EDUCATIONAL COMMENTARY – ANTIMICROBIAL SUSCEPTIBILITY TESTING: RESOURCES AND RECENT UPDATES

Educational commentary is provided through our affiliation with the American Society for Clinical Pathology (ASCP). To obtain FREE CME/CMLE credits click on Earn CE Credits under Continuing Education on the left side of the screen.

Learning Objectives

On completion of this exercise, the participant should be able to

- describe the role of the Clinical Laboratory Standards Institute (CLSI) in developing and publishing guidelines and standards for antimicrobial susceptibility testing of bacteria isolated from clinical specimens;
- discuss the methods of antimicrobial susceptibility testing in the clinical laboratory;
- explain the clinical and nonclinical categories defined in the CLSI M100 standards document;
- compare the main tables utilized in the CLSI M100 standards document; and
- summarize the recent updates to the CLSI M100 standards document.

Introduction

Antimicrobial susceptibility testing (AST) determines the susceptibility of a microorganism (bacteria or yeast/fungus) to an antimicrobial agent via in vitro methods. When a pathogenic microorganism is identified in clinical cultures, the next step is AST on that organism. The goal of AST is to predict the clinical success or failure of a particular antimicrobial agent against a particular organism. The results help clinicians determine the most appropriate antimicrobial therapy for the patient. Antimicrobial susceptibility testing is also an important tool for monitoring the emergence and spread of antimicrobial resistance.

There are a number of AST methods, and corresponding guidelines are published by standards organizations. The most commonly used guidelines in the United States are the Clinical and Laboratory Standards Institute (CLSI) documents. CLSI (formerly the National Committee for Clinical Laboratory Standards [NCCLS]) is a nonprofit organization that has provided guidance for clinical laboratory testing for almost 50 years. This organization produces documents that address various topics in clinical laboratory science, including standards and guidelines for microbiology testing practices and quality control (QC). The CLSI Subcommittee on Antimicrobial Susceptibility Testing is made up of volunteers who are experts in the fields of infectious disease, pharmaceuticals, and clinical microbiology laboratory practices. Documents for routine AST are developed and maintained by this subcommittee; documents for more specialized types of antimicrobial susceptibility testing are produced by separate subcommittees.
CLSI Documents for Antimicrobial Susceptibility Testing

CLSI standards provide all the information necessary to perform an antimicrobial susceptibility test, from media selection to QC. Each CLSI document is given a specific scheme and number. For example, in M02-A12, M denotes that it is a microbiology document, 2 is the number assigned to the specific document for disk diffusion, A means an approved document, and 12 indicates that it is the 12th edition of the M2 document. Documents for routine antimicrobial susceptibility testing include the following:

- M02 Performance Standards for Antimicrobial Disk Susceptibility Tests
- M07 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically
- M11 Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria
- M23 Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters
- M39 Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data
- M45 Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria
- M52 Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems
- M100 Performance Standards for Antimicrobial Susceptibility Testing

Documents for specialized antimicrobial susceptibility testing include the following:

- M21 Methodology for the Serum Bactericidal Test
- M24 Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes
- M26 Methods for Determining Bactericidal Activity of Antimicrobial Agents
- M27 Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts
- M38 Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi
- M44 Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts
- M51 Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi
- Veterinary practice AST documents

The CLSI documents and companion tools can be found and purchased at http://www.clsi.org. CLSI has recently made the critical M100 document available free of charge on the CLSI website in a non-downloadable format. It is updated yearly, typically in January, and contains the most current information for antibiotic selection and interpretation of susceptibility test results for the most common bacterial pathogens. The M100 FREE, traditional M100 document, and searchable M100 PLUS can be accessed at http://clsi.org/m100/.
Use of Commercial AST Systems

When testing a patient’s isolate, a clinical laboratory can either use one of the CLSI reference methods (i.e., disk diffusion, broth microdilution, or agar dilution) as described in the M02 or M07 standards, or a commercial AST system cleared for use by the Food and Drug Administration (FDA). For an antimicrobial susceptibility test instrument to be cleared by the FDA, each antimicrobial agent in the test must be cleared. To obtain FDA-cleared status the manufacturer must demonstrate that results obtained with the commercial system are comparable to results obtained with a CLSI reference method, overall performance of the commercial system meets FDA specifications, and the performance of the commercial system can be reliably monitored in the clinical laboratory by following the manufacturer’s recommended QC procedures.

Before implementing a new antimicrobial susceptibility test system, the laboratory must verify that the system performs according to the manufacturer’s specifications. This is mandated by the Clinical Laboratory Improvement Amendments (CLIA); the regulation can be accessed at http://wwwn.cdc.gov/clia/. The verification process includes reviewing current literature on the new antimicrobial susceptibility test system, reviewing information from the instrument manufacturer, and performing an evaluation in the clinical laboratory by testing control and clinical isolates.

It is also important to recognize the limitations of commercial systems as described in the package insert. For example, a commercial system may not be approved for testing a certain bacterial species that was isolated from the patient’s culture. In this situation, the microbiologist must try to find an alternative commercial susceptibility testing method that is approved for testing this isolate or use a CLSI reference method and breakpoints that are applicable to the species isolated. If the isolate is not in the M100 standard, the microbiologist can check the M45 standard, which describes susceptibility testing methods for infrequently isolated bacteria. If a clinical laboratory uses a commercial system, it must follow the manufacturer’s recommendations precisely.

CLSI M100 Document

The CLSI M02 and M07 standards describe how to perform antimicrobial susceptibility testing. They are to be used in conjunction with the M100 standards document, which provides supplemental tables to be used with disk diffusion and minimum inhibitory concentration (MIC) tests. The MIC is the lowest concentration of an antibiotic that inhibits the visible growth of a microorganism.

The annual update to M100 includes the latest recommendations for detecting resistance of aerobic and anaerobic bacteria, arranged in tabular format. The CLSI Subcommittee on Antimicrobial Susceptibility
Testing reviews data from a variety of sources including in vitro, pharmacokinetics-pharmacodynamics (PK-PD) data, and clinical studies, to establish interpretive criteria. Interpretive criteria are the MIC or zone diameter values used to specify breakpoints. The breakpoints defined in the supplement are specific values on the basis of which bacteria can be assigned to the clinical categories of susceptible (S), intermediate (I), susceptible–dose dependent (SDD), nonsusceptible (NS), or resistant (R). AST interpretations can be different based on the site of infection because an antibiotic may not reach therapeutic concentrations in every body site. It is important for clinical laboratory personnel to be aware of the site of infection so appropriate interpretive criteria can be used and reported.

**Susceptible** indicates that the antimicrobial agent used in the recommended dosage to treat the site of infection is clinically effective for that patient’s isolate. **Resistant** indicates that the isolate is not inhibited by the usually achievable concentrations, likely due to resistance mechanisms, and clinical efficacy has not been shown in treatment studies. **Intermediate** indicates clinical efficacy in body sites where the antimicrobial agent is physiologically concentrated (e.g. quinolones and β-lactams in urine) or when a higher than normal dosage of antimicrobial agent can be used. **Susceptible–dose dependent** indicates that the susceptibility of the patient’s isolate depends on the dose and regimen of the antimicrobial agent, and an altered dosing regimen, usually higher and/or more frequent doses, is needed to reach clinical efficacy. Within this category, the drug label should be consulted for recommended doses and adjustment for organ function.

**Nonsusceptible** is used for isolates that only have a susceptible interpretive criterion designated due to the absence or rare occurrence of resistant strains. Therefore, a resistant category could not be established, and a susceptible-only breakpoint is set. If an organism is in the NS category it does not necessarily mean that the isolate has a resistance mechanism, just that it does not fall within the interpretive category range of susceptible. Organisms in the NS category should have the identification and AST results confirmed.

The MIC values attained through AST may be reported directly to clinicians for patient care purposes but it is essential to include the interpretive category result (S, SDD, I, NS, R) on all reports to facilitate understanding of the AST. The recommended MIC and disk diffusion interpretive criteria are based on usual dosage regimens and routes of administration in the United States. In addition, some of the comments in the tables are denoted with an Rx symbol. This symbol indicates that the comment is related to therapy concerns and can be included on a patient report.
EDUCATIONAL COMMENTARY – ANTIMICROBIAL SUSCEPTIBILITY TESTING: RESOURCES AND RECENT UPDATES (cont.)

It can take several years for the FDA to clear commercial susceptibility testing systems to include revised CLSI breakpoints; this can be a significant barrier for clinical laboratories. However, laboratories that use FDA-cleared systems can implement the new MIC breakpoints after performing appropriate in-house validation studies. The validation pathway allowing the use of alternate breakpoints is outlined in the CLIA Interpretive Guidelines.¹

In addition to the clinical breakpoints, an epidemiologic cutoff value (ECV) may be listed for specific organism/antimicrobial agent combinations. ECVs are not clinical breakpoints, but MIC values that separate bacterial populations into those with resistance mechanisms and those without based on their phenotypic MICs. ECVs are based on in vitro data only and are primarily used to recognize the emergence of strains that possess acquired or mutational resistance mechanisms. The clinical relevance of ECVs is unknown. Breakpoints are established using MIC distributions, PK-PD data, and clinical outcome data. The clinical breakpoints have enough data sets to be considered predictors of clinical outcome and can be used to guide antimicrobial therapy in patients.⁴

There are three main types of tables in the M100. Tables 1A, 1B, and 1C suggest drugs for testing and reporting. Tables 2A through 3I contain criteria used to interpret results. Tables 4A through 5G specify acceptable ranges for QC organisms and describe other QC parameters.

Table 1A suggests drugs for testing and reporting on non-fastidious bacteria. These lists are divided into groups A, B, C, and U. Group A drugs are antibiotics that are considered routine and should be tested and reported for the organisms listed. For group B drugs, testing should be performed, but information included in the patient report should follow selective reporting practices. Selective reporting means a laboratory should list the susceptibility result on a patient report only if the bacterial isolate is resistant to the group A agent, the patient cannot tolerate group A agents, the infection has not responded to group A agents, or a secondary agent would be a better clinical choice for a particular infection. Group C drugs are supplemental antimicrobial agents with very broad-spectrum activity and should be tested and reported selectively. Testing may be warranted for group C agents when institutions have endemic or epidemic strains resistant to several of the primary drugs listed in group A, for treatment of patients allergic to primary drugs, for treatment of unusual organisms, or for reporting to infection control as an epidemiological aid. Group U drugs are antibiotics that are ineffective in treating infections other than urinary tract infections and therefore should only be reported on urine culture isolates. Table 1B addresses fastidious organisms, and 1C addresses anaerobic organisms.
All tables contain important footnotes and comments, which should not be ignored. Glossary I lists individual antimicrobial agents with drug classes, and Glossary II provides antimicrobial agent abbreviations and routes of administration.4

**CLSI M100 Standards Document Recent Updates**

It is critical for every laboratory performing AST to follow the most up-to-date M100 document. The world of clinical microbiology is changing rapidly. Microorganisms are developing mechanisms of resistance, new data may support a breakpoint that is different from what was previously published, new antimicrobial agents are developed and added, previous agents that are no longer recommended are deleted, and definitions to key terms may change. The latest M100 standard is essential to provide clinical laboratories with the most current information for selecting antibiotics and interpreting susceptibility test results. With each update of the M100, the changes are summarized in the front and boldfaced throughout the document for easy identification.4 This includes all deletions, modifications, and/or additions.

Several antibiotics and their breakpoints have been added to the M100 document over the past few years. In 2013, ceftaroline, a β-lactam antibiotic with anti–methicillin-resistant *Staphylococcus aureus* (MRSA) activity, was added. Ceftaroline is a fifth-generation cephalosporin and breakpoints were added for *Staphylococcus* species, *Enterobacteriaceae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Streptococcus pneumoniae*, and β-hemolytic *Streptococcus* species. Also added in 2013 were interpretation criteria for doxycycline with *Streptococcus pneumoniae*.5

In 2016, several antibiotic breakpoints and interpretive criteria were added to M100-S26. These include the following:

- MIC criteria for ceftolozane-tazobactam, a β-lactam/β-lactamase inhibitor antibiotic, for *Enterobacteriaceae* and viridans group streptococci;
- MIC criteria for oritavancin, a lipoglycopeptide, for *S aureus*, vancomycin-susceptible *Enterococcus faecalis*, β-hemolytic *Streptococcus* species, and viridans group streptococci;
- MIC and disk diffusion criteria for telavancin, a lipoglycopeptide, for *S aureus*, vancomycin-susceptible *E faecalis*, and viridans group streptococci; and
- MIC criteria for tedizolid, an oxazolidinone, for *S aureus*, vancomycin-susceptible *E faecalis*, and viridans group streptococci.

M100-S26 also expands definitions for key terms. This includes the definitions for *routine test, supplemental test, screening test, surrogate agent, and equivalent agent*. A *routine* test is a disk diffusion
or broth or agar dilution MIC test used for routine clinical testing. A supplemental test is not routine and detects susceptibility or resistance to a drug or drug class by a method other than noted in routine testing. Some supplemental tests identify specific resistance mechanism(s) and may be required or optional for reporting clinical results. Screening tests provide presumptive results but require additional testing for confirmation of susceptibility or resistance. Surrogate agent testing replaces testing with the antimicrobial agent of interest if the agent of interest cannot be tested due to performance issues or availability, or if the surrogate performs better than the agent of interest. Equivalent agent testing uses an agent that predicts results of closely related agents of the same class or antimicrobial agent. This increases efficiency by limiting the testing of multiple closely related agents.

A significant change to antimicrobial susceptibility testing was published in June 2010 with new MIC and disk diffusion interpretive criteria (breakpoints) for the Enterobacteriaceae. This affected first-generation and third-generation (extended-spectrum) cephalosporins, aztreonam, and three carbapenems (imipenem, meropenem, and ertapenem). These new breakpoints simplified susceptibility testing by eliminating the need for extended-spectrum β-lactamase (ESBL) testing. The change was implemented to enhance detection of strains with low-level cephalosporin resistance. The new MIC breakpoints are 1 to 3 doubling dilutions lower than the original breakpoints, and the new disk diffusion criteria include larger zone diameters than the previous guidelines specified. In effect, organisms that would previously have been categorized as susceptible may now test intermediate or resistant. If a clinical laboratory has implemented the new breakpoints for cephalosporins and carbapenems, the results can be reported out as tested. Confirmatory ESBL testing is not necessary.6

The M100-S26 document has added clarifications for testing of cefazolin against Enterobacteriaceae. The cefazolin breakpoints and interpretive criteria for infections with uncomplicated urinary tract infections (uUTIs) and other infections have been separated. Cefazolin is a surrogate agent and can be used to predict the antimicrobial susceptibility results for the oral agents cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef when used for treatment of uUTIs.

Conclusion

CLSI is one of the main standards organizations throughout the world that publishes and maintains documents detailing AST and other laboratory guidelines. The goal of AST is to provide useful information that assists the clinician in selecting appropriate antimicrobial therapy for the best patient care. The continual refining of the standards and guidelines optimizes patient antimicrobial therapy and detects emerging resistance in microorganisms. Accurate AST and appropriate reporting using the most up-to-date information is critical for optimal patient outcomes. For these reasons, it is good practice to
review the CLSI M100 standards document on a yearly basis and update the laboratory’s AST with the appropriate changes and modifications.

References


© ASCP 2016