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Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline—Second Edition

This document provides guidance to clinical microbiology laboratories for standardized susceptibility testing of infrequently isolated or fastidious bacteria that are not presently included in CLSI documents M02 or M07. The tabular information in this document presents the most current information for drug selection, interpretation, and quality control for the infrequently isolated or fastidious bacterial pathogens included in this guideline.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Approved An approved standard or guideline has achieved consensus within the health care community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (ie, that comments on earlier versions have been satisfactorily addressed), and to identify the need for additional consensus documents.

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Health care professionals in all specialties are urged to volunteer for participation in CLSI projects. Please contact us at customerservice@clsi.org or +610.688.0100 for additional information on committee participation.

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Abstract

If the susceptibility of a bacterial pathogen to antimicrobial agents cannot be predicted based on the identity of the organism alone, *in vitro* antimicrobial susceptibility testing of the organism isolated may be indicated. Susceptibility testing is particularly necessary in those situations in which the etiological agent belongs to a bacterial species for which resistance to commonly used antimicrobial agents has been documented, or could arise.

A variety of laboratory techniques can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. Clinical and Laboratory Standards Institute document M45-A2—*Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline—Second Edition* describes the standard microdilution and agar disk diffusion methods. It also includes a series of procedures designed to standardize test performance. The performance, applications, and limitations of the current CLSI-recommended methods are described.

The tabular information in this document presents the most current information for drug selection, interpretation, and quality control for the infrequently isolated or fastidious bacterial pathogens included in this guideline. As more information becomes available, changes will be incorporated into future revisions of this document.

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Foreword

This document was developed for the purpose of providing guidance to clinical or public health microbiology laboratories regarding the performance of standardized susceptibility testing, when needed, of infrequently isolated or fastidious bacteria that are not presently included in CLSI documents M02[1] or M07.[2] *Helicobacter pylori*, *Vibrio cholerae*, and several potential agents of bioterrorism were moved from CLSI documents M02,[1] M07,[2] and M100[3] to this document because they are fastidious or infrequently encountered in most microbiology laboratories. Some of the organisms included herein are aerobic gram-negative bacilli that are not members of the family *Enterobacteriaceae* but may be tested by the standard CLSI broth microdilution or disk diffusion methods in the same manner as the much more common *Enterobacteriaceae* isolates. Some aerobic gram-positive cocci and bacilli that are encountered periodically by clinical laboratories can likewise be tested reliably by the standard CLSI minimal inhibitory concentration (MIC) or disk diffusion test methods in a manner analogous to *Staphylococcus* or *Enterococcus* spp. In addition, several genera of fastidious gram-positive and gram-negative bacteria can be tested in the same manner as the streptococci, using blood-supplemented Mueller-Hinton media. For the purpose of this document, the term *fastidious* is used to describe bacteria that require media supplemented with blood or blood components and that possibly need an atmosphere other than ambient air (eg, with 5% CO_2) for acceptable growth. Because the standard CLSI media, reagents, and procedures can be used to test the organisms included in this guideline, the quality control procedures, strains, and acceptable zone diameter and MIC limits that have been established through previous rigorous studies can be used for tests with the less common organisms that are included in this document. The working group used a thorough search of the published literature in conjunction with the clinical experience of the members to apply or adapt interpretive criteria or breakpoints from other organisms that could best be applied to the interpretation of tests of the less common organisms in this document. Users of the guideline should be aware that the very extensive microbiological, clinical, and pharmacodynamic databases normally employed for setting breakpoints by CLSI did not exist for the collection of “orphan” organisms described in this document.

Interpretive breakpoints for cefazolin and for ertapenem, imipenem, and meropenem for the *Enterobacteriaceae* were voted to be changed by the Subcommittee on Antimicrobial Susceptibility Testing subsequent to final approval of this edition of M45. It is anticipated that those modified breakpoints will be adopted for use with *Aeromonas* spp. and *Vibrio* spp. in the next edition of M45 or possibly in a future supplement.

It is important for users of M45-A2 to recognize that commercial susceptibility testing devices are not addressed in this guideline. The methods described herein are generic reference procedures that can be used for routine susceptibility testing by clinical laboratories, or that can be used by clinical laboratories to evaluate commercial devices for possible routine use. Results generated by reference methods, such as those contained in CLSI documents, may be used by regulatory authorities to evaluate the performance of commercial systems as part of the approval process. Clearance by a regulatory authority indicates that the commercial susceptibility testing device provides susceptibility results that are substantially equivalent to results generated using the reference methods for the organisms and antimicrobial agents described in the manufacturer’s approved package insert. Some laboratories could find that a commercial dilution, antibiotic gradient, colorimetric, turbidimetric, fluorometric, or other method is suitable for selective or routine use.

Key Words

Agar dilution, antimicrobial agent, antimicrobial susceptibility, broth dilution, disk diffusion, microdilution, minimal inhibitory concentration (MIC), susceptibility testing

Updated Information in This Edition

Below is a summary of the changes in this document, which supersede the information presented in the previous edition of M45. The list includes "major" changes that appear for the first time in this edition of M45, or were modified since publication of M45-A. Other minor or editorial changes that were made to the general formatting are not listed here.

Foreword
Added paragraph from current edition of M100 related to using commercial systems vs CLSI reference methods (p. xi).

Section 1, Scope
Noted that testing of *Haemophilus* included in M100 relates *to H. influenzae* and *H. parainfluenzae* only (p. 1).

Section 2, Introduction
Updated HACEK group designation for *Aggregatibacter* (formerly *Actinobacillus*) (p. 1).

Section 4.2, Definitions
Added definitions for susceptible, intermediate, resistant, and nonsusceptible (p. 4).

Section 4.3, Abbreviations and Acronyms
Added a list of abbreviations and acronyms used in the document (p. 5).

Section 6.3, Interpretive Categories
Deleted only "S" criteria box because the definition of "nonsusceptible" is now in the list of definitions.

Section 9, Quality Control
Expanded statement about selecting quality control strains (p. 7).

Tables 1 through 19A

General
Modified therapy (Rx) comment for rifampin (Tables 3, 5, 7, and 12) (pp. 14, 18, 22, and 30).

Table 2, *Aeromonas* spp. and *Plesiomonas shigelloides*
Clarified incubation conditions for disk diffusion and MIC testing (p. 12).
Added new (revised) breakpoints for cefazolin, cefotaxime, ceftazidime, ceftriaxone, and aztreonam. Also added dosage regimens on which the new breakpoints are based (p. 12).

Table 3, *Bacillus* spp. (not *B. anthracis)*
Added fluoroquinolones to the agents to consider for primary testing box (p. 14).

Table 4, *Campylobacter jejuni/coli*
Clarified incubation conditions for disk diffusion and MIC testing (p. 16).
Added azithromycin and clarithromycin to the examples indicating macrolide resistance (p. 16).

Table 5, *Corynebacterium* spp.
Modified vancomycin breakpoints (p. 18).
Clarified scope of recommendations that apply to *Corynebacterium* spp. and other coryneforms including the genera *Arcanobacterium, Brevibacterium, Cellulomonas, Dermabacter, Leifsonia, Microbacterium, Oerskovia, Rothia,* and *Turicella* (p. 19).

Table 7, HACEK Group
Updated HACEK group designation for *Aggregatibacter* (formerly *Actinobacillus*) (p. 22).

Table 8, *Helicobacter pylori*
Imported table from M100-S20 (p. 24).

Table 9, *Lactobacillus* spp.
Added breakpoints for daptomycin and linezolid (p. 26).
Added intermediate and resistant breakpoints for imipenem (p. 26).
Modified breakpoints for clindamycin and vancomycin (p. 26).
Added comment to ampicillin regarding combined therapy with a penicillin and an aminoglycoside for treatment of serious infections (p. 26).
Updated Derivation of Interpretive Criteria section (p. 27).

Table 10, *Leuconostoc* spp.
Added comment regarding combined therapy with a penicillin and an aminoglycoside for treatment of serious infections (p. 28).

Table 12, *Moraxella catarrhalis*
Modified MIC breakpoints for amoxicillin-clavulanic acid (“S” only), azithromycin, clarithromycin, and erythromycin (p. 30).
Added disk diffusion breakpoints for amoxicillin-clavulanic acid, azithromycin, clarithromycin, erythromycin, tetracycline, and trimethoprim-sulfamethoxazole (p. 30).
Updated Derivation of Interpretive Criteria section (p. 31).

Table 13, *Pasteurella* spp.
Clarified incubation conditions for disk diffusion and MIC testing (p. 32).

Table 15, *Vibrio* spp.
Imported table from M100-S20 (p. 36).
Clarified incubation conditions for disk diffusion and MIC testing (p. 36).
Listed agents to consider for primary testing for *Vibrio* spp. and *V. cholerae* (p. 36).
Added MIC breakpoints for azithromycin for *V. cholerae* (p. 37).
Updated Derivation of Interpretive Criteria section (p. 37).

Table 16, Potential Bacterial Agents of Bioterrorism
Imported table from M100-S20 (p. 38).
Added agents to consider for primary testing for *Brucella* and *Francisella* (p. 38).

Table 17, Summary of Testing Conditions and Quality Control
Added *Helicobacter pylori, V. cholerae,* and potential agents of bioterrorism (pp. 42 and 43).

Quality Control Tables

Table 18
Added ranges for ampicillin and piperacillin for *E. coli* ATCC® 35218 (p. 44).

Table 18A
Added ranges for amoxicillin for *E. coli* ATCC® 35218 (p. 45).

Table 18B
Imported *Campylobacter* quality control from M100-S20 (p. 46).

Table 18C
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Table 18D
Imported potential bacterial agents of bioterrorism quality control from M100-S20 (p. 47).

Table 19
Added ranges for azithromycin, clarithromycin, and erythromycin for *S. aureus* ATCC® 25923 (p. 49).

Glossaries

Glossary I – Added new antimicrobial subclass for ceftaroline and ceftobiprole (p. 51).

Glossaries I and II – Added razupenem to carbapenem subclass (p. 51).
Added sulopenem to penem subclass (p. 51).
Added linopristin-flopristin to streptogramin class (p. 52).
Added mupirocin to pseudomonic acid class (p. 52).

CLSI Subcommittee on Antimicrobial Susceptibility Testing Mission Statement

The CLSI Subcommittee on Antimicrobial Susceptibility Testing is composed of representatives from the professions, government, and industry, including microbiology laboratories, government agencies, health care providers and educators, and pharmaceutical and diagnostic microbiology industries. Using the CLSI voluntary consensus process, the subcommittee develops standards and guidelines that promote accurate antimicrobial susceptibility testing and appropriate reporting.

The mission of the CLSI Subcommittee on Antimicrobial Susceptibility Testing is to:

- Develop standard reference methods for antimicrobial susceptibility tests.
- Provide quality control parameters for standard test methods.
- Establish interpretive criteria for the results of standard antimicrobial susceptibility tests.
- Provide suggestions for testing and reporting strategies that are clinically relevant and cost-effective.
- Continually refine standards and optimize the detection of emerging resistance mechanisms through the development of new or revised methods, interpretive criteria, and quality control parameters.
- Educate users through multimedia communication of standards and guidelines.
- Foster a dialogue with users of these methods and those who apply them.

The ultimate purpose of the subcommittee's mission is to provide useful information to enable laboratories to assist the clinician in the selection of appropriate antimicrobial therapy for patient care. The standards and guidelines are meant to be comprehensive and to include all antimicrobial agents for which the data meet established CLSI guidelines. The values that guide this mission are quality, accuracy, fairness, timeliness, teamwork, consensus, and trust.

Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline—Second Edition

1 Scope

CLSI documents M02,[1] M07,[2] and M100[3] describe standardized methods and interpretive criteria for antimicrobial susceptibility testing of common aerobic bacteria, including some fastidious organisms. However, there are a number of less frequently encountered or fastidious bacteria that are not addressed in those CLSI documents. Some are organisms that may cause serious infections, infections associated with trauma and environmental contamination, or device-associated infections in immunocompromised or postsurgical patients. These latter organisms are addressed in this guideline with the goal of providing recommendations for clinical microbiology laboratories on how and when to determine the susceptibility of these diverse organisms. This edition of the guideline also includes some fastidious or unusual organisms, including those potentially associated with bioterrorism, which were previously included in CLSI document M100.[3]

2 Introduction

Organisms that previously lacked defined methods for susceptibility testing and interpretive criteria included various coryneform bacteria, *Bacillus* spp. (other than *B. anthracis*), *Abiotrophia* and *Granulicatella* spp., several genera of gram-positive bacteria with intrinsic glycopeptide resistance (eg, *Erysipelothrix* spp., *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp.), as well as several species of fastidious gram-negative bacteria (eg, HACEK group organisms and *Pasteurella* spp.). In addition, more detailed guidance for test performance and interpretation were needed, especially breakpoints for *Listeria* spp., *Aeromonas* spp., *Plesiomonas* sp., *Vibrio* spp., *Moraxella catarrhalis*, and *Campylobacter* spp. The lack of test methods or interpretive criteria made it difficult to assess the frequency of acquired resistance in these less frequently isolated or fastidious organisms and discouraged the testing of individual patient isolates by clinical laboratories. However, concerns had been raised that resistance exists in some of these organisms, and that laboratories should be prepared to test them when appropriate.[4,5]

Because infections due to organisms addressed in M45 occur less frequently than many of the organisms presently covered in CLSI documents M02[1] and M07,[2] and because of the fact that many of the antimicrobial agents of interest have been marketed for a number of years, it is not reasonable to expect the intensive CLSI document M23[6] specified studies to be conducted on this special group of organisms. Instead, the goal of this document is to recommend test conditions and interpretive criteria based on a careful review of published microbiological data (distributions of minimal inhibitory concentrations [MICs]), limited animal model studies, the extant clinical literature regarding therapy for these organisms, and in a few instances, a review of existing pharmacokinetic data on the drugs of interest. In some cases, limited *in vitro* studies were performed.

This edition of M45 includes several potential bacterial agents of bioterrorism that could be encountered initially by clinical microbiology laboratories and that should be forwarded to appropriate reference or public health laboratories for identification, confirmation, and possible susceptibility testing. The procedures included in this document are intended for use by those reference or public health laboratories.

It is hoped that this CLSI guideline will assist clinical microbiology laboratories in determining an approach for testing these unusual organisms that is relevant to their individual practice settings.