finally, timely diagnosis and treatment require the selection of drugs that are appropriate for the organism and the specimen site.

The CLSI standards address all of these issues. First, the standards specify which media to use and detail how it is to be prepared and stored. Second, the standards identify which antibiotics may be tested against a particular organism, and they specify the required potency, preparation procedures, and storage conditions for the drugs. Third, the standards explain how to prepare the bacterial inoculum and turbidity
standard and inoculate the test plates or broth, and they define the appropriate incubation conditions (atmosphere, temperature, and duration). Fourth, the standards explain how to evaluate plates or broth for inhibition of growth, and they provide interpretive criteria for zone sizes or dilutions. Finally, the standards specify which bacterial reference strains must be used for quality control (QC); and they provide QC testing algorithms that address frequency of QC testing and corrective actions.

Problem Areas
Despite the consensus that standardization is crucial to ensuring reliable results, studies have shown that many laboratories fail to fully follow CLSI standards for susceptibility testing.\(^1\)\(^-\)\(^5\) This is of greatest concern in testing problem or fastidious bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, and *Enterococcus*,\(^1\)\(^-\)\(^5\) and the problem appears to be particularly acute in smaller laboratories.\(^5\) Researchers have documented\(^1\)\(^-\)\(^5\) serious flaws in every step of the testing process, including:

- Improper inoculum preparation,
- Incorrect incubation time,
- Incorrect media,
- Too many antibiotic disks per plate,
- Incorrect incubation atmosphere,
- Incorrect interpretive criteria for disk zone sizes,
- Improper or absent confirmatory testing procedures, and
- Use of unapproved antibiotics.

Although all of these flaws can cause erroneous test results, the last 2 items—improper or absent confirmatory testing procedures and the use of unapproved antibiotics—are of particular concern because they can delay recognition of resistance and thus cause physicians to prescribe an ineffective antibiotic. In a survey of testing practices in rural hospital laboratories, Stevenson and colleagues found that many laboratories failed to follow recommended procedures to confirm methicillin resistance in *S. aureus*, penicillin resistance in *S. pneumoniae*, and vancomycin resistance in *S. aureus* and *Enterococcus*.\(^5\) This in turn suggests that many rural hospital laboratories may be unable to reliably detect antibiotic resistance in these organisms\(^5\), a deficiency which could significantly impair patient care.

Likewise, many laboratories routinely report susceptibility testing results for antibiotics that have not been approved by CLSI for use with a particular organism. In an analysis of proficiency test data from 2003, American Proficiency Institute found that 48% of laboratories using disk diffusion and 14% of laboratories using MIC methods reported results for at least 1 unapproved drug when performing susceptibility testing on *S. pneumoniae*.\(^2\) These results confirm earlier findings published by Doern and colleagues in 1999 and noted again by Jones in 2001.\(^1\)\(^,\)\(^3\) In an effort to address this problem, the Centers for Medicare and
Medicaid Services now requires proficiency test providers to grade responses to unapproved antibiotics as unacceptable results.

**Sources of Information**

The 2006 standards for susceptibility testing appear in the CLSI publications *M7-A7 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard—Seventh Edition* and *M2-A9 Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard—Ninth Edition*. Information about which drugs may be used for susceptibility testing, which organisms to use for quality control, and interpretive criteria appear in the informational supplement *M100-S16 Performance Standards for Antimicrobial Susceptibility Testing—Sixteenth Informational Supplement*. The informational supplement is updated annually, and it often contains significant changes, so laboratories should acquire and review this information every year and, if necessary, update their procedures.

Information about purchasing the CLSI documents appears on CLSI’s Website at www.nccls.org. Laboratories may also be able to access these publications through their state health department.

Finally, the Multi-level Antimicrobial Susceptibility Testing Resources (MASTER) section of the Centers for Disease Control and Prevention’s website contains information about susceptibility testing for laboratory workers. These pages, available at www.phppo.cdc.gov/dls/master/default.aspx, present information about educational events, case studies, hot papers, and reference materials.

**Conclusion**

Reliable susceptibility test results are critical both to ensure optimal patient care and to protect the public health. Unfortunately, however, studies have shown that many laboratories do not rigorously follow CLSI standards, a finding that has alarmed many healthcare experts. Educational initiatives and more stringent regulations have been proposed to encourage laboratories to follow current standards, but ultimately it is the laboratories themselves that must take responsibility for this problem and solve it. Medical directors, laboratory administrators, and microbiology supervisors must ensure that susceptibility testing procedures are current, especially for problem and fastidious organisms.

**References**

EDUCATIONAL COMMENTARY – ANTIMICROBIAL SUSCEPTIBILITY TESTING (cont.)


**Suggested Reading**


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