CLINICAL AND LABORATORY STANDARDS INSTITUTE®

34th Edition

CLSI M100™

Performance Standards for Antimicrobial Susceptibility Testing

CLSI M100 includes updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards CLSI M02, M07, and M11.

A CLSI supplement for global application.

CLSI M100-Ed34
February 2024
Replaces CLSI M100-Ed33

Performance Standards for Antimicrobial Susceptibility Testing

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Abstract

The data in the tables are valid only if the methodologies in CLSI M02,[1] M07,[2] and M11[3] are followed. These standards contain information about disk diffusion (CLSI M02[1]) and dilution (CLSI M07[2] and CLSI M11[3]) test procedures for aerobic and anaerobic bacteria. Clinicians depend heavily on information from the microbiology laboratory for treating their seriously ill patients. The clinical importance of antimicrobial susceptibility test results demands that these tests be performed under optimal conditions and that laboratories have the capability to provide results for the newest antimicrobial agents. The tables presented in CLSI M100 represent the most current information for drug selection, interpretation, and quality control using the procedures standardized in CLSI M02,[1] M07,[2] and M11.[3] Users should replace previously published tables with these new tables. Changes in the tables since the previous edition appear in boldface type.

Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*. 34th ed. CLSI supplement M100 (ISBN 978-1-68440-220-5 [Print]; ISBN 978-1-68440-221-2 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2024.

The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org.

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Suggested Citation

CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 34th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2024.

Previous Editions:
December 1986, December 1987, December 1991, December 1992, December 1994, December 1995, January 1997, January 1998, January 1999, January 2000, January 2001, January 2002, January 2003, January 2004, January 2005, January 2006, January 2007, January 2008, January 2009, January 2010, June 2010, January 2011, January 2012, January 2013, January 2014, January 2015, January 2016, January 2017, January 2018, January 2019, January 2020, March 2021, February 2022, March 2023

CLSI M100-Ed34
ISBN 978-1-68440-220-5 (Print)
ISBN 978-1-68440-221-2 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic)

Volume 44, Number 5

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Overview of Changes

CLSI M100-Ed34 replaces the previous edition of the supplement, CLSI M100-Ed33, published in 2023. Major additions, reformatting, and/or table relocation changes are summarized below, followed by additional noteworthy changes detailed by section/table. Changes to content since the previous edition appear in boldface type; however, minor editorial or formatting changes are not listed here, nor highlighted in boldface type. To learn more about the organization of CLSI M100-Ed34, check the "Instructions for Use."

CLSI M100 is updated and reviewed annually as new data and new agents become available. Use of outdated documents is strongly discouraged.

Major Additions/Revisions
• Tables 1 and Tables 2 (general): These tables were renumbered so that each Table 1 has a corresponding Table 2. • Table 1I: A new table for suggested drugs to test and report on *Neisseria meningitidis* was added. • Table 1J: A combined table for suggested drugs to test and report on anaerobes was added. Previously, these suggestions were listed in separate gram-negative and gram-positive tables. • Table 2A-2: A new table with breakpoints specific to *Salmonella* and *Shigella* spp. was added. Table 2A-1 no longer addresses these organism groups. • Tables 2, Tables 3, and former Appendix E: In previous editions of CLSI M100, dosage regimens were listed in Tables 2, 3E-2 (now 3F-2), 3E-3 (now 3F-3), and former Appendix E. – Dosage regimens were removed from all Tables 2 and Tables 3F-2 and 3F-3. – Former Appendix E was reformatted, relocated to follow the Tables 2 containing breakpoints, and renamed "Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints" (referred to as "Table 2 Dosages" throughout). • Tables 3 (general): These tables were renumbered to accommodate the addition of new Table 3D. • Table 3D: A new table describing a broth disk elution method for aztreonam plus ceftazidime-avibactam was added. • Table 3F-4: A new table with breakpoints specific to testing *Acinetobacter* spp. directly from positive blood cultures was added. • Table 3H: Former Tables 3G-1 and 3G-2, which described ancillary methods for testing oxacillin and cefoxitin against staphylococci, were condensed into Table 3H, which describes the oxacillin salt agar test only. • Appendixes (general): These sections were relabeled to accommodate the relocation of former Appendix E (now Table 2 Dosages).

Section/Table	Changes
General	
Throughout	**Added:** • "Lower" qualifier to comment that daptomycin should not be routinely reported on organisms isolated from the lower respiratory tract **Revised:** • Suggestion for repeat testing of isolates initially susceptible that may develop resistance after initiation of therapy, from "within 3 to 4 days" to "within a few days"

Overview of Changes (Continued)

Section/Table	Changes
General (Continued)	
CLSI Breakpoint Additions Since 2010	**Added:** • Sulbactam-durlobactam disk diffusion and MIC breakpoints for *Acinetobacter* spp. • Tedizolid disk diffusion breakpoints for *Staphylococcus* spp. (*Staphylococcus aureus* only) • Tedizolid disk diffusion breakpoint for *Streptococcus* spp. β-hemolytic group (*Streptococcus pyogenes* and *Streptococcus agalactiae* only) • Tedizolid disk diffusion breakpoint for *Streptococcus* spp. viridans group (*Streptococcus anginosus* group only)
CLSI Breakpoint Revisions Since 2010	**Revised:** • Minocycline disk diffusion and MIC breakpoints for *Stenotrophomonas maltophilia* • Linezolid disk diffusion breakpoints for *Staphylococcus* spp. ***Deleted:*** • Ceftazidime disk diffusion breakpoints for *Burkholderia cepacia* complex • Meropenem disk diffusion breakpoints for *B. cepacia* complex • Minocycline disk diffusion breakpoints for *B. cepacia* complex • Trimethoprim-sulfamethoxazole disk diffusion breakpoints for *B. cepacia* complex • Ceftazidime MIC breakpoints for *S. maltophilia*
CLSI Archived Resources	**Added:** • Breakpoints that have been eliminated from CLSI M100 (detailed in CLSI Breakpoint Revisions Since 2010) • Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp. table content related to detection of *mecA*-mediated resistance using cefoxitin or oxacillin (former Tables 3G-1 and 3G-2, revised to Table 3H) • QC range that has been eliminated from CLSI M100
CLSI Reference Methods vs Commercial Methods and CLSI vs US Food and Drug Administration Breakpoints	***Deleted:*** • Final paragraph that referenced verification of breakpoints **NOTE:** CLSI now provides or is in the process of updating and developing additional documents for validation and verification of susceptibility breakpoints.
Tables 1. Antimicrobial Agents That Should Be Considered for Testing and Reporting by Microbiology Laboratories	
Table 1B-2. *Acinetobacter* spp.	**Added:** • Sulbactam-durlobactam to Tier 3
Table 1B-4. *Stenotrophomonas maltophilia*	***Deleted:*** • Ceftazidime

Overview of Changes (Continued)

Section/Table	Changes
Tables 1. (Continued)	
Table 1D. *Enterococcus* spp.	**Revised:** • Footnote d regarding susceptibility to penicillin
Table 1H-1. *Streptococcus* spp. β-Hemolytic Group	**Added:** • Reference corresponding to intrapartum prophylaxis recommendations **Revised:** • Footnote b regarding intrapartum prophylaxis recommendations
Table 1I. *Neisseria meningitidis*	New table
Table 1J. Anaerobes	New combined table **Added:** • Footnote a regarding tier placement of ampicillin and penicillin for anaerobes
Tables 2. Zone Diameter and/or MIC Breakpoints	
Introduction to Tables 2A–2J. Zone Diameter and MIC Breakpoints	**Added:** • Introductory text for Tables 2A–2J and Table 2 Dosages
Table 2A-1. Zone Diameter and MIC Breakpoints for Enterobacterales (excluding *Salmonella/Shigella*)	**Added:** • Comment regarding meropenem-vaborbactam and Enterobacterales that harbor OXA-48 • Comment to clarify suggested action when a carbapenemase marker is detected in an Enterobacterales isolate that is cefepime S or SDD
Table 2A-2. Zone Diameter and MIC Breakpoints for *Salmonella* and *Shigella* spp.	New table
Table 2B-2. Zone Diameter and MIC Breakpoints for *Acinetobacter* spp.	**Added:** • General comment regarding using positive blood culture broth as an inoculum for direct disk diffusion testing • Sulbactam-durlobactam disk diffusion and MIC breakpoints
Table 2B-3. MIC Breakpoints for *Burkholderia cepacia* complex	***Deleted*:** • General disk diffusion testing recommendations (disk diffusion no longer recommended for *B. cepacia*) • Ceftazidime disk diffusion breakpoints • Meropenem disk diffusion breakpoints • Minocycline disk diffusion breakpoints • Trimethoprim-sulfamethoxazole disk diffusion breakpoints

Section/Table	Changes
Tables 2. (Continued)	
Table 2B-4. Zone Diameter and MIC Breakpoints for *Stenotrophomonas maltophilia*	**Added:** • Comment regarding trimethoprim-sulfamethoxazole antimicrobial therapy **Revised:** • Minocycline disk diffusion and MIC breakpoints ***Deleted:*** • Ceftazidime MIC breakpoints
Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.	**Added:** • Tedizolid disk diffusion breakpoints (*S. aureus*) **Revised:** • Linezolid disk diffusion breakpoints • Table describing methods or targets for detection of methicillin (oxacillin)-resistant *Staphylococcus* spp. • Text explaining *mecA*, cefoxitin, and oxacillin and associated testing relationships ***Deleted:*** • Comment regarding MIC confirmation requirement for staphylococci resistant to linezolid by disk diffusion and requirement to read disk diffusion zones using transmitted light
Table 2H-1. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. β-Hemolytic Group	**Added:** • Reference corresponding to intrapartum prophylaxis recommendations • Tedizolid disk diffusion breakpoint (*S. pyogenes* and *S. agalactiae* only) **Revised:** • Comment regarding intrapartum prophylaxis recommendations
Table 2H-2. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. Viridans Group	**Added:** • Tedizolid disk diffusion breakpoint (*S. anginosus* group only)
Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints	New table (referred to as "Table 2 Dosages" throughout) **Added:** • Dosage for sulbactam-durlobactam for *Acinetobacter* spp. • Dosage for minocycline for *S. maltophilia* • Dosage for ceftriaxone for MSSA

Overview of Changes (Continued)

Section/Table	Changes
Tables 3. Specialized Resistance Testing	
Table 3B. CarbaNP Test for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*	**Added:** • Reference pertaining to performance of the test
Table 3C. Modified Carbapenem Inactivation Methods for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*	**Revised:** • Test interpretation criterion from positive, negative, "indeterminate" to positive, negative, "inconclusive"
Table 3D. Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution Method	New table
Table 3F-1. Test for Performing Disk Diffusion Directly From Positive Blood Culture Broth	**Added:** • Breakpoint Additions/Revisions Since 2021 table for disk diffusion directly from positive blood culture broth **Revised:** • Table to include testing *Acinetobacter* spp. directly from positive blood cultures
Table 3F-2. Zone Diameter Disk Diffusion Breakpoints for Enterobacterales Direct From Blood Culture	**Added:** • Tobramycin 8-10 hour and 16-18 hour breakpoints **Revised:** • General comment regarding aztreonam, ceftazidime, and tobramycin breakpoints ***Deleted:*** • SDD column in table
Table 3F-3. Zone Diameter Disk Diffusion Breakpoints for *Pseudomonas aeruginosa* Direct From Blood Culture	**Added:** • Cefepime 16-18 hour and tobramycin 8-10 hour and 16-18 hour breakpoints • Comment regarding cefepime confirmatory MIC testing ***Deleted:*** • SDD column in table
Table 3F-4. Zone Diameter Disk Diffusion Breakpoints for *Acinetobacter* spp. Direct From Blood Culture	New table
Table 3H. Oxacillin Salt Agar Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus aureus*	***Deleted:*** • Content related to routine disk diffusion and MIC testing

Section/Table	Changes
Tables 3. (Continued)	
Table 3J. Tests for Detecting Inducible Clindamycin Resistance in *Staphylococcus* spp., *Streptococcus pneumoniae*, and *Streptococcus* spp. β-Hemolytic Group	**Added:** • Reference corresponding to intrapartum prophylaxis recommendations **Revised:** • Footnote b regarding intrapartum prophylaxis recommendations • Reference pertaining to prevention of perinatal group B streptococcal disease updated to the American College of Obstetricians and Gynecologists guidelines
Tables 4. Disk Diffusion QC Ranges and Associated Tables	
Table 4A-1. Disk Diffusion QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-Lactam Combination Agents	**Added:** • Footnote c regarding *S. aureus* ATCC®[a] 43300 as a supplemental QC strain **Revised:** • Linezolid QC range for *S. aureus* ATCC® 25923 • Tedizolid QC range for *S. aureus* ATCC® 25923 ***Deleted:*** • Footnote regarding reading zones of inhibition for linezolid and tedizolid for *S. aureus* ATCC® 25923 using transmitted light
Table 4A-2. Disk Diffusion QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents	**Added:** • Column headers to highlight QC organisms recommended for routine QC testing
Tables 5. MIC QC Ranges and Associated Tables	
Table 5A-1. MIC QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-Lactam Combination Agents	**Added:** • Footnote e regarding *S. aureus* ATCC® 43300 as a supplemental QC strain • Footnote l regarding colistin QC organism alternatives to *P. aeruginosa* ATCC® 27853 • Footnote u regarding polymyxin B QC range for *Escherichia coli* NCTC 13846 • Upleganan QC ranges for *E. coli* ATCC® 25922 and *P. aeruginosa* ATCC® 27853 **Revised:** • Aztreonam QC range for *E. coli* ATCC® 25922 • Colistin QC range for *P. aeruginosa* ATCC® 27853 • Footnote o and associated figures regarding exebacase QC range for *S. aureus* ATCC® 29213 and additional testing guidance ***Deleted:*** • Colistin QC range for *E. coli* ATCC® 25922

Overview of Changes (Continued)

Section/Table	Changes
Tables 5. (Continued)	
Table 5A-2. MIC QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents	**Added:** • Column headers to highlight QC organisms recommended for routine QC testing • Imipenem-funobactam QC ranges for: – *E. coli* ATCC® 25922 – *P. aeruginosa* ATCC® 27853 – *Klebsiella pneumoniae* ATCC® 700603 – *K. pneumoniae* ATCC® BAA-1705™ **Revised:** • Aztreonam QC ranges for: – *E. coli* ATCC® 25922 – *K. pneumoniae* ATCC® 700603
Tables 6. Preparing Antimicrobial Agent Stock Solutions	
Table 6A. Solvents and Diluents for Preparing Stock Solutions of Antimicrobial Agents	**Added:** • Funobactam • Upleganan
Table 6C. Preparing Solutions and Media Containing Combinations of Antimicrobial Agents	**Added:** • Imipenem-funobactam
Appendixes	
Appendix B. Intrinsic Resistance, B1. Enterobacterales	***Deleted:*** • Footnote g regarding *Serratia marcescens* and elevated MICs to tobramycin
Appendix C. QC Strains for Antimicrobial Susceptibility Tests	**Added:** • *E. coli* AR Bank #0348 • Oxacillin MIC testing for MIC tests for *S. aureus* ATCC® 43300
Appendix G. Using Molecular Assays for Resistance Detection	**Revised:** • Table column headers
Table G3. Reporting Results From ESBL Resistance and Carbapenemase Molecular Tests for Enterobacterales	**Added:** • Text to clarify suggested action when a carbapenemase marker is detected in an Enterobacterales isolate that is cefepime S or SDD

Overview of Changes (Continued)

Section/Table	Changes
Appendixes (Continued)	
Appendix H. Cefiderocol Broth Preparation and Reading Broth Microdilution Minimal Inhibitory Concentration End Points, H3. Determining Broth Microdilution End Points	**Added:** • Figures showing determination of broth microdilution end points for cefiderocol **Revised:** • Steps for reading and interpreting broth microdilution end points for cefiderocol, including figures showing determination of end points
Glossaries	
Glossary I (Part 1). β-Lactams: Class and Subclass Designations and Generic Names	**Added:** • Imipenem-funobactam
Glossary I (Part 2). Non β-Lactams: Class and Subclass Designations and Generic Names	**Added:** • Upleganan
Glossary II. Antimicrobial Agent Abbreviations, Routes of Administration, and Drug Class	**Added:** • Imipenem-funobactam • Upleganan

Abbreviations: **AR, antimicrobial resistance;** ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; **MSSA, methicillin (oxacillin) susceptible *Staphylococcus aureus*;** NCTC, National Collection of Type Cultures; QC, quality control**; S, susceptible; SDD, susceptible-dose dependent**.

Footnote

a. ATCC® is a registered trademark of the American Type Culture Collection.

Summary of CLSI Processes for Establishing Breakpoints and QC Ranges

The Clinical and Laboratory Standards Institute (CLSI) is an international, voluntary, not-for-profit, interdisciplinary, standards-developing, and educational organization accredited by the American National Standards Institute that develops and promotes the use of consensus-developed standards and guidelines within the health care community. These consensus standards and guidelines are developed in an open and consensus-seeking forum to cover critical areas of diagnostic testing and patient health care. CLSI is open to anyone or any organization that has an interest in diagnostic testing and patient care. Information about CLSI can be found at www.clsi.org.

The CLSI Subcommittee on Antimicrobial Susceptibility Testing reviews data from a variety of sources and studies (eg, *in vitro*, pharmacokinetics/pharmacodynamics, and clinical studies) to establish antimicrobial susceptibility test methods, breakpoints, and QC parameters. The details of the data necessary to establish breakpoints, QC parameters, and how the data are presented for evaluation are described in CLSI M23.[4]

Over time, a microorganism's susceptibility to an antimicrobial agent may decrease, resulting in a lack of clinical efficacy and/or safety. In addition, microbiological methods and QC parameters may be refined to ensure more accurate and better performance of susceptibility test methods. Because of these types of changes, CLSI continually monitors and updates information in its documents. Although CLSI standards and guidelines are developed using the most current information available at the time, the field of science and medicine is always changing; therefore, standards and guidelines should be used in conjunction with clinical judgment, current knowledge, and clinically relevant laboratory test results to guide patient treatment.

Additional information, updates, and changes in this document are found in the meeting summary minutes of the CLSI Subcommittee on Antimicrobial Susceptibility Testing at https://clsi.org/meetings/ast-file-resources/.

Instructions for Use of Tables

These instructions apply to:

- Tables 1A through 1J: suggested tiers of antimicrobial agents that should be considered for testing and reporting by microbiology laboratories. These suggestions include clinical efficacy, current consensus recommendations for first-choice and alternative drugs, and US Food and Drug Administration (FDA) clinical indications for use. In other countries, placement of antimicrobial agents in Tables 1A through 1J should be based on available drugs approved for clinical use by relevant regulatory organizations.
- Tables 2A through 2J: tables for each organism group that contain:
  - Recommended testing conditions
  - Routine QC recommendations (also see CLSI M02[1] and CLSI M07[2])
  - General comments for testing the organism group and specific comments for testing particular agent/organism combinations
  - Agents that should be considered for routine testing and reporting by medical microbiology laboratories, as specified in Tables 1A through 1J (test/report Tiers 1, 2, 3, and 4), including agents reported only on organisms isolated from the urinary tract (designated by "U")
  - Agents that are appropriate for the respective organism group but are not listed in Tables 1 and would generally not warrant routine testing by a medical microbiology laboratory in the United States (designated with an asterisk as "other"; designated with "Inv." for "investigational" [not yet FDA approved]), including agents reported only on organisms isolated from the urinary tract (designated by "U")
  - Zone diameter and minimal inhibitory concentration (MIC) breakpoints
- Tables 1J and 2J: tables containing specific recommendations for testing and reporting results on anaerobes and some of the information listed in the bullets above
- Tables 3A through 3L: tables describing tests to detect particular resistance types in specific organisms or organism groups

Table 1B-1. *Pseudomonas aeruginosa*

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution.	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ceftazidime	Imipenem Meropenem	Cefiderocol	
Cefepime		Ceftazidime-avibactam	
Piperacillin-tazobactam		Ceftolozane-tazobactam	
		Imipenem-relebactam	
Tobramycin			
Ciprofloxacin Levofloxacin			
			Aztreonam
Urine Only			
	Amikacin		

Abbreviation: MDRO, multidrug-resistant organism.

Table 1B-5. Other Non-Enterobacterales[a,b]

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ceftazidime	Cefepime		
	Imipenem Meropenem		
Gentamicin Tobramycin	Amikacin		
Piperacillin-tazobactam			
Trimethoprim-sulfamethoxazole			
	Aztreonam		
	Ciprofloxacin Levofloxacin		
	Minocycline		
			Cefotaxime Ceftriaxone
Urine Only			
Tetracycline[c]			

Abbreviations: MDRO, multidrug-resistant organism; MIC, minimal inhibitory concentration.

Footnotes

a. Other non-Enterobacterales include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli but exclude *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia*. Refer to each respective Table 1 for suggested antimicrobial agents to test and report.

b. MIC testing only; disk diffusion test is unreliable.

c. Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.

Table 1H-2. *Streptococcus* spp. Viridans Group

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin[a,b] Penicillin[a,b]			
Cefotaxime Ceftriaxone			Cefepime
	Vancomycin		
		Linezolid Tedizolid[c]	
		Dalbavancin[a,c]	
		Oritavancin[a]	
		Telavancin[a]	
			Ceftolozane-tazobactam
			Clindamycin[d]
			Erythromycin[d,e]
			Levofloxacin

Abbreviations: MDRO, multidrug-resistant organism; MIC, minimal inhibitory concentration.

Footnotes

a. MIC testing only; disk diffusion test is unreliable.

b. ***Rx:*** Penicillin- or ampicillin-intermediate isolates may necessitate combined therapy with an aminoglycoside for bactericidal action.

c. Report only on *S. anginosus* group (including *S. anginosus*, *S. intermedius*, and *S. constellatus*).

d. Not routinely reported on organisms isolated from urinary tract.

e. Susceptibility and resistance to azithromycin and clarithromycin can be predicted by testing erythromycin.

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PRINT ISBN 978-1-68440-220-5

ELECTRONIC ISBN 978-1-68440-221-2

CLSI M100-Ed34