CLINICAL AND LABORATORY STANDARDS INSTITUTE®

33rd Edition

M100

Performance Standards for Antimicrobial Susceptibility Testing

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

A CLSI supplement for global application.

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Abstract

The data in the tables are valid only if the methodologies in CLSI documents M02,[1] M07,[2] and M11[3] are followed. These standards contain information about disk diffusion (M02[1]) and dilution (M07[2] and 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 M100 represent the most current information for drug selection, interpretation, and quality control using the procedures standardized in 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.

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Overview of Changes

M100-Ed33 replaces the previous edition of the supplement, M100-Ed32, published in 2022. The major changes in M100-Ed33 are listed below. Other minor or editorial changes were made to the general formatting and to some of the table footnotes and comments. Changes to the tables since the previous edition appear in boldface type. The following are additions or changes unless otherwise noted as "*Deleted.*"

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 Use of outdated documents is strongly discouraged.

Section/Table	Changes
General	
Throughout	Revised text for testing and reporting to clarify relevant institutional stakeholders
CLSI Breakpoint Additions/Revisions Since 2010	**Revised to create 2 separate tables:** • CLSI Breakpoint Additions Since 2010 • CLSI Breakpoint Revisions Since 2010
CLSI Breakpoint Additions Since 2010	**Added:** • Plazomicin disk diffusion and MIC breakpoints for Enterobacterales (p. xxx)
CLSI Breakpoint Revisions Since 2010	**Revised:** • Amikacin – Disk diffusion and MIC breakpoints for Enterobacterales (p. xxxiii) – Disk diffusion and MIC breakpoints for *Pseudomonas aeruginosa* (p. xxxiv) • Gentamicin disk diffusion and MIC breakpoints for Enterobacterales (p. xxxiii) • Piperacillin disk diffusion and MIC breakpoints for *P. aeruginosa* (p. xxxiv) • Piperacillin-tazobactam disk diffusion and MIC breakpoints for *P. aeruginosa* (p. xxxiv) • Tobramycin – Disk diffusion and MIC breakpoints for Enterobacterales (p. xxxiv) – Disk diffusion and MIC breakpoints for *P. aeruginosa* (p. xxxiv) ***Deleted:*** • Gentamicin disk diffusion and MIC breakpoints for *P. aeruginosa* (p. xxxiv)

Overview of Changes (Continued)

Section/Table	Changes
General (Continued)	
CLSI Archived Resources	**Added:** • Former Tables 1A–1C regarding suggested groupings of antimicrobial agents approved by the US Food and Drug Administration for clinical use that should be considered for testing and reporting by microbiology laboratories, which have been replaced by new Tables 1A through 1P (p. xxxvii)
Instructions for Use of Tables	**Added:** • Test/Report Tiers and Additional Designations (pp. 3–5) • Selective and Cascade Reporting (pp. 6–7) **Revised:** • Introductory section to include new Tables 1A through 1P and to update test/report tiers and designations (p. 1) • Appropriate Agents for Routine Testing (p. 2) • Equivalent Agents (pp. 2–3) • Susceptible-dose dependent definition to include extended infusion in the dosage regimen information (p. 9) • Organisms Excluded from Table 2 to clarify *Aeromonas* spp. (p. 11)
Tables 2	**Added (where applicable):** • Urine-only (U) designation and associated footnote • Inv. designation for investigational agents • * designation for "Other" agents not included in Tables 1 ***Deleted:*** • Test/Report column
Tables 1. Antimicrobial Agents That Should Be Considered for Testing and Reporting by Microbiology Laboratories	
Introduction to Tables 1A–1P. Antimicrobial Agents That Should Be Considered for Testing and Reporting by Microbiology Laboratories (new)	**Added:** • Introductory text and warning box for Tables 1A-1P (p. 24)
Table 1A. Enterobacterales (not including inducible AmpC producers and *Salmonella*/*Shigella*) (new table)	**Added:** • Antimicrobial agents for Enterobacterales (not including inducible AmpC producers and *Salmonella*/*Shigella*) (pp. 26–27)
Table 1B. *Salmonella* and *Shigella* spp. (new table)	**Added:** • Antimicrobial agents for *Salmonella* and *Shigella* spp. (p. 28)

Overview of Changes (Continued)

Section/Table	Changes
Tables 1. (Continued)	
Table 1C. *Pseudomonas aeruginosa* (new table)	**Added:** • Antimicrobial agents for *P. aeruginosa* (p. 30)
Table 1D. *Acinetobacter* spp. (new table)	**Added:** • Antimicrobial agents for *Acinetobacter* spp. (p. 32)
Table 1E. *Burkholderia cepacia* complex (new table)	**Added:** • Antimicrobial agents for *B. cepacia* complex (p. 34)
Table 1F. *Stenotrophomonas maltophilia* (new table)	**Added:** • Antimicrobial agents for *S. maltophilia* (p. 36)
Table 1G. Other Non-Enterobacterales (new table)	**Added:** • Antimicrobial agents for other non-Enterobacterales (p. 38)
Table 1H. *Staphylococcus* spp. (new table)	**Added:** • Antimicrobial agents for *Staphylococcus* spp. (pp. 40–41)
Table 1I. *Enterococcus* spp. (new table)	**Added:** • Antimicrobial agents for *Enterococcus* spp. (pp. 42–43)
Table 1J. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (new table)	**Added:** • Antimicrobial agents for *H. influenzae* and *H. parainfluenzae* (pp. 44–45)
Table 1K. *Neisseria gonorrhoeae* (new table)	**Added:** • Antimicrobial agents for *N. gonorrhoeae* (p. 46)
Table 1L. *Streptococcus pneumoniae* (new table)	**Added:** • Antimicrobial agents for *S. pneumoniae* (pp. 48–49)
Table 1M. *Streptococcus* spp. β-Hemolytic Group (new table)	**Added:** • Antimicrobial agents for *Streptococcus* spp. β-hemolytic group (pp. 50–51)
Table 1N. *Streptococcus* spp. Viridans Group (new table)	**Added:** • Antimicrobial agents for *Streptococcus* spp. viridans group (p. 52)
Table 1O. Gram-Negative Anaerobes (new table)	**Added:** • Antimicrobial agents for gram-negative anaerobes (p. 54)
Table 1P. Gram-Positive Anaerobes (new table)	**Added:** • Antimicrobial agents for gram-positive anaerobes (p. 56)

Overview of Changes (Continued)

Section/Table	Changes
Tables 2. Zone Diameter and/or MIC Breakpoints	
Table 2A. Zone Diameter and MIC Breakpoints for Enterobacterales	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 58) • Reference for comments regarding cephems and routine ESBL testing and cephems and third-generation cephalosporin resistance (p. 62) • Comment regarding the accuracy and reproducibility of cefiderocol testing results (p. 64) • Gentamicin, tobramycin, and amikacin combination therapy comment (p. 68) • Gentamicin, tobramycin, and amikacin dosage regimen comments (p. 68) • Plazomicin disk diffusion and MIC breakpoints and associated comments (p. 68) **Revised:** • General comment regarding testing fecal isolates of *Salmonella* and *Shigella* spp. (p. 58) • Comment regarding therapy for oral ampicillin (p. 60) • Comment regarding cephems and routine ESBL testing (p. 62) • Comment regarding cephems and third-generation cephalosporin resistance (p. 62) • Comment regarding carbapenems and elevated MICs (p. 66) • Gentamicin, tobramycin, and amikacin disk diffusion and MIC breakpoints (p. 68) • Gemifloxacin reporting comment (p. 69)

Overview of Changes (Continued)

Section/Table	Changes
Tables 2. (Continued)	
Table 2B-1. Zone Diameter and MIC Breakpoints for *Pseudomonas aeruginosa*	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 74) • Comment regarding the accuracy and reproducibility of cefiderocol testing results (p. 76) • Comment regarding combination therapy for tobramycin and amikacin (p. 78) • Tobramycin and amikacin dosage regimen comments (p. 78) **Revised:** • Piperacillin disk diffusion and MIC breakpoints and associated dosage regimen comment (p. 76) • Piperacillin-tazobactam disk diffusion and MIC breakpoints and associated dosage regimen comment (p. 76) • Tobramycin disk diffusion and MIC breakpoints (p. 78) • U designation for amikacin (p. 78) ***Deleted:*** • Gentamicin disk diffusion and MIC breakpoints
Table 2B-2. Zone Diameter and MIC Breakpoints for *Acinetobacter* spp.	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 80) • Comment regarding the accuracy and reproducibility of cefiderocol testing results (p. 81)

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Tables 2. (Continued)	
Table 2B-3. Zone Diameter and MIC Breakpoints for *Burkholderia cepacia* complex	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 84) **Revised:** • Chloramphenicol reporting comment (p. 85)
Table 2B-4. Zone Diameter and MIC Breakpoints for *Stenotrophomonas maltophilia*	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 86) • Comment regarding the accuracy and reproducibility of cefiderocol testing results (p. 87) • Levofloxacin ***Rx*** monotherapy comment (p. 87) **Revised:** • Chloramphenicol reporting comment (p. 87)
Table 2B-5. MIC Breakpoints for Other Non-Enterobacterales	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 90) **Revised:** • Comment regarding recommendations for testing and reporting *Aeromonas* spp. (p. 90) • Chloramphenicol reporting comment (p. 92)

Overview of Changes (Continued)

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Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 94) **Revised:** • Daptomycin reporting comment (p. 101) • Quinupristin-dalfopristin reporting comment (p. 102)
Table 2D. Zone Diameter and MIC Breakpoints for *Enterococcus* spp.	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 106) **Revised:** • Dalbavancin and daptomycin (*E. faecium* only) reporting comment (p. 109) • Erythromycin and fosfomycin reporting comments (p. 110) • Quinupristin-dalfopristin and tedizolid reporting comments (p. 111)
Table 2E. Zone Diameter and MIC Breakpoints for *Haemophilus influenzae* and *Haemophilus parainfluenzae*	**Added:** • MH-F agar as a medium for disk diffusion to the testing conditions box for *H. influenzae* (p. 112) • MH-F broth as a medium for broth dilution to the testing conditions box for *H. influenzae* (p. 112) • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 112) • General comment regarding the use of MH-F broth vs HTM broth in MIC testing (p. 113) • General comment regarding the use of MH-F agar broth vs HTM broth in disk diffusion testing (p. 113) **Revised:** • Routine QC recommendations box to clarify media for each QC strain (p. 112) • Ceftolozane-tazobactam reporting comment (p. 115)

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Table 2G. Zone Diameter and MIC Breakpoints for *Streptococcus pneumoniae*	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 122) **Revised:** • Medium information for disk diffusion in testing conditions box (p. 122) • General comment regarding MIC testing of cefotaxime, ceftriaxone, meropenem, or penicillin reported with *S. pneumoniae* isolated from CSF (p. 123) • Comment regarding susceptibility to gemifloxacin, levofloxacin, and moxifloxacin (p. 127)
Table 2H-1. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. ß-Hemolytic Group	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 130) **Revised:** • Dalbavancin and daptomycin reporting comments (p. 132) • Erythromycin, azithromycin, clarithromycin, and dirithromycin dosage regimen comment (p. 133) • Tedizolid reporting comment (p. 134)
Table 2H-2. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. Viridans Group	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 136) **Revised:** • Dalbavancin and daptomycin reporting comments (p. 138)
Table 2I. Zone Diameter and MIC Breakpoints for *Neisseria meningitidis*	**Revised:** • Chloramphenicol reporting comment (p. 142)

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Section/Table	Changes
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Table 2J. MIC Breakpoints for Anaerobes	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 144) **Revised:** • Ampicillin and penicillin testing and reporting comment to include test/report tiers (p. 145) • Metronidazole resistance comment to refer users to Appendix D (p. 146)
Tables 3. Specialized Resistance Testing	
Table 3A. Tests for Extended-Spectrum β-Lactamases in *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, and *Proteus mirabilis*	**Added:** • Introductory text regarding reporting of ESBL test results (p. 148) **Revised:** • **NOTE** regarding ESBL testing (p. 148)
Introduction to Tables 3B and 3C. Tests for Carbapenemases in Enterobacterales and *Pseudomonas aeruginosa*	**Revised:** • Introductory text and associated reference (p. 152)
Table 3B. CarbaNP Test for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*	**Revised:** • Indications for when to perform test (p. 154) ***Deleted:*** • **NOTE** regarding the use of former MIC breakpoints for carbapenems
Table 3B-1. Modifications of Table 3B When Using MIC Breakpoints for Carbapenems Described in M100-S20 (January 2010)	***Deleted:*** • Table 3B-1
Table 3C. Modified Carbapenem Inactivation Methods for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*	**Revised:** • Indications for when to perform test (p. 162) ***Deleted:*** • **NOTE** regarding the use of former MIC breakpoints for carbapenems
Table 3C-1. Modifications of Table 3C When Using MIC Breakpoints for Carbapenems Described in M100-S20 (January 2010)	***Deleted:*** • Table 3C-1

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Table 3D. Tests for Colistin Resistance for Enterobacterales and *Pseudomonas aeruginosa*	**Revised:** • "QC recommendations – routine" row (p. 176) • QC strain in Figures 1 and 2 legends (pp. 178-179)
Table 3E-1. Test for Performing Disk Diffusion Directly From Positive Blood Culture Broth	**Revised:** • Incubation length recommendations to refer users to Tables 3E-2 and 3E-3 (p. 180)
Table 3E-2. Zone Diameter Disk Diffusion Breakpoints for Enterobacterales Direct From Blood Culture	**Added:** • General comment regarding organism identification (p. 182) • Breakpoints for ampicillin 8-10 h, meropenem 8-10 h and 16-18 h, and ciprofloxacin excluding *Salmonella* 8-10 h and 16-18 h (p. 183) ***Deleted:*** • Test/Report Group column
Table 3E-3. Zone Diameter Disk Diffusion Breakpoints for *Pseudomonas aeruginosa* Direct From Blood Culture	**Added:** • General comment regarding organism identification (p. 184) • Breakpoints for meropenem 8-10 h (p. 184) ***Deleted:*** • Test/Report Group column
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Overview of Changes (Continued)

Section/Table	Changes
Tables 4. Disk Diffusion QC Ranges and Associated Tables	
Table 4A-2. Disk Diffusion QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents	**Added:** • Organism characteristic OXA-1 for *E. coli* NCTC 13353 (p. 210) • Footnote e regarding colony morphologies for *K. pneumoniae* ATCC® 700603 (p. 212) • Ceftibuten QC range for *E. coli* NCTC 13353 • Ceftibuten-ledaborbactam QC range for *E. coli* NCTC 13353
Table 4B. Disk Diffusion QC Ranges for Fastidious Organisms	**Added:** • Footnote f regarding chloramphenicol QC range for *H. influenzae* ATCC® 49247 in MH-F agar (p. 216) • Gentamicin QC range for *N. gonorrhoeae* ATCC® 49226 (p. 215) • MH-F agar medium for *H. influenzae* (p. 216) **Revised:** • Footnote e regarding *H. influenzae* ATCC® 49247 or 49766 to indicate its use with HTM and to indicate that *H. influenzae* ATCC® 49247 should be used with MH-F (p. 216) • Footnote g regarding QC ranges for delafloxacin, levonadifloxacin, and nafithromycin to include clarithromycin with MH-F agar (p. 216) • Incubation temperatures for *H. influenzae*, *N. gonorrhoeae*, and streptococci and *N. meningitidis* (p. 216)
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 f regarding ceftibuten and tebipenem lack of established equivalency (p. 228) • Footnote j regarding colistin preparation and handling (p. 228) • **NOTE** stating that MIC ranges in table apply to both broth microdilution and agar dilution unless otherwise specified (p. 230) **Revised:** • Ceftibuten QC range for *E. coli* ATCC® 25922 • Footnote i regarding additional colistin QC ranges for *E. coli* NCTC 13846 (p. 228) • Footnote m regarding exebacase QC ranges, including figures showing determination of exebacase *S. aureus* ATCC® 29213 end points (pp. 228-229)

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:** • Organism characteristic OXA-1 for *E. coli* NCTC 13353 (p. 232) • Ceftazidime-avibactam QC ranges – *E. coli* NCTC 13353 – *Klebsiella pneumoniae* ATCC® BAA-1705™ – *K. pneumoniae* ATCC® BAA-2814™ • Ceftibuten QC range – *K. pneumoniae* ATCC® 700603 • Ceftibuten-avibactam QC ranges – *E. coli* ATCC® 25922 – *K. pneumoniae* ATCC® 700603 – *E. coli* NCTC 13353 – *K. pneumoniae* ATCC® BAA-1705™ – *K. pneumoniae* ATCC® BAA-2814™ • Ceftibuten-ledaborbactam QC ranges – *Escherichia coli* NCTC 13353 – *K. pneumoniae* ATCC® BAA-1705™ – *K. pneumoniae* ATCC® BAA-2814™ • Meropenem-xeruborbactam QC ranges – *P. aeruginosa* ATCC® 27853 – *K. pneumoniae* ATCC® BAA-2814™

Overview of Changes (Continued)

Section/Table	Changes
Tables 5. (Continued)	
Table 5A-2. MIC QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents (continued)	**Added (continued):** • Footnote e regarding colony morphologies for *K. pneumoniae* ATCC® 700603 (p. 234) • Footnote h regarding ceftazidime-avibactam, ceftibuten, and ceftibuten-avibactam lack of equivalency (p. 234) • Footnote i regarding meropenem QC range for *P. aeruginosa* ATCC® BAA-3197™ (formerly *P. aeruginosa* PA5257) (p. 234) • Footnote j regarding meropenem-xeruborbactam QC range for *P. aeruginosa* ATCC® BAA-3197™ (formerly *P. aeruginosa* PA5257) (p. 234) • **NOTE** stating that MIC ranges in the table apply to both broth microdilution and agar dilution unless otherwise specified (p. 234) **Revised:** • Ceftibuten QC range for *E. coli* ATCC® 25922 • Piperacillin-tazobactam QC range for *E. coli* ATCC® 25922
Table 5B. MIC QC Ranges for Fastidious Organisms (Broth Dilution Methods)	**Added:** • Tebipenem QC ranges – *H. influenzae* ATCC® 49766 – *S. pneumoniae* ATCC® 49619 • Footnote d regarding ceftazidime-avibactam, ceftibuten, and tebipenem lack of equivalency (p. 239) • Footnote k indicating tebipenem QC range for *H. influenzae* ATCC® 49766 was established with a limited number of media manufacturers (p. 239) • **NOTE** stating that MIC ranges in table apply to both broth microdilution and agar dilution unless otherwise specified (p. 239) **Revised:** • Medium for testing *H. influenzae* to include MH-F broth (p. 238) • Incubation temperature for testing *H. influenzae*, *S. pneumoniae* and streptococci, and *N. meningitidis* (p. 238)

Overview of Changes (Continued)

Section/Table	Changes
Tables 5. (Continued)	
Table 5G. MIC Troubleshooting Guide	**Added:** • Ceftriaxone troubleshooting comments for *P. aeruginosa* ATCC® 27853 (p. 249) • Colistin troubleshooting comments (p. 249) and associated footnote b (p. 252) for *E. coli* ATCC® 25922, *P. aeruginosa* ATCC® 27853, *E. coli* NCTC 13846, and *E. coli* ATCC® BAA-3170™ • Various agents troubleshooting comments for *Enterococcus faecalis* ATCC® 51299 (p. 250) **Revised:** • Carbenicillin troubleshooting comment for *P. aeruginosa* ATCC® 27853 (p. 248) • Various agents troubleshooting comments for *S. pneumoniae* ATCC® 49619 (p. 250)
Tables 6. Preparing Antimicrobial Agent Stock Solutions	
Table 6A. Solvents and Diluents for Preparing Stock Solutions of Antimicrobial Agents	**Added:** • Ledaborbactam (p. 256) • Footnote i regarding exebacase handling instructions (p. 259) • Footnote j regarding exebacase preparation instructions (p. 259) • Xeruborbactam (p. 258) **Revised:** • Ceftibuten solvent and diluent for preparing stock solutions (p. 254) • Exebacase diluent for preparing stock solutions (p. 255) ***Deleted:*** • DMSO as a solvent for ceftibuten
Table 6C. Preparing Solutions and Media Containing Combinations of Antimicrobial Agents	**Added:** • Ceftibuten-avibactam (p. 264) • Ceftibuten-ledaborbactam (p. 264) • Meropenem-xeruborbactam (p. 265)

Overview of Changes (Continued)

Section/Table	Changes
Appendixes	
Appendix A. Suggestions for Confirming Antimicrobial Susceptibility Test Results and Organism Identification for Agents Approved by the US Food and Drug Administration for Clinical Use	**Added:** • Enterobacterales (p. 274) – Imipenem-relebactam – Cefiderocol • *Acinetobacter baumannii* complex (p. 275) – Cefiderocol • *P. aeruginosa* (p. 275) – Ceftazidime-avibactam – Imipenem-relebactam – Cefiderocol • *S. maltophilia* (p. 275) – Cefiderocol • *Bacteroides* spp. and *Parabacteroides* spp. (p. 279) – Imipenem-relebactam
Appendix B. Intrinsic Resistance; B1. Enterobacterales	**Added:** • Footnote g regarding *Serratia marcescens* and tobramycin (p. 284) **Revised:** • **NOTE** regarding agents not listed because there is no intrinsic resistance (p. 285)
Appendix C. QC Strains for Antimicrobial Susceptibility Tests	**Added:** • Organism characteristic OXA-1 for *E. coli* NCTC 13353 (p. 291) • Comment regarding colony morphologies for *K. pneumoniae* ATCC® 700603 (p. 291)

Overview of Changes (Continued)

Section/Table	Changes
Appendixes (Continued)	
Appendix E. Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints	**Added:** • Enterobacterales – Amikacin (p. 302) – Gentamicin (p. 303) – Plazomicin (excluding family Morganellaceae) (p. 304) – Tobramycin (p. 304) • *P. aeruginosa* – Amikacin (p. 304) – Tobramycin (p. 304) **Revised:** • Dosage for piperacillin and piperacillin-tazobactam for *P. aeruginosa* (p. 304)
Appendix I. Cefiderocol Broth Preparation and Reading Broth Microdilution Minimal Inhibitory Concentration End Points	**Revised:** • Step for preparing iron-depleted cation-adjusted Mueller-Hinton broth (p. 335)
Glossaries	
Glossary I (Part 1). B-Lactams: Class and Subclass Designations and Generic Names	**Added:** • Ceftibuten-avibactam • Ceftibuten-ledaborbactam • Meropenem-xeruborbactam
Glossary II. Antimicrobial Agent Abbreviations, Routes of Administration, and Drug Class	**Added:** • Ceftibuten-avibactam • Ceftibuten-ledaborbactam • Meropenem-xeruborbactam

Abbreviations: ATCC®, American Type Culture Collection; CSF, cerebrospinal fluid; MH-F agar, Mueller-Hinton fastidious agar; MH-F broth, Mueller-Hinton fastidious broth; MIC, minimal inhibitory concentration; UTI, urinary tract infection.

Footnote

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

CLSI Breakpoint Additions Since 2010

This table includes the M100 edition in which specific antimicrobial agent breakpoints were added for the first time for a specific organism group.

Antimicrobial Agent	Date of Addition (M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
Enterobacterales				
Azithromycin	January 2015 (M100-S25)	X	X	*S. enterica* ser. Typhi only
	March 2021 (M100-Ed31)	X	X	*Shigella* spp. Previously assigned an ECV
Cefiderocol	January 2019 (M100, 29th ed.)		X	
	January 2020 (M100, 30th ed.)	X		
Ceftaroline	January 2013 (M100-S23)	X	X	
Ceftazidime-avibactam	January 2018 (M100, 28th ed.)	X	X	
Ceftolozane-tazobactam	January 2016 (M100-S26)		X	
	January 2018 (M100, 28th ed.)	X		
Colistin	January 2020 (M100, 30th ed.)		X	Previously assigned an ECV
Doripenem	June 2010 (M100-S20-U)	X	X	
Imipenem-relebactam	March 2021 (M100-Ed31)	X	X	
Meropenem-vaborbactam	January 2019 (M100, 29th ed.)	X	X	
Pefloxacin	January 2015 (M100-S25)	X		*Salmonella* spp. (including *S. enterica* ser. Typhi) Surrogate test for ciprofloxacin
Plazomicin	**March 2023 (M100-Ed33)**	**X**	**X**	
Polymyxin B	January 2020 (M100, 30th ed.)		X	
Pseudomonas aeruginosa				
Cefiderocol	January 2019 (M100, 29th ed.)		X	
	January 2020 (M100, 30th ed.)	X		
Ceftazidime-avibactam	January 2018 (M100, 28th ed.)	X	X	
Doripenem	January 2012 (M100-S22)	X	X	
Imipenem-relebactam	March 2021 (M100-Ed31)	X	X	
***Acinetobacter* spp.**				
Cefiderocol	January 2019 (M100, 29th ed.)		X	
	January 2020 (M100, 30th ed.)	X		
Doripenem	January 2014 (M100-S24)	X	X	

CLSI Breakpoint Additions Since 2010 (Continued)

Antimicrobial Agent	Date of Addition (M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
Stenotrophomonas maltophilia				
Cefiderocol	January 2019 (M100, 29th ed.)		X	
	January 2020 (M100, 30th ed.)	X		
***Staphylococcus* spp.**				
Ceftaroline	January 2013 (M100-S23)	X	X	
Dalbavancin	January 2018 (M100, 28th ed.)		X	
Lefamulin	March 2021 (M100-Ed31)	X	X	
Oritavancin	January 2016 (M100-S26)		X	
Tedizolid	January 2016 (M100-S26)		X	
Telavancin	January 2016 (M100-S26)	X	X	
***Enterococcus* spp.**				
Dalbavancin	January 2018 (M100, 28th ed.)		X	
Oritavancin	January 2016 (M100-S26)		X	
Tedizolid	January 2016 (M100-S26)		X	
Telavancin	January 2016 (M100-S26)	X	X	
Haemophilus influenzae* and *Haemophilus parainfluenzae				
Ceftaroline	January 2013 (M100-S23)	X	X	
Ceftolozane-tazobactam	March 2021 (M100-Ed31)		X	
Doripenem	January 2012 (M100-S22)	X	X	
Lefamulin	March 2021 (M100-Ed31)	X	X	
Neisseria gonorrhoeae				
Azithromycin	January 2019 (M100, 29th ed.)		X	Previously assigned as ECV
	March 2021 (M100-Ed31)	X		
Streptococcus pneumoniae				
Ceftaroline	January 2013 (M100-S23)	X	X	
Doripenem	January 2012 (M100-S22)		X	
Doxycycline	January 2013 (M100-S23)	X	X	
Lefamulin	March 2021 (M100-Ed31)	X	X	

CLSI Breakpoint Additions Since 2010 (Continued)

Antimicrobial Agent	Date of Addition (M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
***Streptococcus* spp. β-Hemolytic Group**				
Ceftaroline	January 2013 (M100-S23)	X	X	
Dalbavancin	January 2018 (M100, 28th ed.)		X	
Doripenem	January 2012 (M100-S22)		X	
Oritavancin	January 2016 (M100-S26)		X	
Tedizolid	January 2016 (M100-S26)		X	
Telavancin	January 2016 (M100-S26)	X	X	
***Streptococcus* spp. Viridans Group**				
Ceftolozane-tazobactam	January 2016 (M100-S26)		X	
Dalbavancin	January 2018 (M100, 28th ed.)		X	
Doripenem	January 2012 (M100-S22)		X	
Oritavancin	January 2016 (M100-S26)		X	
Tedizolid	January 2016 (M100-S26)		X	
Telavancin	January 2016 (M100-S26)	X	X	
Anaerobes				
Doripenem	January 2012 (M100-S22)		X	
Imipenem-relebactam	March 2021 (M100-Ed31)		X	
Piperacillin-tazobactam	January 2017 (M100, 27th ed.)		X	
	January 2018 (M100, 28th ed.)		X	

Abbreviation: ECV, epidemiological cutoff value.

CLSI Breakpoint Revisions Since 2010

This table includes the M100 edition in which specific antimicrobial agent breakpoints were revised or updated for a specific organism group. In some cases, unique breakpoints were added for a specific genus or species previously included within the organism or organism group breakpoints (eg, "*Salmonella* spp. [including *S. enterica* ser. Typhi]" was previously grouped with the organism group breakpoints for Enterobacterales). Previous breakpoints can be found in the edition of M100 that precedes the document listed in the column labeled "Date of Revision (M100 edition)." For example, previous breakpoints for aztreonam are listed in **M100-S20 (January 2010).**

Antimicrobial Agent	Date of Revision (M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
Enterobacterales				
Amikacin	**March 2023 (M100-Ed33)**	**X**	**X**	
Aztreonam	January 2010 (M100-S20)	X	X	
Cefazolin (parenteral)	January 2010 (M100-S20)	X	X	Removed disk diffusion breakpoints January 2010 (M100-S20)
	January 2011 (M100-S21)	X	X	
	January 2016 (M100-S26)	X	X	For uncomplicated UTIs
Cefazolin (oral)	January 2014 (M100-S24)	X	X	Surrogate test for oral cephalosporins and uncomplicated UTIs
Cefepime	January 2014 (M100-S24)	X	X	Revised breakpoints include SDD
Cefiderocol	February 2022 (M100-Ed32)	X		
Cefotaxime	January 2010 (M100-S20)	X	X	
Ceftazidime	January 2010 (M100-S20)	X	X	
Ceftizoxime	January 2010 (M100-S20)	X	X	
Ceftolozane-tazobactam	February 2022 (M100-Ed32)	X		
Ceftriaxone	January 2010 (M100-S20)	X	X	
Ciprofloxacin	January 2012 (M100-S22)	X	X	*Salmonella* spp. (including *S. enterica* ser. Typhi)
	January 2019 (M100, 29th ed.)	X	X	Non-*Salmonella* spp.
Ertapenem	June 2010 (M100-S20-U)	X	X	
	January 2012 (M100-S22)	X	X	
Gentamicin	**March 2023 (M100-Ed33)**	**X**	**X**	
Imipenem	June 2010 (M100-S20-U)	X	X	
Levofloxacin	January 2013 (M100-S23)	X	X	*Salmonella* spp. (including *S. enterica* ser. Typhi)
	January 2019 (M100, 29th ed.)	X	X	Non-*Salmonella* spp.

CLSI Breakpoint Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Revision (M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
Enterobacterales (Continued)				
Meropenem	June 2010 (M100-S20-U)	X	X	
Norfloxacin	January 2020 (M100, 30th ed.)	X	X	Reinstated breakpoints deleted from M100, 29th ed.
Ofloxacin	January 2013 (M100-S23)		X	*Salmonella* spp. (including *S. enterica* ser. Typhi)
Piperacillin	February 2022 (M100-Ed32)		X	Removed disk diffusion breakpoints due to reassessment of disk correlates for revised MIC breakpoints
Piperacillin-tazobactam	February 2022 (M100-Ed32)	X	X	
Tobramycin	**March 2023 (M100-Ed33)**	**X**	**X**	
Pseudomonas aeruginosa				
Amikacin	**March 2023 (M100-Ed33)**	**X**	**X**	**Report only on organisms isolated from the urinary tract**
Ciprofloxacin	January 2019 (M100, 29th ed.)	X	X	
Colistin	January 2017 (M100, 27th ed.)		X	
	January 2020 (M100, 30th ed.)		X	
Gentamicin	**March 2023 (M100-Ed33)**			**Removed disk diffusion and MIC breakpoints March2023 (M100-Ed33)**
Imipenem	January 2012 (M100-S22)	X	X	
Levofloxacin	January 2019 (M100, 29th ed.)	X	X	
Meropenem	January 2012 (M100-S22)	X	X	
Norfloxacin	January 2020 (M100, 30th ed.)	X	X	Reinstated breakpoints deleted from M100, 29th ed.
Piperacillin	January 2012 (M100-S22)	X	X	
	March 2023 (M100-Ed33)	**X**	**X**	
Piperacillin-tazobactam	January 2012 (M100-S22)	X	X	
	March 2023 (M100-Ed33)	**X**	**X**	
Polymyxin B	January 2020 (M100, 30th ed.)		X	
Ticarcillin	January 2012 (M100-S22)	X	X	
Ticarcillin-clavulanate	January 2012 (M100-S22)	X	X	
Tobramycin	**March 2023 (M100-Ed33)**	**X**	**X**	

CLSI Breakpoint Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Revision (M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
***Acinetobacter* spp.**				
Cefiderocol	February 2022 (M100-Ed32)	X		
Colistin	January 2020 (M100, 30th ed.)		X	
Imipenem	January 2014 (M100-S24)	X	X	
Meropenem	January 2014 (M100-S24)	X	X	
Polymyxin B	January 2020 (M100, 30th ed.)		X	
Stenotrophomonas maltophilia				
Cefiderocol	February 2022 (M100-Ed32)	X	X	
Other Non-Enterobacterales				
Norfloxacin	January 2020 (M100, 30th ed.)	X	X	Reinstated breakpoints deleted from M100, 29th ed.
***Staphylococcus* spp.**				
Cefoxitin	January 2019 (M100, 29th ed.)	X		*S. epidermidis* Surrogate test for oxacillin
Ceftaroline	January 2019 (M100, 29th ed.)	X	X	Revised breakpoints include SDD
Norfloxacin	January 2020 (M100, 30th ed.)	X	X	Reinstated breakpoints deleted from M100, 29th ed.
Oxacillin	January 2016 (M100-S26)	X	X	*S. pseudintermedius*
	January 2018 (M100, 28th ed.)	X	X	*S. schleiferi*
	January 2019 (M100, 29th ed.)	X		*S. epidermidis*
	March 2021 (M100-Ed31)		X	*Staphylococcus* spp. except *S. aureus* and *S. lugdunensis*
Telavancin	January 2017 (M100, 27th ed.)			Removed disk diffusion breakpoints January 2017 (M100, 27th ed.)

CLSI Breakpoint Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Revision (M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
***Enterococcus* spp.**				
Daptomycin	January 2019 (M100, 29th ed.)		X	
	January 2020 (M100, 30th ed.)		X	Separated into two sets of breakpoints: • *Enterococcus* spp. other than *Enterococcus faecium* • *E. faecium* (includes SDD)
Norfloxacin	January 2020 (M100, 30th ed.)	X	X	Reinstated breakpoints deleted from M100, 29th ed.
Telavancin	January 2017 (M100, 27th ed.)			Removed disk diffusion breakpoints January 2017 (M100, 27th ed.)
Haemophilus influenzae* and *Haemophilus parainfluenzae				
Amoxicillin-clavulanate	February 2022 (M100-Ed32)		X	Removed disk diffusion breakpoints February 2022 (M100-Ed32)
Lefamulin	February 2022 (M100-Ed32)	X		For *H. influenzae* only
Streptococcus pneumoniae				
Lefamulin	February 2022 (M100-Ed32)	X		
Tetracycline	January 2013 (M100-S23)	X	X	
***Streptococcus* spp. ß-Hemolytic Group**				
Telavancin	January 2017 (M100, 27th ed.)			Removed disk diffusion breakpoints January 2017 (M100, 27th ed.)
***Streptococcus* spp. Viridans Group**				
Telavancin	January 2017 (M100, 27th ed.)			Removed disk diffusion breakpoints January 2017 (M100, 27th ed.)

Abbreviations: SDD, susceptible-dose-dependent; UTI, urinary tract infection.

CLSI Archived Resources

Resource	Web Address for Archived Table
Former Tables 1A-1C regarding suggested groupings of antimicrobial agents approved by the US Food and Drug Administration that should be considered for testing and reporting on nonfastidious, fastidious, and anaerobic organisms have been relocated to the CLSI website.	**https://clsi.org/media/l0hbwxay/m100_archived_tables_1a-1c.pdf**
Breakpoints that have been eliminated from M100 since 2010 have been relocated to the CLSI website.	https://clsi.org/media/pqlom3b5/_m100_archived_drugs_table.pdf
Methods that have been eliminated from M100 have been relocated to the CLSI website.	https://clsi.org/media/nszl4tbc/_m100_archived_methods_table.pdf
QC ranges that have been eliminated from M100 since 2010 have been relocated to the CLSI website.	https://clsi.org/media/r31oari2/_m100_archived_qc_table.pdf
ECVs that have been replaced by breakpoints have been relocated to the CLSI website.	https://clsi.org/media/3mekwxft/_m100_archived_ecvs_table.pdf

Abbreviations: ECV, epidemiological cutoff value; QC, quality control.

NOTE: The content of this document is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

Summary of CLSI Processes for Establishing Breakpoints and Quality Control 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 document 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 Subcommittee on Antimicrobial Susceptibility Testing at https://clsi.org/meetings/ast-file-resources/.

CLSI Reference Methods vs Commercial Methods and CLSI vs US Food and Drug Administration Breakpoints

It is important for users of M02,[1] M07,[2] and M100 to recognize that the standard methods described in CLSI documents are reference methods. These methods may be used for routine antimicrobial susceptibility testing of patient isolates, for evaluating commercial devices that will be used in medical laboratories, or by drug or device manufacturers for testing new agents or systems. Results generated by reference methods, such as those included in CLSI documents, may be used by regulatory authorities to evaluate the performance of commercial susceptibility testing devices as part of the approval process. Clearance by a regulatory authority indicates the commercial susceptibility testing device provides susceptibility results that are substantially equivalent to results generated using reference methods for the organisms and antimicrobial agents described in the device manufacturer's approved package insert.

CLSI breakpoints may differ from those approved by various regulatory authorities for many reasons, including use of different databases, differences in data interpretation, differences in doses used in different parts of the world, and public health policies. Differences also exist because CLSI proactively evaluates the need for changing breakpoints. The reasons why breakpoints may change and the manner in which CLSI evaluates data and determines breakpoints are outlined in CLSI document M23.[4]

Following a decision by CLSI to change an existing breakpoint, regulatory authorities may also review data to determine how changing breakpoints may affect the safety and effectiveness of the antimicrobial agent for the approved indications. If the regulatory authority changes breakpoints, commercial device manufacturers may have to conduct a clinical trial, submit the data to the regulatory authority, and await review and approval. For these reasons, a delay of one or more years may be needed if a breakpoint and interpretive category change is to be implemented by a device manufacturer. In the United States, it is acceptable for laboratories that use US Food and Drug Administration (FDA)–cleared susceptibility testing devices to use existing FDA breakpoints. Either FDA or CLSI susceptibility breakpoints are acceptable to laboratory accrediting organizations in the United States. Policies in other countries may vary. Each laboratory should check with the manufacturer of its antimicrobial susceptibility test system for additional information on the breakpoints and interpretive categories used in its system's software.

Following discussions with **the antimicrobial stewardship team and other relevant institutional** stakeholders, newly approved or revised breakpoints may be implemented by laboratories. Following verification, CLSI disk diffusion test breakpoints may be implemented as soon as they are published in M100. If a device includes antimicrobial test concentrations sufficient to allow interpretation of susceptibility and resistance to an agent using the CLSI breakpoints, a laboratory could choose to, after appropriate verification, interpret and report results using CLSI breakpoints.

Subcommittee on Antimicrobial Susceptibility Testing Mission Statement

The 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 that promote accurate antimicrobial susceptibility testing and appropriate reporting. The mission of the Subcommittee on Antimicrobial Susceptibility Testing is to:

- Develop standard reference methods for antimicrobial susceptibility tests.
- Provide quality control parameters for standard test methods.
- Establish breakpoints and interpretive categories for the results of standard antimicrobial susceptibility tests and provide epidemiological cutoff values when breakpoints are not available.
- Provide suggestions for testing and reporting strategies that are clinically relevant and cost-effective.
- Continually refine standards and optimize detection of emerging resistance mechanisms through development of new or revised methods, breakpoints, 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.

Instructions for Use of Tables

These instructions apply to:

- **Tables 1A through 1P:** 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 1P** should be based on available drugs approved for clinical use by relevant regulatory organizations.

- **Tables 2A through 2I:** tables for each organism group that contain:
  - Recommended testing conditions
  - Routine QC recommendations (also see Chapter 4 in M02[1] and 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 1P** (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 1O, 1P, 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 3K:** tables describing tests to detect particular resistance types in specific organisms or organism groups

I. Selecting Antimicrobial Agents for Testing and Reporting

A. Appropriate Agents for Routine Testing

Selecting the most appropriate antimicrobial agents to test and report is a decision best made by each laboratory in consultation with the antimicrobial stewardship team **and other relevant institutional stakeholders.**

The suggestions for each organism group in Tables 1A-1P include agents of proven efficacy that show acceptable *in vitro* test performance. Considerations in the assignment of agents to specific tiers include:

- **Clinical efficacy**
- **Prevalence of resistance**
- **Minimizing emergence of resistance**
- **FDA clinical indications for use**
- **Current consensus recommendations for first-choice and alternative drugs**
- **Cost**

Tests on selected agents may be useful for infection-prevention purposes (eg, testing ceftazidime for Enterobacterales to indicate potential extended-spectrum ß-lactamase production; see Table 3A).

B. Equivalent Agents

Antimicrobial agents listed together in a single box are agents for which interpretive categories (susceptible, intermediate, susceptible-dose dependent, or resistant) and clinical efficacy are similar**. A laboratory will often test only one agent from a box routinely, typically the agent that is on its formulary. In some cases, a laboratory may not test any agents from a box, depending on institutional needs.**

In some boxes, the agents will be listed with an "or" between them. The "or" identifies agents for which cross-resistance and cross-susceptibility are nearly complete. Results from one agent connected by an "or" can be used to predict results for the other agent (ie, equivalent agents). For example, Enterobacterales susceptible to cefotaxime can be considered susceptible to ceftriaxone. The results obtained from testing cefotaxime could be reported along with a comment that the isolate is also susceptible to ceftriaxone. For drugs connected with an "or," combined major and very major errors are fewer than 3%, and minor errors are fewer than 10%, based on a large population of bacteria tested

(see CLSI document M23[4] for description of error types). In addition, to qualify for an "or," at least 100 strains with resistance to the agents in question must be tested and a result of "resistant" must be obtained with all agents for at least 95% of the strains. "Or" is also used for comparable agents when tested against organisms for which "susceptible-only" breakpoints are provided (eg, cefotaxime or ceftriaxone with *H. influenzae*). When no "or" connects agents within a box, testing of one agent cannot be used to predict results for another, owing either to discrepancies or insufficient data **(see Section VIII, which describes equivalent agent tests).**

C. Test/Report Tiers and Additional Designations

Antimicrobial Agent Test and Report Tiers and Additional Considerations for Agents Listed in Tables 1

Tier	Definition	Test	Report[a]	Additional Testing and Reporting Considerations
1	Antimicrobial agents that are appropriate for routine, primary testing and reporting	Routine	Routine	
2	Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Routine	Cascade[b]	• Report following cascade reporting rules due to resistance to agent(s) in Tier 1. • May be reported routinely based on institution-specific guidelines.
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[c]	Routine or by request	Cascade[b]	Test routinely based on institution-specific guidelines or by clinician request and report following cascade reporting rules due to resistance to agent(s) in Tiers 1 and 2.

Antimicrobial Agent Test and Report Tiers and Additional Considerations for Agents Listed in Tables 1 (Continued)

Tier	Definition	Test	Report[a]	Additional Testing and Reporting Considerations
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	By request	By request	• Test and report by clinician request due to: – Unavailability of preferred drug for clinical use – Patient underlying condition, including allergies – Unusual susceptibility profile of the organism, including resistance to agents in Tiers 1, 2, and 3 – Polymicrobial infection • May also warrant testing as an epidemiological aid (eg, testing ceftazidime for Enterobacterales to indicate potential extended-spectrum ß-lactamase production; see Table 3A).
Urine only	Antimicrobial agents designated by a "(U)" in Tables 2 should be reported only on organisms isolated from the urinary tract.	Routine	Report as appropriate	Agents in Tiers 1, 2, and 3 may also be reported on urine isolates, as appropriate, following the testing and reporting guidance for the respective tiers.

Abbreviations: MDRO, multidrug-resistant organism; UTI, urinary tract infection.

Footnotes

a. Antimicrobial agents should be reported selectively, as appropriate (eg, because it is effective in treating uncomplicated UTIs only, nitrofurantoin would be reported only on isolates from urine). Refer to section D for definition of cascade reporting.

b. Identification of patients at high risk for MDROs will likely be communicated by infection preventionists. For examples of criteria used to identify patients at high risk for MDROs, see https://www.cdc.gov/hai/organisms/ESBL.html and https://www.cdc.gov/mrsa/community/index.html

Antimicrobial Agent Test and Report Designations and Additional Considerations for Agents Not Listed in Tables 1

Designation	Definition	Test	Report[a]	Additional Testing and Reporting Considerations
Other	Antimicrobial agents with established clinical breakpoints designated by an * in Tables 2 that are generally not candidates for testing and reporting in the United States	By request	By request	Test and report only by clinician request and only following consultation with the antimicrobial stewardship team and other relevant institutional stakeholders to ensure appropriateness of the request. Agents with an "Other" designation may not reflect current consensus recommendations for first-choice and alternative drugs for the specific organism or organism group.
Inv.	Antimicrobial agents that are investigational for the organism group designated by "Inv." in Tables 2 have not yet been approved by the FDA for use in the United States.	By request	By request	Test and report only by clinician request and only following consultation with the antimicrobial stewardship team and other relevant institutional stakeholders to ensure appropriateness of the request. These agents would likely be clinically available for compassionate use only.

Abbreviations: FDA, US Food and Drug Administration; UTI, urinary tract infection.

Footnote

a. Antimicrobial agents should be reported selectively, as appropriate (eg, because it is effective in treating uncomplicated UTIs only, nitrofurantoin would be reported only on isolates from urine).

D. Selective and Cascade Reporting

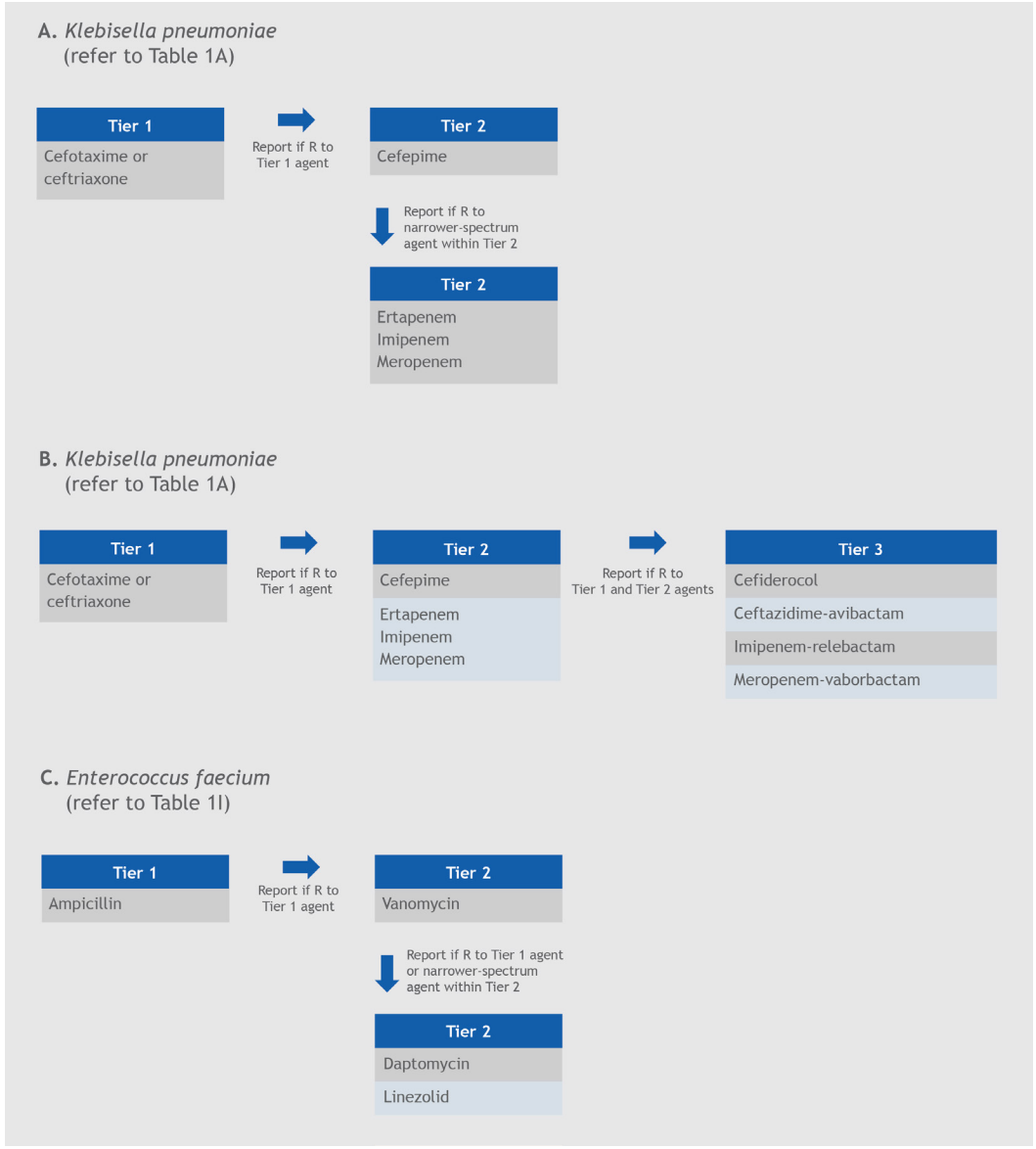
Each laboratory should **consider developing selective and/or cascade reporting rules in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. Selective and cascade reporting is done to encourage appropriate antimicrobial agent use. The positioning of drugs in Tables 1A through 1P can be used to guide development of selective and/or cascade reporting rules.**

Selective reporting involves reporting results for specific antimicrobial agents based on defined criteria unrelated to results obtained from antimicrobial susceptibility testing (AST) (eg, organism identification, body site, clinical setting, or patient demographics). For example, nitrofurantoin would be reported only on isolates from urine because it is effective in treating uncomplicated urinary tract infections (UTIs) only. Daptomycin is not reported for isolates recovered from the respiratory tract because it interacts with pulmonary surfactant, resulting in inhibition of antibacterial activity. First- and second-generation cephalosporins are not reported on *Salmonella* spp. because of their ineffectiveness in treating patients with *Salmonella* infections.

Cascade reporting involves reporting results for specific agents based on the overall antimicrobial susceptibility profile of an isolate. Results for secondary or broader-spectrum agents (eg, Tier 2 or 3) are reported only if the isolate is resistant to primary or narrower-spectrum agents (eg, Tier 1). For example, if a *Klebsiella pneumoniae* isolate is resistant to ceftriaxone, cefepime might be reported. However, cefepime might be suppressed in a ceftriaxone-susceptible *K. pneumoniae* isolate. A "resistant" result for a broader-spectrum agent (eg, Tier 2) should always be reported even if the organism tests "susceptible" to the narrower-spectrum agent (eg, Tier 1). Such unexpected resistant results should be confirmed (see Appendix A, footnote a).

Cascade rules can be created for agents within the same tier or between tiers. Agents listed in the same row between tiers in Tables 1A through 1P can be used as a guide for creating cascade reporting rules. For example, if a *K. pneumoniae* isolate is ceftriaxone resistant (Tier 1), cascade reporting can be initiated for cefepime and/or the carbapenems (Tier 2). If the *K. pneumoniae* isolate is resistant to ceftriaxone, cefepime, and a carbapenem, cascade reporting of cefiderocol, ceftazidime-avibactam, imipenem-relebactam, and/or meropenem-vaborbactam (Tier 3) may be considered (see Figure below, examples A and B). If an *Enterococcus faecium* isolate is ampicillin resistant (Tier 1) and vancomycin resistant (Tier 2), cascade reporting of daptomycin and linezolid (Tier 2) may be considered (see Figure below, example C).

Each laboratory should develop a protocol to **test additional agents on** isolates that are confirmed as resistant to all agents on **their** routine test panels. This protocol should include options for testing additional agents in-house or sending the isolate to a referral laboratory.



Abbreviation: R, resistant.

Cascade Reporting Examples. Cascade reporting within tiers (A, C) and between tiers (A, B, and C).

II. Breakpoint and Interpretive Category Definitions

A. Breakpoint Definition

breakpoint – minimal inhibitory concentration (MIC) or zone diameter value used to categorize an organism as susceptible, susceptible-dose dependent, intermediate, resistant, or nonsusceptible; **NOTE 1:** MIC or zone diameter values generated by a susceptibility test can be interpreted based on established breakpoints; **NOTE 2:** Because breakpoints are largely based on pharmacologically and clinically rich datasets using *in vitro* and *in vivo* data, they are considered robust predictors of likely clinical outcome; **NOTE 3:** Also known as "clinical breakpoint"; **NOTE 4:** See interpretive category.

B. Interpretive Category Definition

interpretive category – category derived from microbiological characteristics, pharmacokinetic/pharmacodynamic parameters, and clinical outcome data, when available; **NOTE 1:** MIC or zone diameter values generated by a susceptibility test can be interpreted based on established breakpoints; **NOTE 2:** See breakpoint.

EXAMPLE:

Interpretive Category	Breakpoints	
	MIC, µg/mL	Zone Diameter, mm
Susceptible	≤4	≥20
Susceptible-dose dependent	8-16	15-19
Intermediate	8-16	15-19
Resistant	≥32	≤14
Nonsusceptible	>1	<17

MIC or zone diameter value breakpoints and interpretive categories are established per CLSI document M23[4] for categories of susceptible, intermediate, and resistant (and susceptible-dose dependent and nonsusceptible, when appropriate).

- **susceptible (S)** – a category defined by a breakpoint that implies that isolates with an MIC at or below or a zone diameter at or above the susceptible breakpoint are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used, resulting in likely clinical efficacy.

- **susceptible-dose dependent (SDD)** – a category defined by a breakpoint that implies that susceptibility of an isolate depends on the dosage regimen that is used in the patient. To achieve levels that are likely to be clinically effective against isolates for which the susceptibility testing results (either MICs or zone diameters) are in the SDD category, it is necessary to use a dosage regimen (ie, higher doses, more frequent doses, or both, **or extended infusion**) that results in higher drug exposure than that achieved with the dose that was used to establish the susceptible breakpoint. Consideration should be given to the maximum, literature-supported dosage regimen, because higher exposure gives the highest probability of adequate coverage of an SDD isolate. Appendix E lists the doses used when establishing SDD categories. The drug label should be consulted for recommended doses and adjustment for organ function; **NOTE:** The SDD category may be assigned when doses well above those used to calculate the susceptible breakpoint are supported by the literature, widely used clinically, and/or approved and for which sufficient data to justify the designation exist and have been reviewed. This category also includes a buffer zone for inherent variability in test methods, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins. See Appendix F for additional information.

- **intermediate (I)** – a category defined by a breakpoint that includes isolates with MICs or zone diameters within the intermediate range that approach usually attainable blood and tissue levels and/or for which response rates may be lower than for susceptible isolates; **NOTE:** An I with a ^ in Tables 2 indicates agents that have the potential to concentrate in the urine. The I^ is for informational use only. The decision to report I^ is best made by each laboratory based on institution-specific guidelines and in consultation with appropriate medical personnel. The I category also includes a buffer zone for inherent variability in test methods, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

- **resistant (R)** – a category defined by a breakpoint that implies that isolates with an MIC at or above or a zone diameter at or below the resistant breakpoint are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs or zone diameters that fall in the range in which specific microbial resistance mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

- **nonsusceptible (NS)** – a category used for isolates for which only a susceptible breakpoint is designated because of the absence or rare occurrence of resistant strains. Isolates for which the antimicrobial agent MICs are above or the zone diameters are below the value indicated for the susceptible breakpoint should be reported as nonsusceptible; **NOTE 1:** An isolate that is interpreted as nonsusceptible does not necessarily mean that the isolate has a resistance mechanism. It is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution after the time the susceptible-only breakpoint was set; **NOTE 2:** The term "nonsusceptible" should not be used when the text is describing an organism/drug category with intermediate and resistant interpretive categories. Isolates that are in the categories of "intermediate" or "resistant" could be called "not susceptible" rather than "nonsusceptible."

C. Example of Breakpoints and Interpretive Categories as Used in Tables 2

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL		
		S	I[a]	R	S	I[a]	R
X	30 µg	≥ 20	15–19	≤ 14	≤ 4	8–16	≥ 32
Y	-	-	-	-	≤ 1	2	≥ 4
Z	10 µg	≥ 16	-	-	≤ 1	-	-

Abbreviations: I, intermediate; R, resistant; S, susceptible; SDD, susceptible-dose dependent.
[a] Or SDD, if appropriate.

For antimicrobial agent X with breakpoints in the table above, the susceptible breakpoint is ≤ 4 µg/mL or ≥ 20 mm and the resistant breakpoint is ≥ 32 µg/mL or ≤ 14 mm. For some antimicrobial agents (eg, antimicrobial agent Y), only MIC breakpoints may be available. For these agents, the disk diffusion zone diameters do not correlate with MIC values or data have not been evaluated as described in CLSI document M23.[4] Technical issues may also preclude the use of the disk diffusion method for some agents. For some antimicrobial agents (eg, antimicrobial agent Z) only a "susceptible" category exists. For these agents, the absence or rare occurrence of resistant strains precludes defining any results categories other than "susceptible." For strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed (see Appendix A). In examples Y and Z, a dash mark (-) indicates a disk is not available or that breakpoints are not applicable.

III. Reporting Results

A. Organisms Included in Tables 2

The MIC values determined as described in M07[2] may be reported directly to clinicians for patient care purposes. However, it is essential that an interpretive category result (S, SDD, I, R, or NS) also be provided routinely to facilitate understanding of the MIC report by clinicians. Zone diameter measurements without an interpretive category should not be reported. Recommended interpretive categories for various MIC and zone diameter values are included in tables for each organism group and are based on the evaluation of data as described in CLSI document M23.[4]

Laboratories should only report results for agents listed in Tables 2 specific to the organism being tested. It is not appropriate to apply disk diffusion or MIC breakpoints borrowed from a table in which the organism is not listed. There may be rare cases for which an agent may be appropriate for an isolate but for which there are no CLSI breakpoints (eg, tigecycline). In these cases, the FDA Susceptibility Test Interpretive Criteria (STIC) website (https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria) and the prescribing information document for the agent should be consulted.

For more information on reporting epidemiological cutoff values in the medical laboratory, see Appendix G.

B. Organisms Excluded From Tables 2

For some organism groups excluded from Tables 2A through 2J, CLSI document M45[5] provides suggestions for standardized methods for AST, including information about drug selection, interpretation, and QC. The organism groups covered in that guideline are *Abiotrophia* and *Granulicatella* spp. (formerly known as nutritionally deficient or nutritionally variant streptococci); *Aerococcus* spp.; *Aeromonas* spp. **(includes members of *A. caviae* complex, *A. hydrophila* complex, and *A. veronii* complex);** *Bacillus* spp. (not *Bacillus anthracis*); *Campylobacter jejuni/coli; Corynebacterium* spp. (including *Corynebacterium diphtheriae*); *Erysipelothrix rhusiopathiae; Gemella* spp.; the HACEK group: *Aggregatibacter* spp. (formerly *Haemophilus aphrophilus, Haemophilus paraphrophilus, Haemophilus segnis,* and *Actinobacillus actinomycetemcomitans*), *Cardiobacterium* spp., *Eikenella corrodens,* and *Kingella* spp.; *Helicobacter pylori; Lactobacillus* spp.; *Lactococcus* spp.; *Leuconostoc* spp.; *Listeria monocytogenes; Micrococcus* spp.; *Moraxella catarrhalis; Pasteurella* spp.; *Pediococcus* spp.; *Rothia mucilaginosa;* potential agents of bioterrorism; and *Vibrio* spp., including *Vibrio cholerae*.

For organisms other than those in the groups mentioned above, studies are not yet adequate to develop reproducible, definitive standards to interpret results. These organisms may need different media or different incubation atmospheres, or they may show marked strain-to-strain variation in growth rate. For these microorganisms, consultation with an infectious diseases specialist is recommended for guidance in determining the need for susceptibility testing and in results interpretation. Published reports in the medical literature and current consensus recommendations for therapy of uncommon microorganisms may preclude the need for testing. If necessary, a dilution method usually is the most appropriate testing method, and this may necessitate submitting the organism to a referral laboratory. Physicians should be informed of the limitations of results and advised to interpret results with caution.

C. Cumulative Antibiograms

Policies regarding the generation of cumulative antibiograms should be developed together with the antimicrobial stewardship team **and other relevant institutional stakeholders.** See CLSI document M39[6] for detailed instructions on generating cumulative antibiograms.

D. MIC Reporting Concentrations

When serial twofold dilution MICs are being prepared and tested, the actual dilution scheme is, eg:

16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 µg/mL, etc. (see Table 7 for additional dilutions).

For convenience only, not because these are the actual concentrations tested, it was decided to use the following values in M100: 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, 0.03 µg/mL, etc.

The values that appear in the tables are equivalent to the actual values tested, eg, 0.12 µg/mL = 0.125 µg/mL, and laboratories should report an MIC of ≤ 0.125 µg/mL as ≤ 0.12 µg/mL.

IV. Therapy-Related Comments and Dosage Regimens

Some comments in the tables relate to therapy concerns. These are denoted with an ***Rx*** symbol. It may be appropriate to include some of these comments (or modifications thereof) on the patient report. An example would be inclusion of a comment when rifampin is being reported stating that "Rifampin should not be used alone for antimicrobial therapy." Antimicrobial dosage regimens often vary widely among **specialists** and institutions. In some cases, the MIC breakpoints rely on pharmacokinetic/pharmacodynamic (PK/PD) data, using specific human dosage regimens. In cases in which specific dosage regimens are important for properly applying breakpoints, the dosage regimen is listed. These dosage regimen comments are not generally intended for use on individual patient reports.

V. Confirmation of Patient Results

Multiple test parameters are monitored by following the QC recommendations described in M100. However, acceptable results derived from testing QC strains do not guarantee accurate results when testing patient isolates. It is important to review all the results obtained from all drugs tested on a patient's isolate before reporting the results. This review should include but not be limited to ensuring that 1) the AST results are consistent with the identification of the isolate; 2) the results from individual agents within a specific drug class follow the established hierarchy of activity rules (eg, in general, third-generation cephems are more active than first- or second-generation cephems against Enterobacterales); and 3) the isolate is susceptible to those agents for which resistance has not been documented (eg, vancomycin and *Streptococcus* spp.) and for which only "susceptible" breakpoints exist in M100.

Unusual or inconsistent results should be confirmed by rechecking various testing parameters detailed in Appendix A. Each laboratory must develop its own policies for confirming unusual or inconsistent antimicrobial susceptibility test results. The list provided in Appendix A emphasizes results that are most likely to affect patient care.

VI. Development of Resistance and Testing of Repeat Isolates

Isolates that are initially susceptible may become intermediate or resistant after therapy is initiated. Therefore, subsequent isolates of the same species from a similar anatomical site should be tested to detect resistance that may have developed. Development of resistance can occur within as little as three to four days and has been noted most frequently in *Enterobacter* (including *Klebsiella* [formerly *Enterobacter*] *aerogenes*), *Citrobacter,* and *Serratia* spp. with third-generation cephalosporins, in *P. aeruginosa* with all antimicrobial agents, and in staphylococci with fluoroquinolones. For *S. aureus,* vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy.

In certain circumstances, the decision to perform susceptibility tests on subsequent isolates necessitates knowledge of the specific situation and the severity of the patient's condition (eg, an isolate of *E. cloacae* complex from a blood culture on a premature infant or methicillin (oxacillin)-resistant *S. aureus* [MRSA] from a patient with prolonged bacteremia). Laboratory guidelines on when to perform susceptibility testing on repeat isolates should be determined after consultation with the medical staff.

VII. Warning

Some of the comments in the tables relate to dangerously misleading results that can occur when certain antimicrobial agents are tested and reported as susceptible against specific organisms. These are denoted with the word **"Warning."**

Locations	Organisms	Antimicrobial Agents
"Warning": The following antimicrobial agent–organism combinations may appear active *in vitro* but are not effective clinically and must not be reported as susceptible.		
Table 2A	*Salmonella* spp., *Shigella* spp.	First- and second-generation cephalosporins, cephamycins, and aminoglycosides
Table 2D	*Enterococcus* spp.	Aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole
"Warning": Do not report the following antimicrobial agents for bacteria isolated from CSF. These are not the drugs of choice and may not be effective for treating CSF infections caused by the bacteria included in Tables 2A through 2J:		
Tables 2A through 2J	Bacteria isolated from CSF	Agents administered by oral route only, first- and second-generation cephalosporins and cephamycins, doripenem, ertapenem, imipenem, clindamycin, lefamulin, macrolides, tetracyclines, and fluoroquinolones

Abbreviation: CSF, cerebrospinal fluid.

VIII. Routine, Supplemental, Screening, Surrogate Agent, and Equivalent Agent Testing to Determine Susceptibility and Resistance to Antimicrobial Agents

The testing categories are defined as follows:

- **Routine test:** disk diffusion or broth or agar dilution MIC tests for routine clinical testing

- **Supplemental (not routine) test:** test that detects susceptibility or resistance to a drug or drug class by method other than routine disk diffusion or broth or agar dilution MIC and does not need additional tests to confirm susceptibility or resistance
  - Some supplemental tests identify a specific resistance mechanism and may be required or optional for reporting specific clinical results.

- **Screening test:** test that provides presumptive results; additional testing typically only needed for a specific result (eg, only if screen is positive)

- **Surrogate agent test:** test performed with an agent that replaces a test performed with the antimicrobial agent of interest and is used when the agent of interest cannot be tested due to unavailability of the agent or performance issues (eg, surrogate agent performs better than the agent of interest)

- **Equivalent agent test:** test performed with an agent that predicts results of closely related agents of the same class and increases efficiency by limiting testing of multiple closely related agents. Equivalent agents are identified by:
  - Listing equivalent agents with an "or" in Tables 1 and 2. "Or" indicates cross-susceptibility and cross-resistance is nearly complete (very major error + major error < 3%; minor error < 10%) and only one agent needs to be tested.

  - Listing agents that are equivalent and results that can be deduced by testing the equivalent agent in a comment (see Tables 1 and 2).

The following tables include tests that fall into the supplemental, screening, surrogate agent, and equivalent agent test categories. The tables for supplemental, screening, and surrogate agent tests are comprehensive. The table for equivalent agent tests includes several examples, and many other equivalent agent tests are described throughout Tables 1 and 2.

Supplemental Tests (Required)

Supplemental Test	Organisms	Test Description	Required for:	Table Location
Inducible clindamycin resistance	• *Staphylococcus* spp. • *S. pneumoniae* • *Streptococcus* spp. Β-hemolytic group	Broth microdilution or disk diffusion with clindamycin and erythromycin tested together	Isolates that test erythromycin resistant and clindamycin susceptible or intermediate before reporting the isolate as clindamycin susceptible	3I
Β-lactamase	*Staphylococcus* spp.	Chromogenic cephalosporin (all staphylococci), penicillin disk diffusion zone-edge test (*S. aureus* only)	Isolates that test penicillin susceptible before reporting the isolate as penicillin susceptible	3F

Supplemental Tests (Optional)

Supplemental Test	Organisms	Test Description	Optional for:	Table Locations
ESBL	• *E. coli* • *K. pneumoniae* • *Klebsiella oxytoca* • *Proteus mirabilis*	Broth microdilution or disk diffusion clavulanate inhibition test for ESBLs	Isolates demonstrating reduced susceptibility to cephalosporins Results that indicate presence or absence of ESBLs	3A
CarbaNP	• Enterobacterales • *P. aeruginosa*	Colorimetric assay for detecting carbapenem hydrolysis	Isolates demonstrating reduced susceptibility to carbapenems Results that indicate presence or absence of certain carbapenemases	3B
mCIM with or without eCIM	• mCIM only: Enterobacterales and *P. aeruginosa* • mCIM with eCIM: Enterobacterales only	Disk diffusion for detecting carbapenem hydrolysis (inactivation) eCIM add-on enables differentiation of metallo-β-lactamases from serine carbapenemases in Enterobacterales isolates that are positive for mCIM	Isolates demonstrating reduced susceptibility to carbapenems Results that indicate presence or absence of certain carbapenemases	3C
Colistin agar test	• Enterobacterales • *P. aeruginosa*	Modified agar dilution	Determining the colistin MIC	3D
Colistin broth disk elution	• Enterobacterales • *P. aeruginosa*	Tube dilution using colistin disks as antimicrobial agent source	Determining the colistin MIC	3D
Oxacillin salt agar	*S. aureus*	Agar dilution; MHA with 4% NaCl and 6 µg/mL oxacillin	Detecting MRSA; see cefoxitin surrogate agent tests, which are preferred	3G-1

Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; ESBL, extended-spectrum β-lactamase; mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRSA, methicillin (oxacillin)-resistant *Staphylococcus aureus*.

Screening Tests

Screening Test	Organisms	Test Description	When to Perform Confirmatory Test	Confirmatory Test	Table Location
Vancomycin agar screen	• *S. aureus* • *Enterococcus* spp.	Agar dilution; BHI with 6 µg/mL vancomycin	If screen positive	Vancomycin MIC	3H
HLAR by disk diffusion	*Enterococcus* spp.	Disk diffusion with gentamicin and streptomycin	If screen inconclusive	Broth microdilution, agar dilution MIC	3K

Abbreviations: BHI, brain heart infusion; HLAR, high-level aminoglycoside resistance; MIC, minimal inhibitory concentration.

Surrogate Agent Tests

Surrogate Agent	Organisms	Test Description	Results	Table Locations
Cefazolin	• *E. coli* • *K. pneumoniae* • *P. mirabilis*	Broth microdilution or disk diffusion	When used for therapy of uncomplicated UTIs, predicts results for the following oral antimicrobial agents: cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef Cefazolin tested as a surrogate may overcall resistance to cefdinir, cefpodoxime, and cefuroxime. If cefazolin tests resistant, test these drugs individually if needed for therapy.	1A, 2A
Cefoxitin	• *S. aureus* • *S. lugdunensis* • *S. epidermidis* • Other *Staphylococcus* spp. (except *S. pseudintermedius* and *S. schleiferi*)	Broth microdilution: *S. aureus* *S. lugdunensis* Disk diffusion: *S. aureus* *S. lugdunensis* Other *Staphylococcus* spp., excluding *S. pseudintermedius* and *S. schleiferi*	Predicts results for *mecA*-mediated methicillin (oxacillin) resistance.	**1H**, 2C, 3G-1, 3G-2
Oxacillin	*S. pneumoniae*	Disk diffusion	Predicts penicillin susceptibility if oxacillin zone is ≥ 20 mm. If oxacillin zone is ≤ 19 mm, penicillin MIC must be performed.	**1L**, 2G
Pefloxacin	*Salmonella* spp.	Disk diffusion	Predicts reduced susceptibility to ciprofloxacin	2A

Abbreviations: MIC, minimal inhibitory concentration; PBP2a, penicillin-binding protein 2a; UTI, urinary tract infection.

Examples of Equivalent Agent Tests

Agents	Organisms	Identified by	Table Locations
Cefotaxime or ceftriaxone	Enterobacterales	"Or"	1A and 2A
Colistin or polymyxin B	Enterobacterales, *P. aeruginosa*, *Acinetobacter baumannii* complex	"Or"	2A, 2B-1, and 2B-2
Azithromycin or clarithromycin or erythromycin	*Staphylococcus* spp.	"Or"	1**H** and 2C
Penicillin-susceptible staphylococci are susceptible to other β-lactam agents with established clinical efficacy for staphylococcal infections (including both penicillinase-labile and penicillinase-stable agents; see Glossary I). Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins.	*Staphylococcus* spp.	Note listed	1**H** and 2C
The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin.	*Haemophilus* spp.	Note listed	1**J** and 2E
The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin.	Anaerobes	Note listed	2J

IX. Quality Control and Verification

Recommendations for QC are included in various tables and appendixes. Acceptable ranges for QC strains are provided in Tables 4A-1 through 4B for disk diffusion and Tables 5A-1 through 5E for MIC testing. Guidance for QC frequency and modifications of AST systems is found in Table 4C for disk diffusion and Table 5F for MIC testing. Guidance for troubleshooting out-of-range results is included in Table 4D for disk diffusion and Table 5G for MIC testing. Additional information is available in Appendix C (eg, QC organism characteristics, QC testing recommendations).

Implementing any new diagnostic test requires verification.[7] Each laboratory that introduces a new AST system or adds a new antimicrobial agent to an existing AST system must verify or establish that, before reporting patient test results, the system meets performance specifications for that system. Verification generally involves testing patient isolates with the new AST system and comparing results to those obtained with an established reference method or a system that has been previously verified. Testing patient isolates may be done concurrently with the two systems. Alternatively, organisms with known MICs or zone sizes may be used for the verification. Guidance on verification studies is not included in this document. Other publications describe AST system verification (eg, CLSI document M52[8] and Patel J, et al.[9]).

X. Abbreviations and Acronyms

AST	antimicrobial susceptibility testing
ATCC®[a]	American Type Culture Collection
BHI	brain heart infusion
BLNAR	β-lactamase negative, ampicillin-resistant
BMHA	blood Mueller-Hinton agar
BSC	biological safety cabinet
BSL-2	biosafety level 2
BSL-3	biosafety level 3
CAMHB	cation-adjusted Mueller-Hinton broth
CAMHB-HSD	**cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4)**
CAT	colistin agar test
CBDE	colistin broth disk elution
CFU	colony-forming unit(s)
CMRNG	chromosomally mediated penicillin-resistant *Neisseria gonorrhoeae*
CSF	cerebrospinal fluid
DMSO	dimethyl sulfoxide
DTT	**DL-dithiothreitol**
ECV	epidemiological cutoff value
eCIM	EDTA-modified carbapenem inactivation method
EDTA	ethylenediaminetetraacetic acid
ESBL	extended-spectrum β-lactamase
FDA	US Food and Drug Administration
HLAR	high-level aminoglycoside resistance
HTM	*Haemophilus* test medium
I	intermediate
ICR	inducible clindamycin resistance
IM	intramuscular
ID	identification
LHB	lysed horse blood
mCIM	modified carbapenem inactivation method

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

MDRO	**multidrug-resistant organism**
MHA	Mueller-Hinton agar
MH-F agar	Mueller-Hinton fastidious agar
MH-F broth	**Mueller-Hinton fastidious broth**
MHB	Mueller-Hinton broth
MIC	minimal inhibitory concentration
MRS	methicillin (oxacillin)-resistant staphylococci
MRSA	methicillin (oxacillin)-resistant *Staphylococcus aureus*
NAD	ß-nicotinamide adenine dinucleotide
NCTC	National Collection of Type Cultures
NS	nonsusceptible
NWT	non-wild-type
PBP2a	penicillin-binding protein 2a
PCR	polymerase chain reaction
PK/PD	pharmacokinetic/pharmacodynamic
pH	negative logarithm of hydrogen ion concentration
QC	quality control
R	resistant
S	susceptible
SDD	susceptible-dose dependent
TSA	tryptic soy agar
TSB	trypticase soy broth
UTI	urinary tract infection
WT	wild-type

References

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*. 9th ed. CLSI standard M11. Clinical and Laboratory Standards Institute; 2018.

4 CLSI. *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*. 5th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute; 2018.

5 CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*. 3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute; 2016.

6 CLSI. *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data*. 5th ed. CLSI guideline M39. Clinical and Laboratory Standards Institute; 2022.

7 Centers for Medicare & Medicaid Services, US Department of Health and Human Services. *Part 493—Laboratory Requirements; Standard: Establishment and verification of performance specifications* (Codified at 42 CFR §493.1253). Office of the Federal Register; published annually.

8 CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.

9 Patel J, Sharp S, Novak-Weekley S. Verification of antimicrobial susceptibility testing methods: a practical approach. *Clin Microbiol Newslett*. 2013;35(13):103-109.

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Introduction to Tables 1A-1P. Antimicrobial Agents That Should Be Considered for Testing and Reporting by Microbiology Laboratories

Selecting the most appropriate antimicrobial agents to test and report is a decision best made by each laboratory in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. The suggestions in these tables:

- **Include agents approved by the US Food and Drug Administration for clinical use**
- **Are directed toward medical laboratories in the United States but may be appropriate in other settings**
- **Are based on the understanding that patient-specific factors (eg, age, body site) or organism-specific factors (eg, overall antimicrobial susceptibility profile) must be considered for testing and reporting of any individual agent**
- **Need to be considered with institutional guidelines when used to develop a laboratory's testing and reporting protocols**

Please review the Instructions for Use of Tables and Section I, Selecting Antimicrobial Agents for Testing and Reporting, for additional guidance regarding antimicrobial agent testing and reporting decisions, including the use of cascade and selective reporting strategies.

"Warning": Do not report the following antimicrobial agents for bacteria isolated from CSF. These are not the drugs of choice and may not be effective for treating CSF infections caused by the bacteria included in Tables 2A through 2J:

- **Agents administered by oral route only**
- **First- and second-generation cephalosporins and cephamycins**
- **Doripenem, ertapenem, and imipenem**
- **Clindamycin**
- **Lefamulin**
- **Macrolides**
- **Tetracyclines**
- **Fluoroquinolones**

Refer to Glossary I for individual agents within the drug classes listed above.

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Table 1A. Enterobacterales (not including inducible AmpC producers and *Salmonella/Shigella*)[a]

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			
Cefazolin	Cefuroxime		
Cefotaxime or ceftriaxone[b]	Cefepime[c]		
	Ertapenem Imipenem Meropenem	Cefiderocol	
		Ceftazidime-avibactam	
		Imipenem-relebactam	
		Meropenem-vaborbactam	
Amoxicillin-clavulanate Ampicillin-sulbactam			
Piperacillin-tazobactam			
Gentamicin	Tobramycin	Plazomicin	
	Amikacin		
Ciprofloxacin Levofloxacin			
Trimethoprim-sulfamethoxazole			
	Cefotetan Cefoxitin		
	Tetracycline[d]		
			Aztreonam
			Ceftaroline[b]
			Ceftazidime[b]
			Ceftolozane-tazobactam
Urine Only			
Cefazolin (surrogate for uncomplicated UTI)[e]			
Nitrofurantoin			
		Fosfomycin[f] (*Escherichia coli*)	

Abbreviations: MDRO, multidrug-resistant organism; UTI, urinary tract infection.

Table 1A. Enterobacterales (Continued)

Footnotes

a. See Appendix B for species-specific intrinsic resistance profiles. If an antimicrobial agent-organism combination that is defined as intrinsically resistant is tested, the result hould be reported as resistant. Consideration may be given to adding comments regarding intrinsic resistance of agents not tested.

b. *Citrobacter freundii* complex, *Enterobacter cloacae* complex, *Hafnia alvei*, *Klebsiella* (formerly *Enterobacter) aerogenes*, *Morganella morganii*, *Providencia* spp., *Serratia marcescens*, and *Yersinia enterocolitica* may test susceptible to ceftriaxone, cefotaxime, ceftazidime, and ceftaroline, but these agents may be ineffective against these genera within a few days after initiation of therapy due to derepression of inducible AmpC β-lactamase. The risk of AmpC derepression during therapy is moderate to high with *C. freundii* complex, *E. cloacae complex*, and *K. aerogenes* and appears to be less frequent with *M. morganii*, *Providencia* spp., and *S. marcescens*.[1] Therefore, isolates that are initially susceptible may become resistant. Testing subsequent isolates may be warranted if clinically indicated.

c. Cefepime should be considered a Tier 1 agent for testing and/or reporting of *C. freundii* complex, *E. cloacae* complex, *H. alvei*, *K. aerogenes*, *M. morganii*, *Providencia* spp., *S. marcescens*, and *Y. enterocolitica* (see footnote b).[1]

d. 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 or minocycline, or both.

e. See cefazolin comments in Table 2A for using cefazolin as a surrogate test for oral cephalosporins and for reporting cefazolin when used for therapy in uncomplicated UTIs.

f. Report only on *E. coli* isolated from the urinary tract.

NOTE: Information in black boldface type is new or modified since the previous edition.

Reference for Table 1A

[1] Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. IDSA Guidance on the treatment of antimicrobial-restant gram-negative infections: version 2.0. Infectious Diseases Society of America; 2022. Accessed 10 January 2023. https://www.idsociety.org/practice-guideline/amr-guidance-2.0/

Table 1B. *Salmonella* and *Shigella* spp.[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
Ampicillin			
Ciprofloxacin Levofloxacin			
Trimethoprim-sulfamethoxazole			
Cefotaxime or ceftriaxone			Ertapenem[c] Imipenem[c] Meropenem[c]
	Azithromycin[d]		
			Tetracycline[e]

Abbreviation: MDRO, multidrug-resistant organism.

Footnotes

a. Table 2A should be used for interpreting antimicrobial susceptibility testing results for *Salmonella* and *Shigella* spp.

b. WARNING: For *Salmonella* spp. and *Shigella* spp., aminoglycosides, first- and second-generation cephalosporins, and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible. Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. However, susceptibility testing is indicated for all *Shigella* isolates. When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. In addition, for extraintestinal isolates of *Salmonella* spp., a third-generation cephalosporin should be tested and reported. Azithromycin may be tested and reported per institutional guidelines.

c. Ertapenem, imipenem, and/or meropenem might be considered for testing and/or reporting for isolates resistant to all agents in Tiers 1 and 2, although there are limited clinical data suggesting their effectiveness for treating salmonellosis or shigellosis.[1]

d. Report only on *Salmonella enterica* ser. Typhi and *Shigella* spp.

e. 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.

NOTE: Information in black boldface type is new or modified since the previous edition.

Reference for Table 1B

1 CDC Health Alert Network. Extensively drug-resistant *Salmonella* typhi infections among US residents without international travel. Accessed 10 January 2023. http://emergency.cdc.gov/han/2021/han00439.asp

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Table 1C. *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.

NOTE: Information in black boldface type is new or modified since the previous edition.

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Table 1D. *Acinetobacter* spp.

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-sulbactam			
Ceftazidime	Imipenem	Cefiderocol	
Cefepime	Meropenem		
Ciprofloxacin Levofloxacin			
Gentamicin Tobramycin	Amikacin		
	Piperacillin-tazobactam		
	Trimethoprim-sulfamethoxazole		
	Minocycline		Doxycycline
			Cefotaxime Ceftriaxone
			Colistin or polymyxin B
Urine only			
Tetracycline[a]			

Abbreviation: MDRO, multidrug-resistant organism.

Footnote

a. 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.

NOTE: Information in black boldface type is new or modified since the previous edition.

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Table 1E. *Burkholderia cepacia* complex

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			
Meropenem			
Levofloxacin[a]			
Minocycline			
Trimethoprim-sulfamethoxazole			

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

Footnote

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

NOTE: Information in black boldface type is new or modified since the previous edition.

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Table 1F. *Stenotrophomonas maltophilia*

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
Levofloxacin			
Minocycline			
Trimethoprim-sulfamethoxazole			
		Cefiderocol	
			Ceftazidime[a]

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

Footnote

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

NOTE: Information in black boldface type is new or modified since the previous edition.

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Table 1G. 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.

NOTE: Information in black boldface type is new or modified since the previous edition.

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Table 1H. *Staphylococcus* spp.

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
Azithromycin or clarithromycin or erythromycin[a]			
Clindamycin[a]			
Oxacillin[b,c,d,e] Cefoxitin[b,c,d] (surrogate for oxacillin)		Ceftaroline[f]	
Doxycycline Minocycline[a] Tetracycline[g]			
Trimethoprim-sulfamethoxazole			
Vancomycin[h]			
	Penicillin [b,i]		
	Daptomycin[h,j]		
	Linezolid	Tedizolid[f]	
		Rifampin[h,k]	
		Lefamulin[a,f]	
			Ciprofloxacin or levofloxacin Moxifloxacin
			Dalbavancin[f,h]
			Oritavancin[f,h]
			Telavancin[f,h]
			Gentamicin[l]
Urine Only			
Nitrofurantoin			

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

Table 1H. *Staphylococcus* spp. (Continued)

Footnotes

a. Not routinely reported on organisms isolated from the urinary tract.

b. Penicillin-susceptible staphylococci are also susceptible to other β-lactam agents with established clinical efficacy for staphylococcal infections. Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins. Methicillin (oxacillin)-resistant staphylococci are resistant to all currently available β-lactam antimicrobial agents, with the exception of ceftaroline. Thus, susceptibility or resistance to a wide array of β-lactam antimicrobial agents may be determined from testing only penicillin and either cefoxitin or oxacillin. Routine testing of other β-lactam agents, except ceftaroline, is not advised.

c. If a penicillinase-stable penicillin is tested, oxacillin is the preferred agent, and results can be applied to the other penicillinase-stable penicillins (refer to Glossary I). Detection of methicillin (oxacillin) resistance in staphylococci is achieved by using specific methods, as described in Tables 2C, 3G-1, and 3G-2.

d. See oxacillin and cefoxitin comments in Table 2C for using cefoxitin as a surrogate test for oxacillin.

e. For *S. aureus*, *S. lugdunensis*, and other *Staphylococcus* spp. (except *S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi*), only MIC testing, not disk diffusion testing, is acceptable; see exceptions in Table 2C.

f. For *S. aureus* only, including methicillin (oxacillin)-resistant *S. aureus*.

g. 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.

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

i. If penicillin is tested, report results when confirmed susceptible (see Table 2C comment [11], and Table 3F).

j. Not routinely reported on organisms isolated from the respiratory tract.

k. *Rx*: Rifampin should not be used alone for antimicrobial therapy.

l. For staphylococci that test susceptible, gentamicin is used only in combination with other active agents that test susceptible.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 1I. *Enterococcus* spp.

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] Penicillin[b]			
	Vancomycin		
	Gentamicin[c] (high-level resistance testing only)	Streptomycin[c] (high-level resistance testing only)	
	Daptomycin[d,e]		
	Linezolid	Tedizolid	
			Dalbavancin[d,f]
			Oritavancin[d,f]
			Telavancin[d,f]
Urine only			
Nitrofurantoin			
	Ciprofloxacin Levofloxacin		
		Fosfomycin[g]	
		Tetracycline[h]	

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

Footnotes

a. The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. Ampicillin results may be used to predict susceptibility to amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam among non-ß-lactamase-producing enterococci. Ampicillin susceptibility can be used to predict imipenem susceptibility, provided the species is confirmed to be *Enterococcus faecalis*.

b. Enterococci susceptible to penicillin are predictably susceptible to ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin-tazobactam for non-ß-lactamase-producing enterococci. However, enterococci susceptible to ampicillin cannot be assumed to be susceptible to penicillin. If penicillin results are needed, testing of penicillin is required. *Rx*: Combination therapy with high-dosage parenteral ampicillin, amoxicillin, penicillin, or vancomycin (for susceptible strains only) plus an aminoglycoside is usually indicated for serious enterococcal infections such as endocarditis, unless high-level resistance to both gentamicin and streptomycin is documented; such combinations are predicted to result in synergistic killing of enterococci.

c. See additional testing and reporting information in Table 3K.

Table 1I. *Enterococcus* spp. (Continued)

d. **MIC testing only; disk diffusion test is unreliable.**

e. **Not routinely reported on organisms isolated from the respiratory tract.**

f. **Report only on vancomycin-susceptible *E. faecalis*.**

g. **Report only on *E. faecalis* urinary tract isolates.**

h. 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.

WARNING: For *Enterococcus* spp., aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole may appear active *in vitro*, but they are not effective clinically, and isolates should not be reported as susceptible.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 1J. *Haemophilus influenzae* and *Haemophilus parainfluenzae*

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]	Cefotaxime or ceftazidime or ceftriaxone[a]	Meropenem[a]	Ertapenem or imipenem
	Ampicillin-sulbactam Amoxicillin-clavulanate[c]		
	Ciprofloxacin or levofloxacin or moxifloxacin		
	Trimethoprim-sulfamethoxazole		
			Azithromycin[c] Clarithromycin[c]
			Aztreonam
			Cefaclor[c] Cefprozil[c]
			Cefdinir or cefixime or cefpodoxime[c]
			Ceftolozane-tazobactam[d]
			Ceftaroline[d]
			Cefuroxime[c]
			Lefamulin[d]
			Rifampin[e]
			Tetracyline[f]

Abbreviations: CSF, cerebrospinal fluid; MDRO, multidrug-resistant organism.

Table 1J. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Footnotes

a. For isolates of *H. influenzae* from CSF, only results of testing with ampicillin, any of the third-generation cephalosporins listed, and meropenem are appropriate to report.

b. The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. The majority of *H. influenzae* isolates that are resistant to ampicillin and amoxicillin produce a TEM-type ß-lactamase. In most cases, a ß-lactamase test can provide a rapid means of detecting resistance to ampicillin and amoxicillin.

c. Amoxicillin-clavulanate, azithromycin, cefaclor, cefdinir, cefixime, cefpodoxime, cefprozil, cefuroxime, and clarithromycin are used as empiric therapy for respiratory tract infections due to *Haemophilus* spp. The results of susceptibility tests with these antimicrobial agents are often not necessary for managing individual patients.

d. Report only on *H. influenzae*.

e. May be appropriate only for prophylaxis of case contacts. Refer to Table 2E.

f. Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 1K. *Neisseria gonorrhoeae*[a]

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
Azithromycin			
Ceftriaxone **Cefixime**			
Ciprofloxacin			
Tetracycline			

Abbreviation: MDRO, multidrug-resistant organism.

Footnote

a. **Culture and susceptibility testing of *N. gonorrhoeae* should be considered in cases of treatment failure. Antimicrobial agents recommended for testing include, at a minimum, the agents listed in Tier 1. The most current guidelines for treatment and testing are available from the Centers for Disease Control and Prevention.[1]**

NOTE: Information in black boldface type is new or modified since the previous edition.

Reference for Table 1K

[1] **Centers for Disease Control and Prevention. Gonorrhea: CDC basic fact sheet. Accessed 10 January 2023. http://cdc.gov/std/gonorrhea/stdfact-gonorrhea.htm**

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Table 1L. *Streptococcus pneumoniae*

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
Erythromycin[a,b]			
Penicillin[c]			Amoxicillin[d] Amoxicillin-clavulanate[d]
Trimethoprim-sulfamethoxazole			
Cefotaxime[c,d] Ceftriaxone[c,d]			Cefepime[d]
			Ceftaroline
	Meropenem[c,d]		Ertapenem[d] Imipenem[d]
	Clindamycin[b]		
	Doxycycline Tetracycline[e]		
	Levofloxacin[f] Moxifloxacin[f]		
	Vancomycin[c]		
			Lefamulin[b]
			Linezolid
			Cefuroxime[d]
			Rifampin[g]

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

Table 1L. *Streptococus pneumoniae* (Continued)

Footnotes

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

b. Not routinely reported on organisms isolated from the urinary tract.

c. Penicillin and cefotaxime, ceftriaxone, or meropenem should be tested by a reliable MIC method (such as that described in M07[1]) and reported routinely with *S. pneumoniae* isolated from CSF. Such isolates can also be tested against vancomycin using the MIC or disk diffusion method. With isolates from other sites, the oxacillin disk test may be used. If the oxacillin zone size is ≤ 19 mm, cefotaxime, ceftriaxone, meropenem, or penicillin MICs should be determined.

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

e. 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.

f. Organisms that are susceptible to levofloxacin are also considered susceptible to gemifloxacin and moxifloxacin. However, some organisms that are intermediate or resistant to levofloxacin may be susceptible to gemifloxacin, moxifloxacin, or both.

g. *Rx*: Rifampin should not be used alone for antimicrobial therapy.

NOTE: Information in black boldface type is new or modified since the previous edition.

Reference for Table 1L

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

Table 1M. *Streptococcus* spp. ß-Hemolytic 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
Clindamycin[a,b]			
Erythromycin[a,b,c]			
Penicillin[d] or ampicillin[d]		Cefotaxime or ceftriaxone	Cefepime
			Ceftaroline
	Tetracycline[e]		
		Vancomycin	
			Linezolid
			Tedizolid[f]
			Daptomycin[f,g,h]
			Levofloxacin
			Dalbavancin[h,i]
			Oritavancin[h]
			Telavancin[h]

Abbreviations: ICR, inducible clindamycin resistance; MDRO, multidrug-resistant organism.

Footnotes

a. Not routinely reported for organisms isolated from urinary tract.

b. *Rx:* Recommendations for intrapartum prophylaxis for group B streptococci are penicillin or ampicillin. Although cefazolin is recommended for penicillin-allergic women at low risk for anaphylaxis, those at high risk for anaphylaxis may receive clindamycin. Group B streptococci are susceptible to ampicillin, penicillin, and cefazolin but may be resistant to erythromycin and clindamycin. When a group B *Streptococcus* is isolated from a pregnant woman with severe penicillin allergy (high risk for anaphylaxis), erythromycin and clindamycin (including ICR) should be tested and only clindamycin should be reported. Erythromycin should be tested for ICR determination but should not be reported. See Table 3I.

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

d. Penicillin and ampicillin are drugs of choice for treating ß-hemolytic streptococcal infections. Susceptibility testing of penicillins and other ß-lactams approved by the US Food and Drug Administration for treatment of ß-hemolytic streptococcal infections does not need to be performed routinely, because nonsusceptible isolates (ie, penicillin MICs > 0.12 and ampicillin MICs > 0.25 µg/mL) are extremely rare in any ß-hemolytic streptococci and have not been reported for *S. pyogenes*. If testing is performed, any ß-hemolytic streptococcal isolate found to be nonsusceptible should be re-identified, retested, and if confirmed, submitted to a public health laboratory (see Appendix A for additional instructions).

Table 1M. *Streptococcus* spp. ß-Hemolytic Group (Continued)

e. 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.

f. Report only on *S. pyogenes* and *S. agalactiae*.

g. Not routinely reported on organisms isolated from the respiratory tract.

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

i. Report only on *S. pyogenes*, *S. agalactiae*, and *S. dysgalactiae*.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 1N. *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 (includes *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.

NOTE: Information in black boldface type is new or modified since the previous edition.

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Table 10. Gram-Negative Anaerobes

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
Amoxicillin-clavulanate **Ampicillin-sulbactam** **Piperacillin-tazobactam**			
Clindamycin			
Ertapenem **Imipenem**[a] **Meropenem**			**Imipenem-relebactam**
Metronidazole			
			Penicillin[b] **Ampicillin**
			Cefotetan **Cefoxitin**
			Ceftriaxone
			Moxifloxacin

Abbreviation: MDRO, multidrug-resistant organism.

Footnotes

a. Organisms that test susceptible to imipenem are also considered susceptible to imipenem-relebactam. However, organisms that test susceptible to imipenem-relebactam cannot be assumed to be susceptible to imipenem.

b. Penicillin retains good *in vitro* activity against most *Fusobacterium* spp. and may be considered for primary testing and reporting with this genus.

NOTE 1: Most anaerobic infections are polymicrobial, including both ß-lactamase-positive and ß-lactamase-negative strains. Testing may not be necessary for isolates associated with polymicrobial anaerobic infections. However, if susceptibility testing is requested, only the organism most likely to be resistant (eg, *Bacteroides* spp. and *Parabacteroides* spp.) should be tested and results reported (see Appendix D).

NOTE 2: Information in black boldface type is new or modified since the previous edition.

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Table 1P. Gram-Positive Anaerobes

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] Penicillin[a]			
Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam			
Clindamycin			
Ertapenem Imipenem[b] Meropenem			Imipenem-relebactam
Metronidazole[c]			
			Cefotetan Cefoxitin
			Ceftriaxone
			Moxifloxacin
			Tetracycline

Abbreviation: MDRO, multidrug-resistant organism.

Footnotes

a. If ß-lactamase positive, report as resistant to ampicillin and penicillin. ß-lactamase-negative isolates may be resistant to ampicillin and penicillin by other mechanisms.

b. Organisms that test susceptible to imipenem are also considered susceptible to imipenem-relebactam. However, organisms that test susceptible to imipenem-relebactam cannot be assumed to be susceptible to imipenem.

c. Many non-spore-forming, gram-positive anaerobic rods are resistant to metronidazole (see Appendix D).

NOTE 1: Most anaerobic infections are polymicrobial, including both ß-lactamase-positive and ß-lactamase-negative strains. Testing may not be necessary for isolates associated with polymicrobial anaerobic infections. However, if susceptibility testing is requested, only the organism most likely to be resistant (eg, *Finegoldia magna*) should be tested and results reported (see Appendix D).

NOTE 2: Specific *Clostridium* spp. (eg, *Clostridium septicum*, *Paeniclostridium sordellii*) may be the singular cause of infection and are typically susceptible to penicillin and ampicillin. Penicillin and clindamycin resistance have been reported in *Clostridium perfringens*. Agents in Tier 1 of Table 1P should be tested and reported for *Clostridium* spp.

NOTE 3: Information in black boldface type is new or modified since the previous edition.

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Table 2A. Zone Diameter and MIC Breakpoints for Enterobacterales

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix I)[1]
Agar dilution: MHA

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [6]).

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours
Dilution methods: 16–20 hours

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®[a] 25922
Pseudomonas aeruginosa ATCC® 27853 (for carbapenems)
Staphylococcus aureus ATCC® 25923 (for disk diffusion) or *S. aureus* ATCC® 29213 (for dilution methods) when testing azithromycin against *Salmonella enterica* ser. Typhi *or Shigella* spp.
Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

Refer to Tables 3A, 3B, and 3C for additional testing, reporting, and QC for Enterobacterales.

General Comments

(1) Refer to Tables 1A-1B for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[2] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see the *M02 Disk Diffusion Reading Guide*[3]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., ignore the thin veil of swarming growth in an otherwise obvious zone of growth inhibition. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(3) When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. Data regarding whether amoxicillin should be used to treat shigellosis are conflicting. When reporting ampicillin results, state that treatment of shigellosis with amoxicillin might **have poorer efficacy compared with treatment with ampicillin.** In addition, for extraintestinal isolates of *Salmonella* spp., a third-generation cephalosporin should be tested and reported, and chloramphenicol may be tested and reported if requested. Susceptibility testing is indicated for typhoidal *Salmonella* (*S. enterica* ser. Typhi and *S. enterica* ser. Paratyphi A–C) isolated from extraintestinal and intestinal sources. Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. In contrast, susceptibility testing is indicated for all *Shigella* isolates.

Table 2A. Enterobacterales (Continued)

(4) The dosage regimens shown in the comments column below are those needed to achieve plasma drug exposures (in adults with normal renal and hepatic functions) on which breakpoints were based. When implementing new breakpoints, it is strongly recommended that laboratories share this information with the antimicrobial stewardship team **and other relevant institutional stakeholders.**

(5) An intermediate (I) with a ^ in Tables 2 indicates agents that have the potential to concentrate in the urine. The I^ is for informational use only. The decision to report I^ is best made by each laboratory based on institution-specific guidelines and in consultation with appropriate medical personnel.

(6) Positive blood culture broth can be used as the inoculum for direct disk diffusion testing of select antimicrobial agents against Enterobacterales (using methods described in Table 3E-1 and applying breakpoints in Table 3E-2). For antimicrobial agents not listed in Table 3E-2 for Enterobacterales, CLSI has not yet evaluated this direct disk diffusion method.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
PENICILLINS										
Ampicillin	10 µg	≥ 17	-	14-16^	≤ 13	≤ 8	-	16^	≥ 32	**(7)** Results of ampicillin testing can be used to predict results for amoxicillin. **(8)** Breakpoints are based on an ampicillin dosage regimen of 2 g parenterally administered every 4-6 h or an amoxicillin dosage regimen of 1-2 g parenterally administered every 6 h. **(9)** Breakpoints when oral ampicillin is used **only** for therapy of **salmonellosis, shigellosis, or** uncomplicated UTIs due to *E. coli* **and** *P. mirabilis* are based on an ampicillin dosage regimen of 500 mg orally administered every 6 h or an amoxicillin dosage regimen of 250 mg orally administered every 8 h or 500 mg every 12 h. See general comment (3).
Piperacillin*		-	-	-	-	≤ 8	16	-	≥ 32	**(10)** Disk diffusion breakpoints have been removed because no disk correlate data are available for the revised piperacillin MIC breakpoints. Disk diffusion breakpoints will be reassessed if data become available.
Mecillinam*	10 µg	≥ 15	-	12-14^	≤ 11	≤ 8	-	16^	≥ 32	**(11)** For testing and reporting of *E. coli* urinary tract isolates only.

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
β-LACTAM COMBINATION AGENTS										
(12) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test SDD, intermediate, or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.										
Amoxicillin-clavulanate	20/10 µg	≥ 18	-	14-17^	≤ 13	≤8/4	-	16/8^	≥ 32/16	**(13)** Breakpoints are based on a dosage regimen of 1.2 g IV administered every 6 h. **(14)** Breakpoints when amoxicillin-clavulanate is used for therapy of uncomplicated UTIs or for completion of therapy for systemic infection are based on a dosage regimen of either 875/125 mg administered orally every 12 h or 500/125 mg every 8 h.
Ampicillin-sulbactam	10/10 µg	≥ 15	-	12-14^	≤ 11	≤ 8/4	-	16/8^	≥ 32/16	**(15)** Breakpoints are based on a dosage regimen of 3 g administered parenterally every 6 h.
Ceftolozane-tazobactam	30/10 µg	≥ 22	-	19-21^	≤ 18	≤ 2/4	-	4/4^	≥ 8/4	**(16)** Breakpoints are based on a dosage regimen of 3 g administered every 8 h for pneumonia and 1.5 g administered every 8 h for other indications.
Ceftazidime-avibactam	30/20 µg	≥ 21	-	-	≤ 20	≤ 8/4	-	-	≥ 16/4	**(17)** Breakpoints are based on a dosage regimen of 2.5 g every 8 h administered over 2 h. **(18)** Confirmatory MIC testing is indicated for isolates with zones of 20–22 mm to avoid reporting false-susceptible or false-resistant results.
Imipenem-relebactam	10/25 µg	≥ 25	-	21-24^	≤ 20	≤ 1/4	-	2/4^	≥ 4/4	**(19)** Breakpoints are based on a dosage regimen of 1.25 g administered every 6 h. **(20)** Breakpoints do not apply to the family Morganellaceae, which includes but is not limited to the genera *Morganella*, *Proteus*, and *Providencia*.

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
ß-LACTAM COMBINATION AGENTS (Continued)										
Meropenem-vaborbactam	20/10 µg	≥ 18	-	15-17^	≤ 14	≤ 4/8	-	8/8^	≥ 16/8	**(21)** Breakpoints are based on a dosage regimen of 4 g every 8 h administered over 3 h.
Piperacillin-tazobactam	100/10 µg	≥ 25	21-24		≤ 20	≤ 8/4	16/4		≥ 32/4	**(22)** Breakpoints for susceptible are based on a dosage regimen of 3.375-4.5 g administered every 6 h as a 30-minute infusion. Breakpoints for SDD are based on a dosage regimen of 4.5 g administered every 6 h as a 3-h infusion or 4.5 g administered every 8 h as a 4-h infusion.
Ticarcillin-clavulanate*	75/10 µg	≥ 20	-	15-19^	≤ 14	≤ 16/2	-	32/2-64/2^	≥ 128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)										
(23) WARNING: For *Salmonella* spp. and *Shigella* spp., first- and second-generation cephalosporins and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible. **(24)** Following evaluation of PK/PD properties, limited clinical data, and MIC distributions, revised breakpoints for cephalosporins (cefazolin, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone) and aztreonam were first published in January 2010 (M100-S20) and are listed in this table. Cefuroxime (parenteral) was also evaluated; however, no change in breakpoints was necessary for the dosage indicated below. When using current breakpoints, routine ESBL testing is not necessary before reporting results. However, **in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders, laboratories may decide to perform phenotypic or genotypic testing for ESBLs, and the results may be used to guide therapeutic management or** for epidemiological or infection prevention purposes. **Limitations of phenotypic and genotypic methods must be considered (see Table 3A introductory text).[4]** Breakpoints for drugs with limited availability in many countries (eg, moxalactam, cefonicid, cefamandole, and cefoperazone) were not evaluated. If considering use of these drugs for *E. coli*, ***K. pneumoniae*** **and** ***K. oxytoca*****,** or *Proteus* spp., ESBL testing should be performed (see Table 3A). If isolates test ESBL positive, the results for moxalactam, cefonicid, cefamandole, and cefoperazone should be reported as resistant. **(25) Some Enterobacterales may develop resistance during therapy with third-generation cephalosporins as a result of derepression of AmpC ß-lactamase. This derepression is most commonly seen with *Citrobacter freundii* complex, *Enterobacter cloacae* complex, and *Klebsiella* (formerly *Enterobacter*) *aerogenes*.** Isolates that are initially susceptible may become resistant within **a few days** after initiation of therapy. Testing **subsequent** isolates may be warranted **if clinically indicated. The approach to reporting AST results for these organisms should be determined in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. See Table 1A, footnotes b and c.[4]**									

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)										
Cefazolin	30 µg	≥ 23	-	20-22	≤ 19	≤ 2	-	4	≥ 8	**(26)** Breakpoints when cefazolin is used for therapy of infections other than uncomplicated UTIs due to *E. coli*, *K. pneumoniae*, and *P. mirabilis*. Breakpoints are based on a dosage regimen of 2 g administered every 8 h. See comment (24).
Cefazolin **(U)**[b]	30 µg	≥ 15	-	-	≤ 14	≤ 16	-	-	≥ 32	**(27)** Breakpoints when cefazolin is used for therapy of uncomplicated UTIs due to *E. coli*, *K. pneumoniae*, and *P. mirabilis*. Breakpoints are based on a dosage regimen of 1 g administered every 12 h. See additional information in CEPHEMS (ORAL).
Ceftaroline	30 µg	≥ 23	-	20-22^	≤ 19	≤ 0.5	-	1^	≥ 2	**(28)** Breakpoints are based on a dosage regimen of 600 mg administered every 12 h.
Cefepime	30 µg	≥ 25	19-24	-	≤ 18	≤ 2	4-8	-	≥ 16	**(29)** The breakpoint for susceptible is based on a dosage regimen of 1 g administered every 12 h. The breakpoint for SDD is based on dosage regimens that result in higher cefepime exposure, either higher doses or more frequent doses or both, up to approved maximum dosage regimens. See Appendix E for more information about breakpoints and dosage regimens. Also see the definition of SDD in the Instructions for Use of Tables section.
Cefotaxime or ceftriaxone	30 µg 30 µg	≥ 26 ≥ 23	-	23-25^ 20-22^	≤ 22 ≤ 19	≤ 1 ≤ 1	-	2^ 2^	≥ 4 ≥ 4	**(30)** Breakpoints are based on a dosage regimen of 1 g administered every 24 h for ceftriaxone and 1 g administered every 8 h for cefotaxime. See comment (24).

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)										
Cefotetan	30 µg	≥ 16	-	13-15^	≤ 12	≤ 16	-	32^	≥ 64	
Cefoxitin	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	**(31)** Breakpoints are based on a dosage regimen of at least 8 g per day (eg, 2 g administered every 6 h).
Cefuroxime (parenteral)	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	**(32)** Breakpoints are based on a dosage regimen of 1.5 g administered every 8 h. See comment (24).
Ceftazidime	30 µg	≥ 21	-	18-20^	≤ 17	≤ 4	-	8^	≥ 16	**(33)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h. See comment (24).
Cefamandole*	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	See comment (24).
Cefmetazole*	30 µg	≥ 16	-	13-15^	≤ 12	≤ 16	-	32^	≥ 64	**(34)** Insufficient new data exist to reevaluate breakpoints listed here.
Cefonicid*	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	See comment (24).
Cefoperazone*	75 µg	≥ 21	-	16-20	≤ 15	≤ 16	-	32	≥ 64	See comment (24).
Ceftizoxime*	30 µg	≥ 25	-	22-24^	≤ 21	≤ 1	-	2^	≥ 4	**(35)** Breakpoints are based on a dosage regimen of 1 g administered every 12 h. See comment (24).
Moxalactam*	30 µg	≥ 23	-	15-22^	≤ 14	≤ 8	-	16-32^	≥ 64	See comment (24).
Cefiderocol	30 µg	≥ 16	-	9-15^	≤ 8	≤ 4	-	8^	≥ 16	**(36)** Breakpoints are based on a dosage regimen of 2 g every 8 h administered over 3 h. **(37) The accuracy and reproducibility of cefiderocol testing results by disk diffusion and broth microdilution are markedly affected by iron concentration and inoculum preparation and may vary by disk and media manufacturer. Depending on the type of variance observed, false-resistant or false-susceptible results may occur. Testing subsequent isolates is encouraged. Discussion with prescribers and antimicrobial stewardship members regarding the potential for inaccuracies is recommended.**

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
CEPHEMS (ORAL)										
Cefuroxime (oral)	30 µg	≥ 23	-	15-22^	≤ 14	≤ 4		8-16^	≥ 32	See comment (38).
Cefazolin **(U)**[b] (surrogate test for oral cephalosporins and uncomplicated UTIs)	30 µg	≥ 15	-	-	≤ 14	≤ 16	-	-	≥ 32	**(38)** Breakpoints are for cefazolin when used as a surrogate test to predict results for the oral agents cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef when used for therapy of uncomplicated UTIs due to *E. coli*, *K. pneumoniae*, and *P. mirabilis*. Cefazolin tested as a surrogate may overcall resistance to cefdinir, cefpodoxime, and cefuroxime. If cefazolin tests resistant, test these drugs individually if needed for therapy.
Loracarbef*****	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	**(39)** Do not test *Citrobacter*, *Providencia*, or *Enterobacter* spp. with cefdinir or loracarbef by disk diffusion because false-susceptible results have been reported. See comment (38).
Cefaclor*****	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	See comment (38).
Cefdinir*****	5 µg	≥ 20	-	17-19^	≤ 16	≤ 1	-	2^	≥ 4	See comments (38) and (39).
Cefixime*****	5 µg	≥ 19	-	16-18^	≤ 15	≤ 1	-	2^	≥ 4	**(40)** Do not test *Morganella* spp. with cefixime, cefpodoxime, or cefetamet by disk diffusion.
Cefpodoxime*****	10 µg	≥ 21	-	18-20^	≤ 17	≤ 2	-	4^	≥ 8	See comments (38) and (40).
Cefprozil*****	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	**(41)** Do not test *Providencia* spp. with cefprozil by disk diffusion because false-susceptible results have been reported. See comment (38).
Cefetamet **(Inv.)**	10 µg	≥ 18	-	15-17^	≤ 14	≤ 4	-	8^	≥ 16	See comment (40).
Ceftibuten **(U, Inv.)**[b]	30 µg	≥ 21	-	18-20^	≤ 17	≤ 8	-	16^	≥ 32	

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
MONOBACTAMS										
Aztreonam	30 µg	≥ 21	-	18-20^	≤ 17	≤ 4	-	8^	≥ 16	**(42)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h. See comment (24).
CARBAPENEMS										
(43) Following evaluation of PK/PD properties, limited clinical data, and MIC distributions that include recently described carbapenemase-producing strains, revised breakpoints for carbapenems were first published in June 2010 (M100-S20-U) and are listed below. Because of limited treatment options for infections caused by organisms with carbapenem MICs or zone diameters in the intermediate range, clinicians may wish to design carbapenem dosage regimens that use maximum recommended doses and possibly prolonged intravenous infusion regimens, as has been reported in the literature.[5-8] Consultation with an infectious diseases **specialist** is recommended for isolates for which the carbapenem MICs or zone diameter results from disk diffusion testing are in the intermediate or resistant ranges. **Institutional treatment guidelines, infection prevention procedures, or epidemiological investigations may necessitate identification of carbapenemase-producing Enterobacterales. Isolates with elevated carbapenem MICs (intermediate or resistant) can be tested for carbapenemase production by a phenotypic and/or a molecular assay (refer to Tables 3B and 3C for methods). See Appendix H, Table H3 regarding suggestions for reporting when mechanism of resistance-based testing (molecular and phenotypic methods) is discordant with phenotypic AST.** The following information is provided as background on carbapenemases in Enterobacterales that are largely responsible for MICs and zone diameters in the intermediate and resistant ranges, and thus the rationale for setting revised carbapenem breakpoints: • The clinical effectiveness of carbapenem treatment of infections produced by isolates for which the carbapenem MIC or disk diffusion test results are within the intermediate range is uncertain due to lack of controlled clinical studies. Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (eg, MICs in the intermediate or resistant range) than meropenem or doripenem MICs. These isolates may have elevated imipenem MICs by mechanisms other than production of carbapenemases.									
Doripenem*	10 µg	≥ 23	-	20-22^	≤ 19	≤ 1	-	2^	≥ 4	**(44)** Breakpoints are based on a dosage regimen of 500 mg administered every 8 h.
Ertapenem	10 µg	≥ 22	-	19-21^	≤ 18	≤ 0.5	-	1^	≥ 2	**(45)** Breakpoints are based on a dosage regimen of 1 g administered every 24 h.
Imipenem	10 µg	≥ 23	-	20-22^	≤ 19	≤ 1	-	2^	≥ 4	**(46)** Breakpoints are based on a dosage regimen of 500 mg administered every 6 h or 1 g every 8 h.
Meropenem	10 µg	≥ 23	-	20-22^	≤ 19	≤ 1	-	2^	≥ 4	**(47)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h.

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
LIPOPEPTIDES										
(48) WARNING: Clinical and PK/PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained. Alternative agents are strongly preferred. Colistin and polymyxin B should be used in combination with one or more active antimicrobial agents. Consultation with an infectious diseases specialist is recommended. **(49)** Several species are intrinsically resistant to the lipopeptides (colistin and polymyxin B). Refer to Appendix B.										
Colistin or polymyxin B*		- -	- -	- -	- -	- -	- -	≤ 2 ≤ 2	≥ 4 ≥ 4	**(50)** Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see International Consensus Guidelines[9]). **(51)** Polymyxin B should be given with a loading dose and maximum recommended doses (see International Consensus Guidelines[9]). **(52)** When colistin or polymyxin B is given systemically, neither is likely to be effective for pneumonia. **(53)** For colistin, broth microdilution, CBDE, and CAT MIC methods are acceptable. For polymyxin B, broth microdilution is the only approved method. Disk diffusion and gradient diffusion methods should not be performed (see Table 3D).

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
AMINOGLYCOSIDES										
(54) WARNING: For *Salmonella* spp. and *Shigella* spp., aminoglycosides may appear active *in vitro* but are not effective clinically and should not be reported as susceptible. **(55) Breakpoints for gentamicin, tobramycin, and amikacin are based on population distributions of various species, PK/PD target attainment analyses with an end point of net bacterial stasis and limited clinical data. Clinical outcomes data for aminoglycosides as monotherapy for systemic infections are limited and have resulted in worse treatment outcomes (for infections outside of the urinary tract) compared with other therapies. Combination therapy for most indications other than UTIs should be considered. Consultation with an infectious diseases specialist is recommended.**										
Gentamicin	10 µg	**≥ 18**	- -	**15-17^**	**≤ 14**	**≤ 2**	- -	**4^**	**≥ 8**	**(56) Breakpoints are based on a dosage regimen of 7 mg/kg parenterally administered every 24 h.**
Tobramycin	10 µg	**≥ 17**	-	13-**16^**	≤ 12	**≤ 2**	-	**4^**	**≥ 8**	**(57) Breakpoints are based on a dosage regimen of 7 mg/kg parenterally administered every 24 h.**
Amikacin	30 µg	**≥ 20**	-	**17-19^**	**≤ 16**	**≤ 4**	-	**8^**	**≥ 16**	**(58) Breakpoints are based on a dosage regimen of 15 mg/kg parenterally administered every 24 h.**
Plazomicin	**30 µg**	**≥ 18**	**-**	**15-17^**	**≤ 14**	**≤ 2**	**-**	**4^**	**≥ 8**	**(59) Breakpoints are based on a dosage regimen of 15 mg/kg every 24 h over 30 minutes.** **See comment (20).**
Kanamycin*	30 µg	≥ 18	-	14-17^	≤ 13	≤ 16	-	32^	≥ 64	
Netilmicin*	30 µg	≥ 15	-	13-14^	≤ 12	≤ 8	-	16^	≥ 32	
Streptomycin*	10 µg	≥ 15	-	12-14^	≤ 11	-	-	-	-	
MACROLIDES										
Azithromycin	15 µg	≥ 13	-	-	≤ 12	≤ 16	-	-	≥ 32	**(60)** *S. enterica* ser. Typhi only: breakpoints are based on MIC distribution data and limited clinical data. **(61)** Breakpoints are based on a dosage regimen of 500 mg administered daily.
		≥ 16	-	11-15	≤ 10	≤ 8	-	16	≥ 32	**(62)** *Shigella* spp. only: azithromycin disk diffusion zones can be hazy and difficult to measure, especially *S. sonnei*. If an isolate has a zone of inhibition that is difficult to measure, an MIC method is recommended. Media source may affect the clarity of the end points for disk diffusion tests. See comment (61).

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
TETRACYCLINES										
(63) 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.										
Tetracycline	30 µg	≥ 15	-	12-14	≤ 11	≤ 4	-	8	≥ 16	
Doxycycline*	30 µg	≥ 14	-	11-13	≤ 10	≤ 4	-	8	≥ 16	
Minocycline*	30 µg	≥ 16	-	13-15	≤ 12	≤ 4	-	8	≥ 16	
QUINOLONES AND FLUOROQUINOLONES for Enterobacterales except *Salmonella* spp. (Please refer to Glossary I.)										
Ciprofloxacin	5 µg	≥ 26	-	22-25^	≤ 21	≤ 0.25	-	0.5^	≥ 1	**(64)** Breakpoints for ciprofloxacin are based on a dosage regimen of 400 mg IV or 500 mg orally administered every 12 h.
Levofloxacin	5 µg	≥ 21		17-20^	≤ 16	≤ 0.5	-	1^	≥ 2	**(65)** Breakpoints for levofloxacin are based on a dosage regimen of 750 mg administered every 24 h.
Cinoxacin* **(U)**[b]	100 µg	≥ 19	-	15-18^	≤ 14	≤ 16	-	32^	≥ 64	
Enoxacin* **(U)**[b]	10 µg	≥ 18	-	15-17^	≤ 14	≤ 2	-	4^	≥ 8	
Gatifloxacin*	5 µg	≥ 18	-	15-17^	≤ 14	≤ 2	-	4^	≥ 8	
Gemifloxacin*	5 µg	≥ 20	-	16-19	≤ 15	≤ 0.25	-	0.5	≥ 1	**(66) Report only on** *K. pneumoniae*.
Grepafloxacin*	5 µg	≥ 18	-	15-17	≤ 14	≤ 1	-	2	≥ 4	
Lomefloxacin*	10 µg	≥ 22	-	19-21^	≤ 18	≤ 2	-	4^	≥ 8	
Nalidixic acid* **(U)**[b]	30 µg	≥ 19	-	14-18	≤ 13	≤ 16	-	-	≥ 32	
Norfloxacin* **(U)**[b]	10 µg	≥ 17	-	13-16	≤ 12	≤ 4	-	8	≥ 16	
Ofloxacin*	5 µg	≥ 16	-	13-15^	≤ 12	≤ 2	-	4^	≥ 8	
Fleroxacin **(Inv.)**	5 µg	≥ 19	-	16-18^	≤ 15	≤ 2	-	4^	≥ 8	

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
QUINOLONES AND FLUOROQUINOLONES for *Salmonella* spp. (Please refer to Glossary I.)										
(67) For testing and reporting of *Salmonella* spp. (including *S. enterica* ser. Typhi and *S. enterica* ser. Paratyphi A-C). Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. **(68)** The preferred test for assessing fluoroquinolone susceptibility or resistance in *Salmonella* spp. is a ciprofloxacin MIC test. A levofloxacin or ofloxacin MIC test can be performed if either agent, respectively, is the fluoroquinolone of choice in a specific facility. If a ciprofloxacin, levofloxacin, or ofloxacin MIC or ciprofloxacin disk diffusion test cannot be done, pefloxacin disk diffusion may be used as surrogate test to predict ciprofloxacin susceptibility. **(69)** No single test detects resistance resulting from all possible fluoroquinolone resistance mechanisms that have been identified in *Salmonella* spp.										
Ciprofloxacin Levofloxacin	5 µg -	≥ 31 -	- -	21-30^ -	≤ 20 -	≤ 0.06 ≤ 0.12	- -	0.12-0.5 ^ 0.25-1^	≥ 1 ≥ 2	**(70)** Isolates of *Salmonella* spp. that test not susceptible to ciprofloxacin, levofloxacin, ofloxacin, or pefloxacin may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with salmonellosis.
Ofloxacin	-	-	-	-	-	≤ 0.12	-	0.25-1^	≥ 2	
Pefloxacin **(Inv.)** (surrogate test for ciprofloxacin)	5 µg	≥ 24	-	-	≤ 23	-	-	-	-	**(71)** Report results as ciprofloxacin susceptible or resistant based on the pefloxacin test result. Pefloxacin will not detect resistance in *Salmonella* spp. due to *aac(6')-Ib-cr*. Pefloxacin disks are not available in the United States. See comment (69).
FOLATE PATHWAY ANTAGONISTS										
Trimethoprim-sulfamethoxazole	1.25/ 23.75 µg	≥ 16	-	11-15	≤ 10	≤ 2/38	-	-	≥ 4/76	See general comment (3).
Sulfonamides***** **(U)**[b]	250 or 300 µg	≥ 17	-	13-16	≤ 12	≤ 256	-	-	≥ 512	**(72)** Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
Trimethoprim***** **(U)**[b]	5 µg	≥ 16	-	11-15	≤ 10	≤ 8	-	-	≥ 16	
PHENICOLS										
Chloramphenicol*****	30 µg	≥ 18	-	13-17	≤ 12	≤ 8	-	16	≥ 32	**(73)** Not routinely reported on isolates from the urinary tract.

Table 2A. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
FOSFOMYCINS										
Fosfomycin **(U)**[b]	200 µg	≥ 16	-	13-15	≤ 12	≤ 64	-	128	≥ 256	**(74)** Disk diffusion and MIC breakpoints apply only to *E. coli* urinary tract isolates and should not be extrapolated to other species of Enterobacterales. **(75)** The 200-µg fosfomycin disk contains 50 µg glucose-6-phosphate. **(76)** The only approved MIC method for testing is agar dilution using agar media supplemented with 25 µg/mL of glucose-6-phosphate. Broth dilution MIC testing should not be performed.
NITROFURANS										
Nitrofurantoin **(U)**[b]	300 µg	≥ 17	-	15-16	≤ 14	≤ 32	-	64	≥ 128	

Abbreviations: **AST, antimicrobial susceptibility testing;** ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CAT, colistin agar test; CBDE, colistin broth disk elution; eCIM, EDTA-modified carbapenem inactivation method; ESBL, extended-spectrum β-lactamase; I, intermediate; **Inv., investigational agent;** IV, intravenous; mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control; R, resistant; S, susceptible; SDD, susceptible-dose dependent; **U, urine;** UTI, urinary tract infection.

Symbols: ^, designation for agents that have the potential to concentrate in the urine; ***, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.**

Footnotes

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

b. Report only on organisms isolated from the urinary tract.

Table 2A. Enterobacterales (Continued)

References for Table 2A

1 Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis*. 2019;94(4):321-325.

2 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

4 Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. IDSA Guidance on the treatment of antimicrobial-restant gram-negative infections: version 2.0. Infectious Diseases Society of America; 2022. Accessed 10 January 2023. https://www.idsociety.org/practice-guideline/amr-guidance-2.0/

5 Perrott J, Mabasa VH, Ensom MH. Comparing outcomes of meropenem administration strategies based on pharmacokinetic and pharmacodynamic principles: a qualitative systematic review. *Ann Pharmacother*. 2010;44(3):557-564.

6 Cirillo I, Vaccaro N, Turner K, Solanki B, Natarajan J, Redman R. Pharmacokinetics, safety, and tolerability of doripenem after 0.5-, 1-, and 4-hour infusions in healthy volunteers. *J Clin Pharmacol*. 2009;49(7):798-806.

7 Sakka SG, Glauner AK, Bulitta JB, et al. Population pharmacokinetics and pharmacodynamics of continuous versus short-term infusion of imipenem-cilastatin in critically ill patients in a randomized, controlled trial. *Antimicrob Agents Chemother*. 2007;51(9):3304-3310.

8 Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. *N Engl J Med*. 2010;362(19):1804-1813.

9 Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy*. 2019;39(1):10-39.

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Table 2B-1. Zone Diameter and MIC Breakpoints for *Pseudomonas aeruginosa*

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix I)[1]
Agar dilution: MHA

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [7]).

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours
Dilution methods: 16–20 hours

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Pseudomonas aeruginosa ATCC®[a] 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Refer to Table 1C for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[2] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see the *M02 Disk Diffusion Reading Guide*[3]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(3) The susceptibility of *P. aeruginosa* isolated from patients with cystic fibrosis can be reliably determined by disk diffusion or dilution methods but may need extended incubation for up to 24 hours before reporting as susceptible.

(4) *P. aeruginosa* may develop resistance during prolonged therapy with all antimicrobial agents. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing of repeat isolates may be warranted.

(5) The dosage regimens shown in the comments column below are those necessary to achieve plasma drug exposures (in adults with normal renal and hepatic functions) on which breakpoints were derived. When implementing new breakpoints, it is strongly recommended that laboratories share this information with the antimicrobial stewardship team **and other relevant institutional stakeholders.**

(6) An intermediate (I) with a ^ in Tables 2 indicates agents that have the potential to concentrate in the urine. The I^ is for informational use only. The decision to report I^ is best made by each laboratory based on institution-specific guidelines and in consultation with appropriate medical personnel.

Table 2B-1. *Pseudomonas aeruginosa* (Continued)

(7) Positive blood culture broth can be used as the inoculum for direct disk diffusion testing of select antimicrobial agents against *P. aeruginosa* (using methods described in Table 3E-1 and applying breakpoints in Table 3E-3). For antimicrobial agents not listed in Table 3E-3 for *P. aeruginosa*, CLSI has not yet evaluated this direct disk diffusion method.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2B-1. *Pseudomonas aeruginosa* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Piperacillin*	100 µg	**≥22**	**18-21^**	**≤17**	≤16	**32^**	**≥64**	**(8)** Breakpoints for piperacillin (alone or with tazobactam) are based on a piperacillin dosage regimen of **4 g administered every 6 h over 30 minutes or over 3 h.**
β-LACTAM COMBINATION AGENTS								
(9) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Piperacillin-tazobactam	100/10 µg	**≥22**	**18-21^**	**≤17**	≤16/4	**32/4**	**≥64**/4	**(10) Breakpoints for susceptible are based on a dosage regimen of 4.5 g administered every 6 h over 30 minutes or over 3 h. Breakpoints for intermediate are only to provide a buffer zone to prevent small uncontrolled technical factors from causing major discrepancies in interpretations.**
Ceftazidime-avibactam	30/20 µg	≥21	-	≤20	≤8/4	-	≥16/4	**(11)** Breakpoints are based on a dosage regimen of 2.5 g administered every 8 h over 2 h.
Ceftolozane-tazobactam	30/10 µg	≥21	17-20^	≤16	≤4/4	8/4^	≥16/4	**(12)** Breakpoints are based on a dosage regimen of 3 g administered every 8 h for pneumonia and 1.5 g administered every 8 h for other indications.
Imipenem-relebactam	10/25 µg	≥23	20-22^	≤19	≤2/4	4/4^	≥8/4	**(13)** Breakpoints are based on a dosage regimen of 1.25 g administered every 6 h.
Ticarcillin-clavulanate*	75/10 µg	≥24	16-23^	≤15	≤16/2	32/2-64/2^	≥128/2	**(14)** Breakpoints for ticarcillin (alone or with clavulanate) are based on a ticarcillin dosage regimen of at least 3 g administered every 6 h.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftazidime	30 µg	≥18	15-17^	≤14	≤8	16^	≥32	**(15)** Breakpoints are based on a dosage regimen of 1 g administered every 6 h or 2 g administered every 8 h.
Cefepime	30 µg	≥18	15-17^	≤14	≤8	16^	≥32	**(16)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h or 2 g administered every 12 h.
Cefiderocol	30 µg	≥18	13-17^	≤12	≤4	8^	≥16	**(17)** Breakpoints are based on a dosage regimen of 2 g every 8 h administered over 3 h. **(18) The accuracy and reproducibility of cefiderocol testing results by disk diffusion and broth microdilution are markedly affected by iron concentration and inoculum preparation and may vary by disk and media manufacturer. Depending on the type of variance observed, false-resistant or false-susceptible results may occur. Testing subsequent isolates is encouraged. Discussion with prescribers and antimicrobial stewardship members regarding the potential for inaccuracies is recommended.**

Table 2B-1. *Pseudomonas aeruginosa* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
MONOBACTAMS								
Aztreonam	30 µg	≥22	16-21^	≤15	≤8	16^	≥32	**(19)** Breakpoints are based on a dosage regimen of 1 g administered every 6 h or 2 g administered every 8 h.
CARBAPENEMS								
Doripenem*	10 µg	≥19	16-18^	≤15	≤2	4^	≥8	**(20)** Breakpoints for doripenem are based on a dosage regimen of 500 mg administered every 8 h.
Imipenem	10 µg	≥19	16-18^	≤15	≤2	4^	≥8	**(21)** Breakpoints for imipenem are based on a dosage regimen of 1 g administered every 8 h or 500 mg administered every 6 h.
Meropenem	10 µg	≥19	16-18^	≤15	≤2	4^	≥8	**(22)** Breakpoints for meropenem are based on a dosage regimen of 1 g administered every 8 h.
LIPOPEPTIDES								
(23) WARNING: Clinical and PK/PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained. Alternative agents are strongly preferred. Colistin and polymyxin B should be used in combination with one or more active antimicrobial agents. Consultation with an infectious diseases specialist is recommended.								
Colistin or polymyxin B*	- -	- -	- -	- -	- -	≤2 ≤2	≥4 ≥4	**(24)** Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see International Consensus Guidelines[4]). **(25)** Polymyxin B should be given with a loading dose and maximum recommended doses (see International Consensus Guidelines[4]). **(26)** When colistin or polymyxin B is given systemically, neither is likely to be effective for pneumonia. **(27)** For colistin, broth microdilution, CBDE, and CAT MIC methods are acceptable. For polymyxin B, broth microdilution is the only approved method. Disk diffusion and gradient diffusion methods should not be performed (see Table 3D).

Table 2B-1. *Pseudomonas aeruginosa* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
AMINOGLYCOSIDES								
(28) Breakpoints for tobramycin and amikacin are based on population distributions of various species, PK/PD target attainment analyses with an end point of net bacterial stasis, and limited clinical data. Clinical outcomes data for aminoglycosides as monotherapy for systemic infections are limited and have resulted in worse treatment outcomes (for infections outside of the urinary tract) compared with other therapies. Combination therapy for most indications other than urinary tract infections should be considered. Consultation with an infectious diseases specialist is recommended.								
Tobramycin	10 µg	**≥19**	13-**18**^	≤12	**≤1**	**2**^	≥**4**	**(29) Breakpoints are based on a dosage regimen of 7 mg/kg parenterally administered every 24 h.** **(30) Tobramycin does not predict susceptibility to gentamicin.**
Amikacin **(U)**[b]	30 µg	≥17	15-16^	≤14	≤16	32^	≥64	**(31) Breakpoints are based on a dosage regimen of 15 mg/kg parenterally administered every 24 h.**
Netilmicin*	30 µg	≥15	13-14^	≤12	≤8	16^	≥32	
FLUOROQUINOLONES								
Ciprofloxacin	5 µg	≥25	19-24^	≤18	≤ 0.5	1^	≥2	**(32)** Breakpoints are based on a dosage regimen of 400 mg IV administered every 8 h.
Levofloxacin	5 µg	≥22	15-21^	≤14	≤ 1	2^	≥4	**(33)** Breakpoints are based on a dosage regimen of 750 mg administered every 24 h.
Lomefloxacin* **(U)**[b]	10 µg	≥22	19-21^	≤18	≤ 2	4^	≥8	
Norfloxacin* **(U)**[b]	10 µg	≥17	13-16	≤12	≤ 4	8	≥16	
Ofloxacin*	5 µg	≥16	13-15^	≤12	≤ 2	4^	≥8	
Gatifloxacin*	5 µg	≥18	15-17^	≤14	≤ 2	4^	≥8	

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CAT, colistin agar test; CBDE, colistin broth disk elution; I, intermediate; IV, intravenous; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control; R, resistant; S, susceptible; **U, urine.**
Symbols: ^, designation for agents that have the potential to concentrate in the urine; ***, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.**

Footnotes

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

b. Report only on organisms isolated from the urinary tract.

Table 2B-1. *Pseudomonas aeruginosa* (Continued)

References for Table 2B-1

1. Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis*. 2019;94(4):321-325.

2. CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

3. CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

4. Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective *Pharmacology* (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy*. 2019;39(1):10-39.

Table 2B-2. Zone Diameter and MIC Breakpoints for *Acinetobacter* spp.

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix I)[1]
Agar dilution: MHA

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C±2°C; ambient air; 20–24 hours, all methods

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®[a] 25922 (for tetracyclines and trimethoprim-sulfamethoxazole)
Pseudomonas aeruginosa ATCC® 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Refer to Table 1D for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[2] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see the *M02 Disk Diffusion Reading Guide*[3]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2B-2. *Acinetobacter* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Piperacillin*	100 µg	≥21	18-20	≤17	≤16	32-64	≥128	
Β-LACTAM COMBINATION AGENTS								
(3) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Ampicillin-sulbactam	10/10 µg	≥15	12-14	≤11	≤8/4	16/8	≥32/16	
Piperacillin-tazobactam	100/10 µg	≥21	18-20	≤17	≤16/4	32/4-64/4	≥128/4	
Ticarcillin-clavulanate*	75/10 µg	≥20	15-19	≤14	≤16/2	32/2-64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftazidime	30 µg	≥18	15-17	≤14	≤8	16	≥32	
Cefepime	30 µg	≥18	15-17	≤14	≤8	16	≥32	
Cefotaxime Ceftriaxone	30 µg 30 µg	≥23 ≥21	15-22 14-20	≤14 ≤13	≤8 ≤8	16-32 16-32	≥64 ≥64	
Cefiderocol	30 µg	≥15	-	-	≤4	8	≥16	**(4)** Breakpoints are based on a dosage regimen of 2 g every 8 h administered over 3 h. Disk diffusion zone diameters ≤14 mm should not be interpreted or reported because zone diameters ≤14 mm occur with resistant, intermediate, and susceptible isolates. For isolates with zone diameters ≤14 mm, do not report cefiderocol without performing an MIC test. **(5)** For testing and reporting against *Acinetobacter baumannii* complex only. **(6) The accuracy and reproducibility of cefiderocol testing results by disk diffusion and broth microdilution are markedly affected by iron concentration and inoculum preparation and may vary by disk and media manufacturer. Depending on the type of variance observed, false-resistant or false-susceptible results may occur. Testing subsequent isolates is encouraged. Discussion with prescribers and antimicrobial stewardship members regarding the potential for inaccuracies is recommended.**

Table 2B-2. *Acinetobacter* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
CARBAPENEMS								
Doripenem*	10 µg	≥18	15-17	≤14	≤2	4	≥8	**(7)** Breakpoints for doripenem are based on a dosage regimen of 500 mg administered every 8 h.
Imipenem	10 µg	≥22	19-21	≤18	≤2	4	≥8	**(8)** Breakpoints for imipenem are based on a dosage regimen of 500 mg administered every 6 h.
Meropenem	10 µg	≥18	15-17	≤14	≤2	4	≥8	**(9)** Breakpoints for meropenem are based on a dosage regimen of 1 g administered every 8 h or 500 mg administered every 6 h.
LIPOPEPTIDES								
(10) WARNING: Clinical and PK/PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained. Alternative agents are strongly preferred. Colistin and polymyxin B should be used in combination with one or more active antimicrobial agents. Consultation with an infectious diseases specialist is recommended.								
Colistin or polymyxin B	- -	- -	- -	- -	- -	≤2 ≤2	≥4 ≥4	**(11)** Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see International Consensus Guidelines[4]). **(12)** Polymyxin B should be given with a loading dose and maximum recommended doses (see International Consensus Guidelines[4]). **(13)** When colistin or polymyxin B is given systemically, the drug is unlikely to be effective for pneumonia. **(14)** The only approved MIC method is broth microdilution. CBDE, CAT, disk diffusion, and gradient diffusion should not be performed. See comment (5).
AMINOGLYCOSIDES								
Gentamicin	10 µg	≥15	13-14	≤12	≤4	8	≥16	
Tobramycin	10 µg	≥15	13-14	≤12	≤4	8	≥16	
Amikacin	30 µg	≥17	15-16	≤14	≤16	32	≥64	
Netilmicin*	-	-	-	-	≤8	16	≥32	
TETRACYCLINES								
(15) 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.								
Doxycycline	30 µg	≥13	10-12	≤9	≤4	8	≥16	
Minocycline	30 µg	≥16	13-15	≤12	≤4	8	≥16	
Tetracycline **(U)**[b]	30 µg	≥15	12-14	≤11	≤4	8	≥16	

Table 2B-2. *Acinetobacter* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
FLUOROQUINOLONES								
Ciprofloxacin	5 µg	≥21	16-20	≤15	≤1	2	≥4	
Levofloxacin	5 µg	≥17	14-16	≤13	≤2	4	≥8	
Gatifloxacin*	5 µg	≥18	15-17	≤14	≤2	4	≥8	
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥ 16	11-15	≤10	≤2/38	-	≥4/76	

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CAT, colistin agar test; CBDE, colistin broth elution test; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control; R, resistant; S, susceptible, **U, urine.**

Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Footnotes

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

b. Report only on organisms isolated from the urinary tract.

References for Table 2B-2

1 Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis*. 2019;94(4):321-325.

2 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

4 Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy*. 2019;39(1):10-39.

Table 2B-3. Zone Diameter and MIC Breakpoints for *Burkholderia cepacia* complex

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB
Agar dilution: MHA

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C±2°C; ambient air; 20-24 hours, all methods

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®[a] 25922 (for chloramphenicol, minocycline, and trimethoprim-sulfamethoxazole)
Pseudomonas aeruginosa ATCC® 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Refer to Table 1E for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[1] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see the *M02 Disk Diffusion Reading Guide*[2]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2B-3. *Burkholderia cepacia* complex (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
β-LACTAM COMBINATION AGENTS								
Ticarcillin-clavulanate*	-	-	-	-	≤16/2	32/2-64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftazidime	30 µg	≥21	18-20	≤17	≤8	16	≥32	
CARBAPENEMS								
Meropenem	10 µg	≥20	16-19	≤15	≤4	8	≥16	
TETRACYCLINES								
Minocycline	30 µg	≥19	15-18	≤14	≤4	8	≥16	
FLUOROQUINOLONES								
Levofloxacin	-	-	-	-	≤2	4	≥8	
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11-15	≤10	≤2/38	-	≥4/76	
PHENICOLS								
Chloramphenicol*	-	-	-	-	≤8	16	≥32	**(3)** Not routinely reported on **organisms isolated** from the urinary tract.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Footnote

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

References for Table 2B-3

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

Table 2B-4. Zone Diameter and MIC Breakpoints for *Stenotrophomonas maltophilia*

Testing Conditions	
Medium:	Disk diffusion: MHA Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix I)[1] Agar dilution: MHA
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	35°C±2°C; ambient air; 20–24 hours, all methods

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®[a] 25922 (for chloramphenicol, minocycline, and trimethoprim-sulfamethoxazole)
Pseudomonas aeruginosa ATCC® 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comment

(1) Refer to Table 1F for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[2] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see the *M02 Disk Diffusion Reading Guide*[3]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2B-4. *Stenotrophomonas maltophilia* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
B-LACTAM COMBINATION AGENTS								
Ticarcillin-clavulanate*	-	-	-	-	≤16/2	32/2-64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftazidime	-	-	-	-	≤8	16	≥32	
Cefiderocol	30 µg	≥15	-	-	≤1	-	-	(3) Breakpoints are based on a dosage regimen of 2 g every 8 h administered over 3 h. Breakpoints are based on PK/PD properties, MIC distributions, and limited clinical data. **(4) The accuracy and reproducibility of cefiderocol testing results by disk diffusion and broth microdilution are markedly affected by iron concentration and inoculum preparation and may vary by disk and media manufacturer. Depending on the type of variance observed, false-resistant or false-susceptible results may occur. Testing subsequent isolates is encouraged. Discussion with prescribers and antimicrobial stewardship members regarding the potential for inaccuracies is recommended.**
TETRACYCLINES								
Minocycline	30 µg	≥19	15-18	≤14	≤4	8	≥16	
FLUOROQUINOLONES								
Levofloxacin	5 µg	≥17	14-16	≤13	≤2	4	≥8	**(5) *Rx:* Levofloxacin should not be used alone for antimicrobial therapy.**
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11-15	≤10	≤2/38	-	≥4/76	
PHENICOLS								
Chloramphenicol*	-	-	-	-	≤8	16	≥32	(6) Not routinely reported on **organisms isolated** from the urinary tract.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; PK/PD, pharmacokinetic/pharmacodynamic; R, resistant; S, susceptible.

Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Table 2B-4. *Stenotrophomonas maltophilia* (Continued)

Footnote

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

References for Table 2B-4

[1] Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis*. 2019;94(4):321-325.

[2] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

[3] CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

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Table 2B-5. MIC Breakpoints for Other Non-Enterobacterales (Refer to General Comment [2])

Testing Conditions

Medium: Broth dilution: CAMHB
Agar dilution: MHA

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air; 16–20 hours

Routine QC Recommendations (see Table 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®[a] 25922 (for chloramphenicol, tetracyclines, sulfonamides, and trimethoprim-sulfamethoxazole)
Pseudomonas aeruginosa ATCC® 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Refer to Table 1G for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) Other non-Enterobacterales include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli but exclude *P. aeruginosa*, *Acinetobacter* spp., *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia* (refer to Tables 2B-2, 2B-3, and 2B-4, respectively). Recommendations for testing and reporting *Aeromonas* **spp. (includes members of *A. caviae* complex,** *A. hydrophila* complex, **and *A. veronii* complex),** *Burkholderia mallei*, *Burkholderia pseudomallei*, and *Vibrio* spp. (including *V. cholerae)* are found in CLSI document M45.[1]

(3) For other non-Enterobacterales, the disk diffusion method has not been systematically studied. Therefore, for this organism group, disk diffusion testing is not recommended.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2B-5. Other Non-Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Piperacillin*	-	-	-	-	≤16	32-64	≥128	
β-LACTAM COMBINATION AGENTS								
(4) Organisms that test susceptible to the ß-lactam agent alone are also considered susceptible to the ß-lactam combination agent. However, organisms that test susceptible to the ß-lactam combination agent cannot be assumed to be susceptible to the ß-lactam agent alone. Similarly, organisms that test intermediate or resistant to the ß-lactam agent alone may be susceptible to the ß-lactam combination agent.								
Piperacillin-tazobactam	-	-	-	-	≤16/4	32/4-64/4	≥128/4	
Ticarcillin-clavulanate*	-	-	-	-	≤16/2	32/2-64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftazidime	-	-	-	-	≤8	16	≥32	
Cefepime	-	-	-	-	≤8	16	≥32	
Cefotaxime Ceftriaxone	- -	- -	- -	- -	≤8 ≤8	16-32 16-32	≥64 ≥64	
Cefoperazone*	-	-	-	-	≤16	32	≥64	
Ceftizoxime*	-	-	-	-	≤8	16-32	≥64	
Moxalactam*	-	-	-	-	≤8	16-32	≥64	
MONOBACTAMS								
Aztreonam	-	-	-	-	≤8	16	≥32	
CARBAPENEMS								
Imipenem	-	-	-	-	≤4	8	≥16	
Meropenem	-	-	-	-	≤4	8	≥16	
AMINOGLYCOSIDES								
Gentamicin	-	-	-	-	≤4	8	≥16	
Tobramycin	-	-	-	-	≤4	8	≥16	
Amikacin	-	-	-	-	≤16	32	≥64	
Netilmicin*	-	-	-	-	≤8	16	≥32	
TETRACYCLINES								
(5) 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.								
Tetracycline **(U)**[b]	-	-	-	-	≤4	8	≥16	
Doxycycline*	-	-	-	-	≤4	8	≥16	
Minocycline	-	-	-	-	≤4	8	≥16	

Table 2B-5. Other Non-Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
FLUOROQUINOLONES								
Ciprofloxacin	-	-	-	-	≤1	2	≥4	
Levofloxacin	-	-			≤2	4	≥8	
Gatifloxacin*	-	-	-	-	≤2	4	≥8	
Lomefloxacin*	-	-	-	-	≤2	4	≥8	
Norfloxacin* **(U)**[b]	-	-	-	-	≤4	8	≥16	
Ofloxacin*	-	-	-	-	≤2	4	≥8	
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	-	-	-	-	≤2/38	-	≥4/76	
Sulfonamides **(U)**[b]	-	-	-	-	≤256	-	≥512	**(6)** Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
PHENICOLS								
Chloramphenicol*	-	-	-	-	≤8	16	≥32	**(7)** Not routinely reported on **organisms isolated** from the urinary tract.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible, **U, urine.**
Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Footnotes

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

b. Report only on organisms isolated from the urinary tract.

Reference for Table 2B-5

1 CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*. 3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute; 2016.

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Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; CAMHB + 2% NaCl for oxacillin; CAMHB supplemented to 50 µg/mL calcium for daptomycin.
Agar dilution: MHA; MHA + 2% NaCl for oxacillin.
NOTE: Agar dilution has not been validated for daptomycin.

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours; 24 hours (for cefoxitin when testing *Staphylococcus* spp., except *S. aureus*, *S. lugdunensis*, *S. pseudintermedius*, and *S. schleiferi*)
Dilution methods: 16–20 hours; 24 hours for oxacillin and vancomycin
Testing at temperatures above 35°C may not detect MRS.

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Disk diffusion:
S. aureus ATCC®[a] 25923

Dilution methods:
S. aureus ATCC® 29213

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Refer to Table 1H for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[1] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see the *M02 Disk Diffusion Reading Guide*[2]). Hold the Petri plate a few inches above a black background illuminated with reflected light, except for linezolid, which should be read with transmitted light (plate held up to light source). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter. For linezolid, any discernible growth within the zone of inhibition is indicative of resistance to the respective agent.

(3) *S. aureus* complex consists of the coagulase-positive species *S. aureus*, *Staphylococcus argenteus*, and *Staphylococcus schweitzeri*. If *S. argenteus* is identified by MALDI-TOF MS or sequencing, it is recommended that it be reported as "*S. aureus* complex (*S. argenteus*)," and *S. aureus* phenotypic testing method recommendations, breakpoints, and interpretive categories should be used. Human infections with *S. schweitzeri* have yet to be reported.[3]

Table 2C. Staphylococcus spp. (Continued)

(4) For staphylococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[4] Figures 3 and 4). With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is ≥ 80% reduction in growth compared with the control (see M07,[4] Figure 5).

(5) Routine testing of urine isolates of *Staphylococcus saprophyticus* is not advised, because infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute, uncomplicated UTIs (eg, nitrofurantoin, trimethoprim-sulfamethoxazole, or a fluoroquinolone).

(6) Historically, resistance to the penicillinase-stable penicillins (see Glossary I) has been referred to as "methicillin resistance" or "oxacillin resistance." MRSA are strains of *S. aureus* that express *mecA*, *mecC*, or another mechanism of methicillin (oxacillin) resistance, such as changes in affinity of penicillin-binding proteins for oxacillin (modified *S. aureus* strains).

(7) Most methicillin (oxacillin) resistance is mediated by *mecA*, encoding PBP2a (also called PBP2′). Tests for *mecA* and PBP2a are the most definitive tests for detection of methicillin (oxacillin) resistance for *Staphylococcus* spp. Isolates that test positive for *mecA* or PBP2a or resistant by any of the recommended phenotypic methods should be reported as methicillin (oxacillin) resistant (see Appendix H and the table below).

Detection of methicillin (oxacillin) resistance in staphylococci is achieved by using specific methods as listed in Table 2C and further described in Tables 3G-1 and 3G-2.

	Phenotypic Methods for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp.				
Organism	Cefoxitin MIC	Cefoxitin disk diffusion	Oxacillin MIC	Oxacillin disk diffusion	Oxacillin salt agar
S. aureus	Yes (16-20 h)	Yes (16-18 h)	Yes (24 h)	No	Yes (24 h)
S. lugdunensis	Yes (16-20 h)	Yes (16-18 h)	Yes (24 h)	No	No
S. epidermidis	No	Yes (24 h)	Yes (24 h)	Yes (16-18 h)	No
S. pseudintermedius	No	No	Yes (24 h)	Yes (16-18 h)	No
S. schleiferi	No	No	Yes (24 h)	Yes (16-18 h)	No
Staphylococcus spp. (not listed above or not identified to the species level)	No	Yes[a] (24 h)	Yes[a] (24 h)	No	No

Abbreviations: h, hour(s); MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant staphylococci; PBP2a, penicillin-binding protein 2a.
[a] For isolates that fall into the category of *Staphylococcus* spp (not listed above or not identified to the species level) from serious infections for which the oxacillin MICs are 1–2 µg/mL, tests for *mecA* or PBP2a should be considered, because these are the most definitive tests for detection of methicillin (oxacillin) resistance (see comment [19]). Recent data suggest that the cefoxitin disk diffusion test may not perform reliably for all species (eg, *S. haemolyticus*) that fall into the category of "*Staphylococcus* spp. (not listed above or not identified to the species level)."[5]

Mechanisms of methicillin (oxacillin) resistance other than *mecA* are rare and include a novel *mecA* homologue, *mecC*.[6] MICs for strains with *mecC* are typically cefoxitin resistant and oxacillin susceptible; *mecC* resistance cannot be detected by tests directed at *mecA* or PBP2a.

Table 2C. *Staphylococcus* spp. (Continued)

(8) MRS, as defined by cefoxitin or oxacillin testing, as appropriate to the species, are considered resistant to other β-lactam agents, ie, penicillins, β-lactam combination agents, cephems (with the exception of ceftaroline), and carbapenems. This is because most cases of documented MRS infections have responded poorly to β-lactam therapy or because convincing clinical data that document clinical efficacy for those agents have not been presented.

(9) For tests for β-lactamase production, methicillin (oxacillin) resistance and *mecA*-mediated methicillin (oxacillin) resistance using cefoxitin, reduced susceptibility to vancomycin, ICR, and high-level mupirocin resistance (*S. aureus* only), refer to Tables 3F, 3G-1, 3G-2, 3H, and 3J, respectively.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINASE-LABILE PENICILLINS											
(10) Penicillin-susceptible staphylococci are susceptible to other ß-lactam agents with established clinical efficacy for staphylococcal infections (including both penicillinase-labile and penicillinase-stable agents; see Glossary I). Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins. **(11)** Penicillin should be used to test the susceptibility of all staphylococci to penicillinase-labile penicillins (see Glossary I). Penicillin-resistant strains of staphylococci produce ß-lactamase. Perform a test(s) to detect ß-lactamase production on staphylococci for which the penicillin MICs are ≤ 0.12 µg/mL or zone diameters ≥ 29 mm before reporting the isolate as penicillin susceptible. Rare isolates of staphylococci that contain genes for ß-lactamase production may appear negative by ß-lactamase tests. Consequently, for serious infections requiring penicillin therapy, laboratories should perform MIC tests and ß-lactamase testing on all subsequent isolates from the same patient. PCR testing of the isolate for the *blaZ* ß-lactamase gene may be considered. See Table 3F.										
Penicillin	All staphylococci	10 units	≥29	-	-	≤28	≤0.12		-	≥0.25	**(12)** For MRS, report penicillin as resistant or do not report.
PENICILLINASE-STABLE PENICILLINS											
(13) Cefoxitin is tested as a surrogate for oxacillin for some species of *Staphylococcus*. Isolates that test resistant by cefoxitin or oxacillin, when using the appropriate test method for the species, should be reported as methicillin (oxacillin) resistant. If testing only cefoxitin, report as methicillin (oxacillin) susceptible or resistant based on the cefoxitin result. **(14)** Oxacillin (or cefoxitin) results can be applied to the other penicillinase-stable penicillins (cloxacillin, dicloxacillin, methicillin, and nafcillin). For agents with established clinical efficacy and considering site of infection and appropriate dosing, methicillin (oxacillin)-susceptible staphylococci can be considered susceptible to: • ß-lactam combination agents (amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam) • Oral cephems (cefaclor, cefdinir, cephalexin, cefpodoxime, cefprozil, cefuroxime, loracarbef) • Parenteral cephems including cephalosporins I, II, III, and IV (cefamandole, cefazolin, cefepime, cefmetazole, cefonicid, cefoperazone, cefotaxime, cefotetan, ceftizoxime, ceftriaxone, cefuroxime, ceftaroline, moxalactam) • Carbapenems (doripenem, ertapenem, imipenem, meropenem) Methicillin (oxacillin)-resistant staphylococci are resistant to all currently available ß-lactam antimicrobial agents, with the exception of ceftaroline. Thus, susceptibility or resistance to a wide array of ß-lactam antimicrobial agents may be deduced from testing only penicillin and either cefoxitin or oxacillin. Testing of other ß-lactam agents, except ceftaroline, is not advised. See general comments (7) and (8). Additional explanation on the use of cefoxitin for prediction of *mecA*-mediated methicillin (oxacillin) resistance can be found in Subchapter 3.12 of M07[4] and Subchapter 3.9 of M02.[1]										

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINASE-STABLE PENICILLINS (Continued)											
Oxacillin	*S. aureus* and *S. lugdunensis*	-	-	-	-	-	≤ 2 (oxacillin)	-	-	≥ 4 (oxacillin)	**(15)** Oxacillin disk testing is not reliable for *S. aureus* and *S. lugdunensis*. **(16)** For isolates of *S. aureus* that do not grow well on CAMHB or unsupplemented MHA (eg, small-colony variants), testing on other media (eg, BMHA) does not reliably detect *mecA*-mediated resistance. Testing for PBP2a using induced growth (ie, growth taken from the zone margin surrounding a cefoxitin disk on either BMHA or a blood agar plate after 24 hours incubation in 5% CO_2) or *mecA* should be done. See general comments (7) and (8) and comments (10), (13), and (14).
		30 µg cefoxitin (surrogate test for oxacillin)	≥ 22	-	-	≤ 21	≤ 4 (cefoxitin)	-	-	≥ 8 (cefoxitin)	
Oxacillin	*S. epidermidis*	1 µg oxacillin	≥ 18 (oxacillin)	-	-	≤ 17 (oxacillin)	≤ 0.5 (oxacillin)	-	-	≥ 1 (oxacillin)	See general comments (7) and (8) and comments (10), (13), and (14). **(17)** Cefoxitin MIC testing is not reliable for detecting *mecA*-mediated resistance in *S. epidermidis*.
		30 µg cefoxitin (surrogate test for oxacillin)	≥ 25 (cefoxitin)	-	-	≤ 24 (cefoxitin)	-	-	-	–	
	S. pseudintermedius and *S. schleiferi*	1 µg oxacillin	≥ 18	-	-	≤ 17	≤ 0.5	-	-	≥ 1	**(18)** Neither cefoxitin MIC nor cefoxitin disk tests are reliable for detecting *mecA*-mediated resistance in *S. pseudintermedius* and *S. schleiferi*. See general comments (7) and (8) and comments (10), (13), and (14).

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINASE-STABLE PENICILLINS (Continued)											
Oxacillin	*Staphylococcus* spp., except: *S. aureus* *S. lugdunensis* *S. epidermidis* *S. pseudintermedius* *S. schleiferi*	30 µg cefoxitin (surrogate test for oxacillin)	≥ 25 (cefoxitin)	-	-	≤ 24 (cefoxitin)	≤ 0.5 (oxacillin)	-	-	≥ 1 (oxacillin)	**(19)** Oxacillin MIC breakpoints may overcall resistance, and some isolates for which the oxacillin MICs are 1–2 µg/mL may be *mecA* negative. Isolates from serious infections for which oxacillin MICs are 1–2 µg/mL may be tested for *mecA* or for PBP2a. Isolates that test *mecA* or PBP2a negative should be reported as methicillin (oxacillin) susceptible. See general comments (7) and (8) and comments (10), (13), and (14).
CEPHEMS (PARENTERAL)											
Ceftaroline	*S. aureus*, including MRSA	30 µg	≥ 25	20–24		≤ 19	≤ 1	2–4	-	≥ 8	**(20)** The breakpoint for susceptible is based on a dosage regimen of 600 mg administered every 12 h. **(21)** The breakpoint for SDD is based on a dosage of 600 mg every 8 h administered over 2 h.

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
GLYCOPEPTIDES											
(22) MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, -intermediate, and -resistant isolates of *Staphylococcus* spp. other than *S. aureus*, all of which give similar size zones of inhibition.											
Vancomycin	*S. aureus*, including MRSA	-	-	-	-	-	≤2	-	4-8	≥16	**(23)** For *S. aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy. **(24)** Send any *S. aureus* for which the vancomycin is ≥8 µg/mL to a referral laboratory. See Appendix A. Also refer to Table 3G-1 for *S. aureus*, Subchapter 3.12 in M07,[4] and Subchapter 3.9 in M02.[1]
	Staphylococcus spp. other than *S. aureus*	-	-	-	-	-	≤4	-	8-16	≥32	**(25)** Send any *Staphylococcus* spp. other than *S. aureus* for which the vancomycin MIC is ≥32 µg/mL to a referral laboratory. See Appendix A. See also Subchapter 3.12 in M07[4] and Subchapter 3.9 in M02.[1]
LIPOGLYCOPEPTIDES											
Dalbavancin	*S. aureus*, including MRSA	-	-	-	-	-	≤0.25	-	-	-	**(26)** Breakpoints are based on a dosage regimen of 1500 mg (single dose) or 1000 mg (two doses) IV administered over 30 minutes followed one week later by 500 mg IV administered over 30 minutes.
Oritavancin		-	-	-	-	-	≤0.12	-	-	-	**(27)** Breakpoints are based on a dosage regimen of 1200 mg IV administered once.
Telavancin		-	-	-	-	-	≤0.12	-	-	-	**(28)** Breakpoints are based on a dosage regimen of 10 mg/kg administered every 24 h.
Teicoplanin **(Inv.)**	All staphylococci	-	-	-	-	-	≤8	-	16	≥32	

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
LIPOPEPTIDES											
Daptomycin	All staphylococci	-	-	-	-	-	≤1	-	-	-	**(29) Not routinely** reported **on organisms** isolated from the respiratory tract.
AMINOGLYCOSIDES											
(30) For staphylococci that test susceptible, gentamicin is used only in combination with other active agents that test susceptible.											
Gentamicin	All staphylococci	10 µg	≥15	-	13-14	≤12	≤4	-	8	≥16	
MACROLIDES											
(31) Not routinely reported on organisms isolated from the urinary tract.											
Azithromycin	All staphylococci	15 µg	≥18	-	14-17	≤13	≤2	-	4	≥8	
or clarithromycin or erythromycin		15 µg	≥18		14-17	≤13	≤2		4	≥8	
		15 µg	≥23		14-22	≤13	≤0.5		1-4	≥8	
Dirithromycin*		15 µg	≥19	-	16-18	≤15	≤2	-	4	≥8	
TETRACYCLINES											
(32) 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.											
Tetracycline	All staphylococci	30 µg	≥19	-	15-18	≤14	≤4	-	8	≥16	
Doxycycline		30 µg	≥16	-	13-15	≤12	≤4	-	8	≥16	
Minocycline		30 µg	≥19	-	15-18	≤14	≤4	-	8	≥16	See comment (31).
FLUOROQUINOLONES											
(33) *Staphylococcus* spp. may develop resistance during prolonged therapy with quinolones. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing of repeat isolates may be warranted.											
Ciprofloxacin or levofloxacin	All staphylococci	5 µg	≥21	-	16-20	≤15	≤1	-	2	≥4	
Moxifloxacin		5 µg	≥19	-	16-18	≤15	≤1	-	2	≥4	
		5 µg	≥24	-	21-23	≤20	≤0.5	-	1	≥2	
Enoxacin* **(U)**[b]		10 µg	≥18	-	15-17	≤14	≤2	-	4	≥8	
Gatifloxacin*		5 µg	≥23	-	20-22	≤19	≤0.5	-	1	≥2	
Grepafloxacin*		5 µg	≥18	-	15-17	≤14	≤1	-	2	≥4	
Lomefloxacin*		10 µg	≥22	-	19-21	≤18	≤2	-	4	≥8	
Norfloxacin* **(U)**[b]		10 µg	≥17	-	13-16	≤12	≤4	-	8	≥16	
Ofloxacin*		5 µg	≥18	-	15-17	≤14	≤1	-	2	≥4	
Sparfloxacin*		5 µg	≥19	-	16-18	≤15	≤0.5	-	1	≥2	
Fleroxacin **(Inv.)**		5 µg	≥19	-	16-18	≤15	≤2	-	4	≥8	

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
NITROFURANS											
Nitrofurantoin **(U)**[b]	All staphylococci	300 µg	≥17	-	15-16	≤14	≤32	-	64	≥128	
LINCOSAMIDES											
Clindamycin	All staphylococci	2 µg	≥21	-	15-20	≤14	≤0.5	-	1-2	≥4	**(34)** For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR by disk diffusion using the D-zone test or by broth microdilution is required before reporting clindamycin (see Table 3I, Subchapter 3.9 in M02,[1] and Subchapter 3.12 in M07[4]). See comment (31).
FOLATE PATHWAY ANTAGONISTS											
Trimethoprim-sulfamethoxazole	All staphylococci	1.25/23.75 µg	≥16	-	11-15	≤10	≤2/38	-	-	≥4/76	
Sulfonamides **(U)**[b]	All staphylococci	250 or 300 µg	≥17	-	13-16	≤12	≤256	-	-	≥512	**(35)** Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
Trimethoprim **(U)**[b]	All staphylococci	5 µg	≥16	-	11-15	≤10	≤8	-	-	≥16	
PHENICOLS											
Chloramphenicol*	All staphylococci	30 µg	≥18	-	13-17	≤12	≤8	-	16	≥32	See comment (31).
ANSAMYCINS											
Rifampin	All staphylococci	5 µg	≥20	-	17-19	≤16	≤1	-	2	≥4	**(36)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
STREPTOGRAMINS											
Quinupristin-dalfopristin*	*S. aureus*	15 µg	≥19	-	16-18	≤15	≤1	-	2	≥4	**(37) Report only on** methicillin (oxacillin)-susceptible *S. aureus*.

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
OXAZOLIDINONES											
(38) *S. aureus* that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that test resistant to linezolid may be susceptible to tedizolid.											
Linezolid	All staphylococci	30 µg	≥21	-	-	≤20	≤4	-	-	≥8	**(39)** When testing linezolid, disk diffusion zones should be examined using transmitted light. Organisms with resistant results by disk diffusion should be confirmed using an MIC method.
Tedizolid	*S. aureus*, including MRSA	-	-	-	-	-	≤0.5	-	1	≥2	**(40)** Breakpoints are based on a dosage regimen of 200 mg administered every 24 h.
PLEUROMUTILINS											
Lefamulin	*S. aureus*, including MRSA	20 µg	≥23	-	-	-	≤0.25	-	-	-	**(41)** The breakpoints for susceptible are based on a dosage regimen of 150 mg IV or 600 mg orally administered every 12 h. **(42)** Not routinely reported on organisms isolated from the urinary tract.

Abbreviations: ATCC®, American Type Culture Collection; BMHA, blood Mueller-Hinton agar; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; ICR, inducible clindamycin resistance; **Inv., investigational agent;** IV, intravenous; MALDI-TOF MS; matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant staphylococci; MRSA, methicillin (oxacillin)-resistant *S. aureus*; PBP2a, penicillin-binding protein 2a; PCR, polymerase chain reaction; QC, quality control; R, resistant; S, susceptible; SDD, susceptible-dose dependent; **U, urine;** UTI, urinary tract infection.

Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Footnotes

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

b. Report only on organisms isolated from the urinary tract.

Table 2C. *Staphylococcus* spp. (Continued)

References for Table 2C

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

3 Becker K, Schaumburg F, Kearns A, et al. Implications of Identifying the recently defined members of the *Staphylococcus aureus* complex S. *argenteus* and *S. schweitzeri*: a position paper of members of the ESCMID Study Group for Staphylococci and Staphylococcal Diseases (ESGS). *Clin Microbiol Infect*. 2019;25(9):1064-1070.

4 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

5 Humphries RM, Magnano P, Burnham CA, et al. Evaluation of surrogate tests for the presence of *mecA*-mediated methicillin resistance in *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis* and *Staphylococcus warneri*. *J. Clin Microbiol*. 2020;59(1):e02290-20.

6 García-Álvarez L, Holden MT, Lindsay H, et al. Methicillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infect Dis*. 2011;11(8):595-603.

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Table 2D. Zone Diameter and MIC Breakpoints for *Enterococcus* spp.

Testing Conditions	
Medium:	Disk diffusion: MHA Broth dilution: CAMHB; CAMHB supplemented to 50 µg/mL calcium for daptomycin Agar dilution: MHA; agar dilution has not been validated for daptomycin
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	35°C ± 2°C; ambient air Disk diffusion: 16–18 hours Dilution methods: 16–20 hours All methods: 24 hours for vancomycin

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Disk diffusion:
S. aureus ATCC®[a] 25923

Dilution methods:
E. faecalis ATCC® 29212

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

Refer to Tables 3H and 3K for additional testing recommendations, reporting suggestions, and QC.

General Comments

(1) Refer to Table 1I for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[1] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see the *M02 Disk Diffusion Reading Guide*[2]). Hold the Petri plate a few inches above a black background illuminated with reflected light, except for vancomycin, which should be read with transmitted light (plate held up to light source). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Any discernible growth within the zone of inhibition indicates vancomycin resistance.

(3) For enterococci when testing chloramphenicol, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[3] Figures 3 and 4).

(4) WARNING: For *Enterococcus* spp., aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole may appear active *in vitro,* but they are not effective clinically, and isolates should not be reported as susceptible.

(5) Synergy between ampicillin, penicillin, or vancomycin and an aminoglycoside can be predicted for enterococci by using a high-level aminoglycoside (gentamicin and streptomycin) test (see Table 3K).

(6) An intermediate (I) with a ^ in Tables 2 indicates agents that have the potential to concentrate in the urine. The I^ is for informational use only. The decision to report I^ is best made by each laboratory based on institution-specific guidelines and in consultation with appropriate medical personnel.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2D. *Enterococcus* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	I	R	S	SDD	I	R	
PENICILLINS									
Penicillin	10 units	≥ 15	-	≤ 14	≤ 8	-	-	≥ 16	**(7)** The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. Ampicillin results may be used to predict susceptibility to amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam among non-β-lactamase-producing enterococci. Ampicillin susceptibility can be used to predict imipenem susceptibility, providing the species is confirmed to be *E. faecalis*. **(8)** Enterococci susceptible to penicillin are predictably susceptible to ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin-tazobactam for non-β-lactamase-producing enterococci. However, enterococci susceptible to ampicillin cannot be assumed to be susceptible to penicillin. If penicillin results are needed, testing of penicillin is required. **(9)** ***Rx:*** Combination therapy with high-dosage parenteral ampicillin, amoxicillin, penicillin, or vancomycin (for susceptible strains only), plus an aminoglycoside, is usually indicated for serious enterococcal infections, such as endocarditis, unless high-level resistance to both gentamicin and streptomycin is documented; such combinations are predicted to result in synergistic killing of enterococci. **(10)** Breakpoints are based on an ampicillin dosage regimen of 2 g parenterally administered every 4–6 h or an amoxicillin dosage regimen of 1-2 g parenterally administered every 6 h. **(11)** Breakpoints when oral ampicillin is used for therapy of uncomplicated UTIs only are based on an ampicillin dosage regimen of 500 mg orally administered every 6 h or amoxicillin dosage regimen of 250 mg orally administered every 8 h or 500 mg every 12 h.
Ampicillin	10 µg	≥ 17	-	≤ 16	≤ 8	-	-	≥ 16	

Table 2D. *Enterococcus* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	I	R	S	SDD	I	R	
PENICILLINS (Continued)									
Penicillin Ampicillin	10 units 10 µg	≥15 ≥17	- -	≤14 ≤16	≤8 ≤8	- -	- -	≥16 ≥16	**(12)** Penicillin or ampicillin resistance among enterococci due to ß-lactamase production has been reported very rarely. Penicillin or ampicillin resistance due to ß-lactamase production is not reliably detected with routine disk or dilution methods but is detected using a direct, nitrocefin-based ß-lactamase test. Because of the rarity of ß-lactamase–positive enterococci, this test does not need to be performed routinely but can be used in selected cases. A positive ß-lactamase test predicts resistance to penicillin as well as amino- and ureidopenicillins (see Glossary I).
GLYCOPEPTIDES									
Vancomycin	30 µg	≥17	15–16	≤14	≤4	-	8–16	≥32	**(13)** When testing vancomycin against enterococci, plates should be held a full 24 hours for accurate detection of resistance. Zones should be examined using transmitted light; the presence of a haze or any growth within the zone of inhibition indicates resistance. Organisms with intermediate zones should be tested by an MIC method as described in M07.[3] For isolates for which the vancomycin MICs are 8–16 µg/mL, perform biochemical tests for identification as listed under the "Vancomycin MIC ≥ 8 µg/mL" test found in Table 3H. See general comment (5) and comment (9).

Table 2D. *Enterococcus* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	I	R	S	SDD	I	R	
LIPOGLYCOPEPTIDES									
Dalbavancin	-	-	-	-	≤0.25	-	-	-	**(14) Report only on** vancomycin-susceptible *E. faecalis*. **(15)** Breakpoints are based on a dosage regimen of 1500 mg (single dose) or 1000 mg (two doses) IV administered over 30 minutes followed one week later by 500 mg IV administered over 30 minutes.
Oritavancin	-	-	-	-	≤0.12	-	-	-	**(16)** Breakpoints are based on a dosage regimen of 1200 mg administered IV once. See comment (14).
Telavancin	-	-	-	-	≤0.25	-	-	-	**(17)** Breakpoints are based on a dosage regimen of 10 mg/kg administered every 24 h. See comment (14).
Teicoplanin **(Inv.)**	30 µg	≥14	11-13	≤10	≤8	-	16	≥32	
LIPOPEPTIDES									
Daptomycin *E. faecium* only	-	-	-	-	-	≤ 4	-	≥ 8	**(18) Not routinely** reported **on organisms isolated** from the respiratory tract. **(19)** The breakpoint for SDD is based on a dosage regimen of 8-12 mg/kg administered every 24 h and is intended for serious infections due to *E. faecium*. Consultation with an infectious diseases specialist is recommended.
Daptomycin *Enterococcus* spp. other than *E. faecium*	-	-	-	-	≤2	-	4	≥ 8	**(20)** The breakpoint for susceptible is based on a dosage regimen of 6 mg/kg administered every 24 h. See comment (18).

Table 2D. *Enterococcus* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	I	R	S	SDD	I	R	
MACROLIDES									
Erythromycin*	15 µg	≥23	14-22	≤13	≤0.5	-	1-4	≥8	**(21)** Not routinely reported on **organisms isolated** from the urinary tract.
TETRACYCLINES									
(22) 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.									
Tetracycline **(U)**[b]	30 µg	≥19	15-18	≤14	≤4	-	8	≥16	
Doxycycline*	30 µg	≥16	13-15	≤12	≤4	-	8	≥16	
Minocycline*	30 µg	≥19	15-18	≤14	≤4	-	8	≥16	
FLUOROQUINOLONES									
Ciprofloxacin **(U)**[b] Levofloxacin **(U)**[b]	5 µg 5 µg	≥21 ≥17	16-20^ 14-16^	≤15 ≤13	≤1 ≤2	-	2^ 4^	≥4 ≥8	
Gatifloxacin*	5 µg	≥18	15-17^	≤14	≤2	-	4^	≥8	
Norfloxacin* **(U)**[b]	10 µg	≥17	13-16	≤12	≤4	-	8	≥16	
NITROFURANS									
Nitrofurantoin **(U)**[b]	300 µg	≥17	15-16	≤14	≤32	-	64	≥128	
ANSAMYCINS									
Rifampin*	5 µg	≥20	17-19	≤16	≤1	-	2	≥4	**(23)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
FOSFOMYCINS									
Fosfomycin **(U)**[b]	200 µg	≥16	13-15	≤12	≤64	-	128	≥256	**(24) Report only on *E. faecalis*.** **(25)** The approved MIC testing method is agar dilution. Agar media should be supplemented with 25 µg/mL of glucose-6-phosphate. Broth dilution testing should not be performed. **(26)** The 200-µg fosfomycin disk contains 50 µg glucose-6-phosphate.

Table 2D. *Enterococcus* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	I	R	S	SDD	I	R	
PHENICOLS									
Chloramphenicol*	30 µg	≥18	13-17	≤12	≤8	-	16	≥32	See comment (21).
STREPTOGRAMINS									
Quinupristin-dalfopristin*	15 µg	≥19	16-18	≤15	≤1	-	2	≥4	**(27) Report only on** vancomycin-resistant *E. faecium*.
OXAZOLIDINONES									
(28) *E. faecalis* that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that are intermediate or resistant to linezolid may be susceptible to tedizolid.									
Linezolid	30 µg	≥23	21-22	≤20	≤2	-	4	≥8	
Tedizolid	-	-	-	-	≤0.5	-	-	-	**(29) Report only on** *E. faecalis*. **(30)** Breakpoints are based on a dosage regimen of 200 mg administered every 24 h.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; **Inv., investigational agent;** MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible; SDD, susceptible-dose dependent; **U, urine;** UTI, urinary tract infection.
Symbols: ^, designation for agents that have the potential to concentrate in the urine; ***, designation for "Other" agents not included in Tables 1 but have established clinical breakpoints.**

Footnotes

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

b. Report only on organisms isolated from the urinary tract.

References for Table 2D

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

Table 2E. Zone Diameter and MIC Breakpoints for *Haemophilus influenzae* and *Haemophilus parainfluenzae*

Testing Conditions

Medium: Disk diffusion: HTM **(for all agents when testing *H. influenzae* or *H. parainfluenzae*) or MH-F agar (MHA with 5% mechanically defibrinated horse blood and 20 µg/mL NAD) (for selected agents when testing *H. influenzae*)**

Broth dilution: HTM broth **(for all agents when testing *H. influenzae* or *H. parainfluenzae*) or MH-F broth (for selected agents when testing *H. influenzae*)**

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard prepared using colonies from an overnight (preferably 20- to 24-hour) chocolate agar plate (see general comment [3])

Incubation: 35°C ± 2°C
Disk diffusion: 5% CO_2; 16–18 hours
Broth dilution: ambient air; 20–24 hours

Routine QC Recommendations (see Tables 4A-1, 4B, 5A-1, and 5B for acceptable QC ranges)

H. influenzae ATCC®[a] 49247 **(for HTM or MH-F agar/broth)**
H. influenzae ATCC® 49766 **(for HTM)**

Use either *H. influenzae* ATCC® 49247 or *H. influenzae* ATCC® 49766 or both of these strains, based on the antimicrobial agents to be tested. Neither strain has QC ranges for all agents that might be tested against *H. influenzae* or *H. parainfluenzae*.

E. coli ATCC® 35218 (when testing amoxicillin-clavulanate)

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Refer to Table 1J for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) *Haemophilus* spp., as used in this table, includes only *H. influenzae* and *H. parainfluenzae*. See CLSI document M45[1] for testing and reporting recommendations for other species of *Haemophilus*.

(3) The 0.5 McFarland suspension contains approximately 1 to 4×10^8 CFU/mL. Use care in preparing this suspension, because higher inoculum concentrations may lead to false-resistant results with some ß-lactam antimicrobial agents, particularly when ß-lactamase–producing strains of *H. influenzae* are tested.

(4) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

(5) For isolates of *H. influenzae* from CSF, only results of testing with ampicillin, any of the third-generation cephalosporins listed below, chloramphenicol, and meropenem are appropriate to report.

(6) Amoxicillin-clavulanate, azithromycin, cefaclor, cefdinir, cefixime, cefpodoxime, cefprozil, cefuroxime, and clarithromycin are used as empiric therapy for respiratory tract infections due to *Haemophilus* spp. The results of susceptibility tests with these antimicrobial agents are often not necessary for management of individual patients.

(7) To make HTM: Prepare a fresh hematin stock solution by dissolving 50 mg of hematin powder in 100 mL of 0.01 mol/L NaOH with heat and stirring until the powder is thoroughly dissolved. Add 30 mL of the hematin stock solution and 5 g of yeast extract to 1 L of MHA, and autoclave. After autoclaving and cooling, add 3 mL of an NAD stock solution (50 mg NAD dissolved in 10 mL distilled water, filter sterilized) aseptically.

(8) For MIC testing with *H. influenzae*, results for ampicillin, amoxicillin-clavulanate, cefotaxime, ceftriaxone, cefuroxime, clarithromycin, chloramphenicol, levofloxacin, meropenem, rifampin, tetracycline, and trimethoprim-sulfamethoxazole were equivalent when HTM or MH-F broth and testing conditions and MIC breakpoints in Table 2E were used. MICs obtained for cefuroxime and rifampin using MH-F broth may show a one-doubling dilution bias toward more resistance compared with HTM broth. The comparative study showed ≥ 90% essential agreement of MICs between MH-F broth and HTM broth for all agents listed above. MIC QC ranges for *H. influenzae* ATCC® 49247 in Table 5B apply to testing using either HTM or MH-F broth.

(9) For disk diffusion testing with *H. influenzae*, results for ampicillin, ceftriaxone, cefuroxime, clarithromycin, chloramphenicol, levofloxacin, and tetracycline were equivalent when HTM or MH-F agar and the disk contents, testing conditions, and zone diameter breakpoints in Table 2E were used. Results with trimethoprim-sulfamethoxazole were not equivalent between media, and HTM agar should be used if this agent is tested. Disk diffusion QC ranges for *H. influenzae* ATCC® 49247 in Table 4B apply to testing using either HTM or MH-F agar, with the exception of trimethoprim-sulfamethoxazole, which must be tested on HTM agar, not MH-F agar.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Ampicillin	10 µg	≥22	19-21	≤18	≤1	2	≥4	See general comment (5). **(10)** Breakpoints when ampicillin is used for therapy of meningitis are based on a dosage regimen of 2 g IV administered every 4 h. **(11)** The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. The majority of isolates of *H. influenzae* that are resistant to ampicillin and amoxicillin produce a TEM-type ß-lactamase. In most cases, a ß-lactamase test can provide a rapid means of detecting resistance to ampicillin and amoxicillin. **(12)** Rare BLNAR strains of *H. influenzae* should be considered resistant to amoxicillin-clavulanate, ampicillin-sulbactam, cefaclor, cefamandole, cefetamet, cefonicid, cefprozil, cefuroxime, loracarbef, and piperacillin-tazobactam, despite apparent *in vitro* susceptibility of some BLNAR strains to these agents.

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
β-LACTAM COMBINATION AGENTS								
(13) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Ampicillin-sulbactam	10/10 µg	≥20	-	≤19	≤2/1	-	≥4/2	See comment (12). **(14)** Breakpoints are based on a dosage regimen of 3 g IV administered every 6 h.
Amoxicillin-clavulanate	20/10 µg	-	-	-	≤2/1	4/2	≥8/4	**(15)** Breakpoints are based on a dosage regimen of 875/125 mg orally administered every 12 h or 500/125 mg every 8 h. Additional disk correlate data are pending before disk diffusion breakpoints with this dosage regimen can be established. See general comment (6) and comment (12).
Ceftolozane-tazobactam	-	-	-	-	≤ 0.5/4	-	-	**(16)** Breakpoints are based on a dosage regimen of 3 g IV administered every 8 h. **(17) Report only on** *H. influenzae*.
Piperacillin-tazobactam*	100/10 µg	≥21	-	-	≤1/4	-	≥2/4	See comment (12).
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Cefotaxime or ceftazidime or ceftriaxone	30 µg 30 µg 30 µg	≥26 ≥26 ≥26	- - -	- - -	≤2 ≤2 ≤2	- - -	- - -	See general comment (5).
Cefuroxime	30 µg	≥20	17-19	≤16	≤4	8	≥16	See general comments (6) and **(8) and** comment (12).
Ceftaroline	30 µg	≥30	-	-	≤0.5	-	-	See comment (17). **(18)** Breakpoints are based on a dosage regimen of 600 mg administered every 12 h.
Cefonicid*	30 µg	≥20	17-19	≤16	≤4	8	≥16	See comment (12).

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)								
Cefamandole*	-	-	-	-	≤4	8	≥16	See comment (12).
Cefepime*	30 µg	≥26	-	-	≤2	-	-	
Ceftizoxime*	30 µg	≥26	-	-	≤2	-	-	See general comment (5).
CEPHEMS (ORAL)								
Cefaclor	30 µg	≥20	17-19	≤16	≤8	16	≥32	See general comment (6) and comment (12).
Cefprozil	30 µg	≥18	15-17	≤14	≤8	16	≥32	
Cefdinir or	5 µg	≥20	-	-	≤1	-	-	See general comment (6).
cefixime or	5 µg	≥21	-	-	≤1	-	-	
cefpodoxime	10 µg	≥21	-	-	≤2	-	-	
Cefuroxime	30 µg	≥20	17-19	≤16	≤4	8	≥16	See general comment (6) and comment (12).
Loracarbef*	30 µg	≥19	16-18	≤15	≤8	16	≥32	See general comment (6) and comment (12).
Ceftibuten*	30 µg	≥28	-	-	≤2	-	-	
Cefetamet (Inv.)	10 µg	≥18	15-17	≤14	≤4	8	≥16	See comment (12).
MONOBACTAMS								
Aztreonam	30 µg	≥26	-	-	≤2	-	-	
CARBAPENEMS								
Meropenem	10 µg	≥20	-	-	≤0.5	-	-	See general comment (5).
Ertapenem or	10 µg	≥19	-	-	≤0.5	-	-	
imipenem	10 µg	≥16	-	-	≤4	-	-	
Doripenem*	10 µg	≥16	-	-	≤1	-	-	
MACROLIDES								
Azithromycin	15 µg	≥12	-	-	≤4	-	-	See general comment (6).
Clarithromycin	15 µg	≥13	11-12	≤10	≤8	16	≥32	
TETRACYCLINES								
(19) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, resistance to doxycycline and minocycline cannot be inferred from tetracycline resistance.								
Tetracycline	30 µg	≥29	26-28	≤25	≤2	4	≥8	
FLUOROQUINOLONES								
Ciprofloxacin or	5 µg	≥21	-	-	≤1	-	-	
levofloxacin or	5 µg	≥17	-	-	≤2	-	-	
moxifloxacin	5 µg	≥18	-	-	≤1	-	-	
Gemifloxacin*	5 µg	≥18	-	-	≤0.12	-	-	
Gatifloxacin*	5 µg	≥18	-	-	≤1	-	-	
Grepafloxacin*	5 µg	≥24	-	-	≤0.5	-	-	
Lomefloxacin*	10 µg	≥22	-	-	≤2	-	-	
Ofloxacin*	5 µg	≥16	-	-	≤2	-	-	
Sparfloxacin*	-	-	-	-	≤0.25	-	-	

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
FLUOROQUINOLONES (Continued)								
Trovafloxacin*	10 µg	≥22	-	-	≤1	-	-	
Fleroxacin **(Inv.)**	5 µg	≥19	-	-	≤2	-	-	
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11-15	≤10	≤0.5/9.5	1/19-2/38	≥4/76	**See general comment (9).**
PHENICOLS								
Chloramphenicol*	30 µg	≥29	26-28	≤25	≤2	4	≥8	See general comment (5). **(20)** Not routinely reported on organisms isolated from the urinary tract.
ANSAMYCINS								
Rifampin	5 µg	≥20	17-19	≤16	≤1	2	≥4	**See general comment (8).** **(21)** May be appropriate only for prophylaxis of case contacts. These breakpoints do not apply to therapy of patients with invasive *H. influenzae* disease.
PLEUROMUTILINS								
Lefamulin	20 µg	≥18	-	-	≤2	-	-	**(22)** The breakpoints for susceptible are based on a dosage regimen of 150 mg IV or 600 mg orally administered every 12 h. See comments (17) and (20).

Abbreviations: ATCC®, American Type Culture Collection; BLNAR, β-lactamase negative, ampicillin-resistant; CFU, colony-forming unit(s); CSF, cerebrospinal fluid; HTM, *Haemophilus* test medium; I, intermediate; **Inv., investigational agent;** IV, intravenous; **MH-F, Mueller-Hinton fastidious agar; MH-F broth, Mueller-Hinton fastidious broth;** MIC, minimal inhibitory concentration; NAD, β-nicotinamide adenine dinucleotide; QC, quality control; R, resistant; S, susceptible.

Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Footnote

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

Reference for Table 2E

[1] CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.* 3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute; 2016.

Table 2F. Zone Diameter and MIC Breakpoints for *Neisseria gonorrhoeae*

Testing Conditions

Medium: Disk diffusion: GC agar base and 1% defined growth supplement. (The use of a cysteine-free growth supplement is not required for disk diffusion testing.) Agar dilution: GC agar base and 1% defined growth supplement. (The use of a cysteine-free growth supplement is required for agar dilution tests with carbapenems and clavulanate. Cysteine-containing defined growth supplement does not significantly alter dilution test results with other drugs.)

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard prepared in MHB or 0.9% phosphate-buffered saline, pH 7, using colonies from an overnight (20- to 24-hour) chocolate agar plate incubated in 5% CO_2

Incubation: 36°C ± 1°C (do not exceed 37°C); 5% CO_2; all methods, 20–24 hours

Routine QC Recommendations (see Tables 4B and 5C for acceptable QC ranges)

N. gonorrhoeae ATCC®[a] 49226

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Refer to Table 1K for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. For some agents, eg, fluoroquinolones or cephalosporins, only 2 to 3 disks may be tested per plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(3) The clinical effectiveness of cefotetan, cefoxitin, and spectinomycin for treating infections due to organisms that produce intermediate results with these agents is unknown.

(4) For disk diffusion testing of *N. gonorrhoeae*, an intermediate result for an antimicrobial agent indicates either a technical problem that should be resolved by repeat testing or a lack of clinical experience in treating infections due to organisms with these zones. Strains with intermediate zones to agents other than cefotetan, cefoxitin, and spectinomycin have a documented lower clinical cure rate (85% to 95%) compared with > 95% for susceptible strains.

(5) The recommended medium for testing *N. gonorrhoeae* consists of GC agar to which a 1% defined growth supplement (1.1 g L-cystine, 0.03 g guanine HCl, 0.003 g thiamine HCl, 0.013 g para-aminobenzoic acid, 0.01 g B12, 0.1 g cocarboxylase, 0.25 g NAD, 1 g adenine, 10 g L-glutamine, 100 g glucose, 0.02 g ferric nitrate, 25.9 g L-cysteine HCl [in 1 L H_2O]) is added after autoclaving.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2F. *Neisseria gonorrhoeae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Penicillin*	10 units	≥47	27-46	≤26	≤0.06	0.12-1	≥2	See general comment (4). **(6)** A positive β-lactamase test predicts resistance to penicillin, ampicillin, and amoxicillin. **(7)** A β-lactamase test detects one form of penicillin resistance in *N. gonorrhoeae* and also may be used to provide epidemiological information. Strains with chromosomally mediated resistance can be detected only by the disk diffusion method or the agar dilution MIC method. **(8)** Isolates that produce zones of inhibition ≤19 mm around a 10-unit penicillin disk are likely to be β-lactamase-producing strains. However, the β-lactamase test remains preferable to other susceptibility methods for rapid, accurate recognition of this plasmid-mediated penicillin resistance.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftriaxone	30 µg	≥35	-	-	≤0.25	-	-	
Cefoxitin*	30 µg	≥28	24-27	≤23	≤2	4	≥8	See general comment (3).
Cefepime*	30 µg	≥31	-	-	≤0.5	-	-	
Cefotaxime*	30 µg	≥31	-	-	≤0.5	-	-	
Cefotetan*	30 µg	≥26	20-25	≤19	≤2	4	≥8	See general comment (3).
Ceftizoxime*	30 µg	≥38	-	-	≤0.5	-	-	
CEPHEMS (ORAL)								
Cefixime	5 µg	≥ 31	-	-	≤ 0.25	-	-	
Cefpodoxime*	10 µg	≥ 29	-	-	≤0.5	-	-	

Table 2F. *Neisseria gonorrhoeae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
MACROLIDES								
Azithromycin	15 µg	≥ 30	-	-	≤ 1	-	-	**(9)** This breakpoint presumes that azithromycin (1 g single dose) is used in an approved regimen that includes an additional antimicrobial agent (ie, ceftriaxone 250 mg IM single dose).
TETRACYCLINES								
(10) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.								
Tetracycline	30 µg	≥38	31-37	≤30	≤0.25	0.5-1	≥2	**(11)** Isolates with disk zone diameters ≤19 mm usually indicate plasmid-mediated tetracycline resistance. Resistance in these strains should be confirmed by a dilution test (MIC ≥16 µg/mL).
FLUOROQUINOLONES								
See general comment (3).								
Ciprofloxacin	5 µg	≥41	28-40	≤27	≤ 0.06	0.12-0.5	≥1	
AMINOCYCLITOLS								
Spectinomycin*	100 µg	≥18	15-17	≤14	≤ 32	64	≥128	See general comment (3).

Abbreviations: ATCC®, American Type Culture Collection; I, intermediate; IM, intramuscular; MHB, Mueller-Hinton broth; MIC, minimal inhibitory concentration; NAD, β-nicotinamide adenine dinucleotide; pH, negative logarithm of hydrogen ion concentration; QC, quality control; R, resistant; S, susceptible.

Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Footnote

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

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Table 2G. Zone Diameter and MIC Breakpoints for *Streptococcus pneumoniae*

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood or MH-F agar (MHA with 5% **mechanically** defibrinated horse blood and 20 µg/mL NAD)
Broth dilution: CAMHB with LHB (2.5% to 5% v/v) (see M07[1] for instructions for preparation of LHB)
Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard, prepared using colonies from an overnight (18- to 20-hour) sheep blood agar plate

Incubation: 35°C ± 2°C
Disk diffusion: 5% CO_2; 20–24 hours
Dilution methods: ambient air; 20–24 hours (CO_2 if necessary, for growth with agar dilution)

Routine QC Recommendations (see Tables 4B and 5B for acceptable QC ranges)

S. pneumoniae ATCC®[a] 49619

Disk diffusion: deterioration of oxacillin disk content is best assessed with *S. aureus* ATCC® 25923, with an acceptable range of 18–24 mm on unsupplemented MHA.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Refer to Table 1L for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see the *M02 Disk Diffusion Reading Guide*[2]). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not measure the zone of inhibition of hemolysis. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(3) For pneumococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[1] Figures 3 and 4). With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is ≥ 80% reduction in growth compared with the control (see M07,[1] Figure 5).

(4) Amoxicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, cefuroxime, ertapenem, imipenem, and meropenem may be used to treat pneumococcal infections; however, reliable disk diffusion susceptibility tests with these agents do not yet exist. The *in vitro* activity of these agents is best determined using an MIC method.

Table 2G. *Streptococcus pneumoniae* (Continued)

(5) Penicillin and cefotaxime, ceftriaxone, or meropenem should be tested by a reliable MIC method (such as that described in M07[1]) and reported routinely with *S. pneumoniae* isolated from CSF. Such isolates can also be tested against vancomycin using the MIC or disk diffusion method. With isolates from other sites, the oxacillin disk test may be used. If the oxacillin zone size is ≤ 19 mm, cefotaxime, ceftriaxone, meropenem, or penicillin MICs should be determined.

(6) For disk diffusion, results using MHA with 5% sheep blood and MH-F agar were equivalent when disk contents, testing conditions, and zone diameter breakpoints in Table 2G were used. Disk diffusion QC ranges for *S. pneumoniae* ATCC® 49619 in Table 4B apply to testing using either MHA with 5% sheep blood or MH-F agar.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2G. *Streptococcus pneumoniae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
(7) For nonmeningitis isolates, a penicillin MIC of ≤ 0.06 µg/mL (or oxacillin zone ≥ 20 mm) can predict susceptibility to the following ß-lactams: ampicillin (oral or parenteral), ampicillin-sulbactam, amoxicillin, amoxicillin-clavulanate, cefaclor, cefdinir, cefditoren, cefepime, cefotaxime, cefpodoxime, cefprozil, ceftaroline, ceftizoxime, ceftriaxone, cefuroxime, doripenem, ertapenem, imipenem, loracarbef, meropenem. See general comment (5).								
Penicillin	1 µg oxacillin	≥ 20	-	-	-	-	-	**(8)** Isolates of pneumococci with oxacillin zone sizes ≥ 20 mm are susceptible (MIC ≤ 0.06 µg/mL) to penicillin. Penicillin and cefotaxime, ceftriaxone, or meropenem MICs should be determined for isolates with oxacillin zone diameters ≤ 19 mm, because zones ≤ 19 mm occur with penicillin-resistant, -intermediate, or certain -susceptible strains. For isolates with oxacillin zones ≤ 19 mm, do not report penicillin as resistant without performing a penicillin MIC test.
Penicillin parenteral (nonmeningitis)	-	-	-	-	≤ 2	4	≥ 8	**(9)** ***Rx:*** Doses of intravenous penicillin of at least 2 million units every 4 hours in adults with normal renal function (12 million units per day) can be used to treat nonmeningeal pneumococcal infections due to strains with penicillin MICs ≤ 2 µg/mL. Strains with an intermediate MIC of 4 µg/mL may necessitate penicillin doses of 18–24 million units per day. **(10)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
Penicillin parenteral (meningitis)	-	-	-	-	≤ 0.06	-	≥ 0.12	**(11)** ***Rx:*** Use of penicillin in meningitis requires therapy with maximum doses of intravenous penicillin (eg, at least 3 million units every 4 hours in adults with normal renal function). **(12)** For CSF isolates, report only meningitis interpretations. See general comment (5).

Table 2G. *Streptococcus pneumoniae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS (Continued)								
Penicillin (oral penicillin V)	-	-	-	-	≤0.06	0.12-1	≥2	**(13)** Interpretations for oral penicillin may be reported for isolates other than those from CSF.
Amoxicillin (nonmeningitis) Amoxicillin-clavulanate (nonmeningitis)	-	-	-	-	≤2 ≤2/1	4 4/2	≥8 ≥8/4	**(14)** Breakpoints for amoxicillin (alone or with clavulanate) are based on an oral amoxicillin dosage regimen of 500 mg administered every 8 h or 875 mg administered every 12 h.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
See comment (7).								
Cefepime (meningitis)*	-	-	-	-	≤0.5	1	≥2	**(15)** In the United States, for CSF isolates, report only nonmeningitis interpretations. There is not an FDA-approved indication for the use of cefepime for meningitis in the United States.
Cefepime (nonmeningitis)	-	-	-	-	≤1	2	≥4	**(16)** In the United States, report only interpretations for nonmeningitis and include the nonmeningitis notation on the report.
Cefotaxime (meningitis) Ceftriaxone (meningitis)	- -	- -	- -	- -	≤0.5 ≤0.5	1 1	≥2 ≥2	**(17)** For CSF isolates, report only meningitis interpretations. **(18)** ***Rx:*** Use of cefotaxime or ceftriaxone in meningitis requires therapy with maximum doses. See general comment (5).
Cefotaxime (nonmeningitis) Ceftriaxone (nonmeningitis)	- -	- -	- -	- -	≤1 ≤1	2 2	≥4 ≥4	**(19)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
Ceftaroline (nonmeningitis)	30 µg	≥26	-	-	≤0.5	-	-	**(20)** Breakpoints are based on a dosage regimen of 600 mg administered every 12 h.
Cefuroxime (parenteral)	-	-	-	-	≤0.5	1	≥2	

Table 2G. *Streptococcus pneumoniae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
CEPHEMS (ORAL)								
See comment (7).								
Cefuroxime (oral)	-	-	-	-	≤1	2	≥4	**(21)** Interpretations for oral cefuroxime may be reported for isolates other than those from CSF.
Cefaclor*	-	-	-	-	≤1	2	≥4	
Cefdinir*	-	-	-	-	≤0.5	1	≥2	
Cefpodoxime*	-	-	-	-	≤0.5	1	≥2	
Cefprozil*	-	-	-	-	≤2	4	≥8	
Loracarbef*	-	-	-	-	≤2	4	≥8	
CARBAPENEMS								
See comment (7).								
Meropenem	-	-	-	-	≤0.25	0.5	≥1	See general comment (5) and comment (8).
Ertapenem	-	-	-	-	≤1	2	≥4	
Imipenem	-	-	-	-	≤0.12	0.25-0.5	≥1	
Doripenem*	-	-	-	-	≤1	-	-	
GLYCOPEPTIDES								
Vancomycin	30 µg	≥17	-	-	≤1	-	-	See general comment (5).
MACROLIDES								
(22) Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(23)** Not routinely reported **on** organisms isolated from the urinary tract.								
Erythromycin	15 µg	≥21	16-20	≤15	≤0.25	0.5	≥1	
Azithromycin*	15 µg	≥18	14-17	≤13	≤0.5	1	≥2	
Clarithromycin*	15 µg	≥21	17-20	≤16	≤0.25	0.5	≥1	
Dirithromycin*	15 µg	≥18	14-17	≤13	≤0.5	1	≥2	
TETRACYCLINES								
(24) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline. However, resistance to doxycycline cannot be inferred from tetracycline resistance.								
Tetracycline Doxycycline	30 µg 30 µg	≥28 ≥28	25-27 25-27	≤24 ≤24	≤1 ≤0.25	2 0.5	≥4 ≥1	

Table 2G. *Streptococcus pneumoniae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
FLUOROQUINOLONES								
Gemifloxacin*	5 µg	≥23	20-22	≤19	≤0.12	0.25	≥0.5	**(25) Organisms that are susceptible to levofloxacin are also considered susceptible to gemifloxacin and moxifloxacin. However, some organisms that are intermediate or resistant to levofloxacin may be susceptible to gemifloxacin, moxifloxacin, or both.**
Levofloxacin	5 µg	≥17	14-16	≤13	≤2	4	≥8	
Moxifloxacin	5 µg	≥18	15-17	≤14	≤1	2	≥4	
Gatifloxacin*	5 µg	≥21	18-20	≤17	≤1	2	≥4	
Ofloxacin*	5 µg	≥16	13-15	≤12	≤2	4	≥8	
Sparfloxacin*	5 µg	≥19	16-18	≤15	≤0.5	1	≥2	
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥19	16-18	≤15	≤0.5/9.5	1/19-2/38	≥4/76	
PHENICOLS								
Chloramphenicol*	30 µg	≥21	-	≤20	≤4	-	≥8	See comment (23).
ANSAMYCINS								
Rifampin	5 µg	≥19	17-18	≤16	≤1	2	≥4	**(26)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
LINCOSAMIDES								
Clindamycin	2 µg	≥19	16-18	≤15	≤0.25	0.5	≥1	**(27)** For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR by disk diffusion using the D-zone test or by broth microdilution is required before reporting clindamycin (see Table 3I, Subchapter 3.9 in M02,[3] and Subchapter 3.12 in M07[1]). See comment (23).
STREPTOGRAMINS								
Quinupristin-dalfopristin*	15 µg	≥19	16-18	≤15	≤1	2	≥4	
OXAZOLIDINONES								
Linezolid	30 µg	≥21	-	-	≤2	-	-	
PLEUROMUTILINS								
Lefamulin	20 µg	≥19	-		≤0.5	-	-	**(28)** The susceptible breakpoints are based on a dosage regimen of 150 mg IV or 600 mg orally administered every 12 h. See comment (23).

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CSF, cerebrospinal fluid; FDA, US Food and Drug Administration; I, intermediate; ICR, inducible clindamycin resistance; IV, intravenous; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MH-F agar, Mueller-Hinton fastidious agar; MIC, minimal inhibitory concentration; NAD, ß-nicotinamide adenine dinucleotide; QC, quality control; R, resistant; S, susceptible.
Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Table 2G. *Streptococcus pneumoniae* (Continued)

Footnote

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

NOTE: Information in black boldface type is new or modified since the previous edition.

References for Table 2G

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

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Table 2H-1. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. ß-Hemolytic Group

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood
Broth dilution: CAMHB with LHB (2.5% to 5% v/v); the CAMHB should be supplemented to 50 µg/mL calcium for daptomycin (see M07[1] for instructions for preparation of LHB)
Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard, using colonies from an overnight (18- to 20-hour) sheep blood agar plate

Incubation: 35°C ± 2°C
Disk diffusion: 5% CO_2; 20–24 hours
Dilution methods: ambient air; 20–24 hours (CO_2 if necessary, for growth with agar dilution)

Routine QC Recommendations (see Tables 4B and 5B for acceptable QC ranges)

S. pneumoniae ATCC®[a] 49619

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

Refer to Table 3I for additional testing recommendations, reporting suggestions, and QC.

General Comments

(1) Refer to Table 1M for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see the *M02 Disk Diffusion Reading Guide*[2]). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not measure the zone of inhibition of hemolysis. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(3) For ß-hemolytic streptococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[1] Figures 3 and 4).

(4) For this table, the ß-hemolytic group includes the large colony-forming pyogenic strains of streptococci with group A (*S. pyogenes*), C, or G antigens and strains with Group B (*S. agalactiae*) antigen. Small colony-forming ß-hemolytic strains with group A, C, F, or G antigens (*S. anginosus* group, previously *S. milleri*) are considered part of the viridans group, and breakpoints for the viridans group should be used (see Table 2H-2).

Table 2H-1. *Streptococcus* spp. ß-Hemolytic Group (Continued)

(5) Penicillin and ampicillin are drugs of choice for treatment of ß-hemolytic streptococcal infections. Susceptibility testing of penicillins and other ß-lactams approved by the US Food and Drug Administration for treatment of ß-hemolytic streptococcal infections does not need to be performed routinely, because nonsusceptible isolates (ie, penicillin MICs > 0.12 and ampicillin MICs > 0.25 µg/mL) are extremely rare in any ß-hemolytic streptococcus and have not been reported for *S. pyogenes*. If testing is performed, any ß-hemolytic streptococcal isolate found to be nonsusceptible should be re-identified, retested, and, if confirmed, submitted to a public health laboratory. See Appendix A for additional instructions.

(6) Breakpoints for *Streptococcus* spp. ß-hemolytic group are proposed based on population distributions of various species, pharmacokinetics of the antimicrobial agents, previously published literature, and the clinical experience of subcommittee members. Systematically collected clinical data were not available for review with many of the antimicrobial agents in this table.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2H-1. *Streptococcus* spp. ß-Hemolytic Group (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
(7) An organism that is susceptible to penicillin can be considered susceptible to antimicrobial agents listed here when used for approved indications and does not need to be tested against those agents. For groups A, B, C, and G ß-hemolytic streptococci, penicillin is tested as a surrogate for ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefazolin, cefepime, ceftaroline, cephradine, cephalothin, cefotaxime, ceftriaxone, ceftizoxime, imipenem, ertapenem, and meropenem. For group A ß-hemolytic streptococci, penicillin is also a surrogate for cefaclor, cefdinir, cefprozil, ceftibuten, cefuroxime, and cefpodoxime.							
Penicillin or ampicillin	10 units 10 µg	≥24 ≥24	- -	- -	≤0.12 ≤0.25	- -	- -	See general comment (5).
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
See comment (7).								
Cefepime or cefotaxime or ceftriaxone	30 µg 30 µg 30 µg	≥24 ≥24 ≥24	- - -	- - -	≤0.5 ≤0.5 ≤0.5	- - -	- - -	
Ceftaroline	30 µg	≥26	-	-	≤0.5	-	-	**(8)** Breakpoints are based on a dosage regimen of 600 mg administered every 12 h.
CARBAPENEMS								
See comment (7).								
Doripenem*	-	-	-	-	≤0.12	-	-	
Ertapenem*	-	-	-	-	≤1	-	-	
Meropenem*	-	-	-	-	≤0.5	-	-	
GLYCOPEPTIDES								
Vancomycin	30 µg	≥17	-	-	≤1	-	-	
LIPOGLYCOPEPTIDES								
Dalbavancin	-	-	-	-	≤0.25	-	-	**(9) Report only on** *S. pyogenes*, *S. agalactiae*, and *S. dysgalactiae*. **(10)** Breakpoints are based on a dosage regimen of 1500 mg (single dose) or 1000 mg (two doses) IV administered over 30 minutes followed one week later by 500 mg IV administered over 30 minutes.
Oritavancin	-	-	-	-	≤0.25	-	-	**(11)** Breakpoints are based on a dosage regimen of 1200 mg IV administered once.
Telavancin	-	-	-	-	≤0.12	-	-	**(12)** Breakpoints are based on a dosage regimen of 10 mg/kg administered every 24 h.
LIPOPEPTIDES								
Daptomycin	-	-	-	-	≤1	-	-	**(13) Not routinely** reported **on organisms isolated** from the respiratory tract.

Table 2H-1. *Streptococcus* spp. β-Hemolytic Group (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
MACROLIDES								
(14) Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(15)** Not routinely reported on **organisms isolated** from the urinary tract.								
Erythromycin	15 µg	≥21	16-20	≤15	≤0.25	0.5	≥1	**(16)** ***Rx:*** Recommendations for intrapartum prophylaxis for group B streptococci are penicillin or ampicillin. Although cefazolin is recommended for penicillin-allergic women at low risk for anaphylaxis, those at high risk for anaphylaxis may receive clindamycin. Group B streptococci are susceptible to ampicillin, penicillin, and cefazolin, but may be resistant to erythromycin and clindamycin. When a group B *Streptococcus* is isolated from a pregnant woman with severe penicillin allergy (high risk for anaphylaxis), erythromycin and clindamycin (including ICR) should be tested, and only clindamycin should be reported. Erythromycin should be tested for ICR determination only and should not be reported. See Table 3I.
Azithromycin*	15 µg	≥18	14-17	≤13	≤0.5	1	≥2	
Clarithromycin*	15 µg	≥21	17-20	≤16	≤0.25	0.5	≥1	
Dirithromycin*	15 µg	≥18	14-17	≤13	≤0.5	1	≥2	
TETRACYCLINES								
(17) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, resistance to doxycycline and minocycline cannot be inferred from tetracycline resistance.								
Tetracycline	30 µg	≥23	19-22	≤18	≤2	4	≥8	
FLUOROQUINOLONES								
Levofloxacin	5 µg	≥17	14-16	≤13	≤2	4	≥8	
Gatifloxacin*	5 µg	≥21	18-20	≤17	≤1	2	≥4	
Grepafloxacin*	5 µg	≥19	16-18	≤15	≤0.5	1	≥2	
Ofloxacin*	5 µg	≥16	13-15	≤12	≤2	4	≥8	
Trovafloxacin*	10 µg	≥19	16-18	≤15	≤1	2	≥4	
PHENICOLS								
Chloramphenicol*	30 µg	≥21	18-20	≤17	≤4	8	≥16	See comment (15).

Table 2H-1. *Streptococcus* spp. ß-Hemolytic Group (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
LINCOSAMIDES								
Clindamycin	2 µg	≥19	16-18	≤15	≤0.25	0.5	≥1	See comments (15) and (16). **(18)** For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR by disk diffusion using the D-zone test or by broth microdilution is required before reporting clindamycin. See Table 3I, Subchapter 3.9 in M02,[3] and Subchapter 3.12 in M07.[1]
STREPTOGRAMINS								
Quinupristin-dalfopristin*	15 µg	≥19	16-18	≤15	≤1	2	≥4	**(19)** For reporting against *S. pyogenes* only.
OXAZOLIDINONES								
(20) *S. agalactiae* and *S. pyogenes* that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that are nonsusceptible to linezolid may be susceptible to tedizolid.								
Linezolid	30 µg	≥21	-	-	≤2	-	-	
Tedizolid	-	-	-	-	≤0.5	-	-	**(21) Report only on** *S. pyogenes* and *S. agalactiae*. **(22)** Breakpoints are based on a dosage regimen of 200 mg administered every 24 h.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; ICR, inducible clindamycin resistance; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.
Symbol: *, designation for "Other" agents not included in Tables 1 but have established clinical breakpoints.

Footnote

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

References for Table 2H-1

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

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Table 2H-2. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. Viridans Group

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood
Broth dilution: CAMHB with LHB (2.5% to 5% v/v); the CAMHB should be supplemented to 50 µg/mL calcium for daptomycin (see M07[1] for instructions for preparation of LHB)
Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard using colonies from an overnight (18- to 20-hour) sheep blood agar plate

Incubation: 35°C ± 2°C
Disk diffusion: 5% CO_2; 20–24 hours
Dilution methods: ambient air; 20–24 hours (CO_2 if necessary for growth with agar dilution)

Routine QC Recommendations (see Tables 4B and 5B for acceptable QC ranges)

S. pneumoniae ATCC®[a] 49619

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Refer to Table 1N for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not measure the zone of inhibition of hemolysis. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(3) For viridans streptococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[1] Figures 3 and 4).

(4) The viridans group of streptococci includes the following five groups, with several species within each group: *mutans* group, *salivarius* group, *bovis* group, *anginosus* group (previously *S. milleri* group), and *mitis* group. The *anginosus* group includes small colony-forming ß-hemolytic strains with groups A, C, F, and G antigens. For detailed information on the species within the groups, please refer to recent literature.

(5) Breakpoints for *Streptococcus* spp. viridans group are proposed based on population distributions of various species, pharmacokinetics of the antimicrobial agents, previously published literature, and the clinical experience of subcommittee members. Systematically collected clinical data were not available for review with many of the antimicrobial agents in this table.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2H-2. *Streptococcus* spp. Viridans Group (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm S	I	R	Interpretive Categories and MIC Breakpoints, µg/mL S	I	R	Comments
PENICILLINS								
Penicillin	-	-	-	-	≤0.12	0.25-2	≥4	**(6)** Viridans streptococci isolated from normally sterile anatomical sites (eg, CSF, blood, bone) should be tested for penicillin susceptibility using an MIC method. **(7)** A penicillin MIC of ≤0.125 µg/mL is the same as a penicillin MIC of ≤0.12 µg/mL and both should be interpreted as susceptible. Laboratories should report an MIC of ≤0.125 µg/mL as ≤0.12 µg/mL. **(8)** ***Rx:*** Penicillin- or ampicillin-intermediate isolates may necessitate combined therapy with an aminoglycoside for bactericidal action.
Ampicillin					≤0.25	0.5-4	≥8	
β-LACTAM COMBINATION AGENTS								
Ceftolozane-tazobactam	-	-	-	-	≤8/4	16/4	≥32/4	**(9)** Breakpoints are based on a dosage regimen of 1.5 g administered every 8 h.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Cefepime	30 µg	≥24	22-23	≤21	≤1	2	≥4	
Cefotaxime	30 µg	≥28	26-27	≤25	≤1	2	≥4	
Ceftriaxone	30 µg	≥27	25-26	≤24	≤1	2	≥4	
CARBAPENEMS								
Doripenem*	-	-	-	-	≤1	-	-	
Ertapenem*	-	-	-	-	≤1	-	-	
Meropenem*	-	-	-	-	≤0.5	-	-	
GLYCOPEPTIDES								
Vancomycin	30 µg	≥17	-	-	≤1	-	-	

Table 2H-2. *Streptococcus* spp. Viridans Group (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
LIPOGLYCOPEPTIDES								
Dalbavancin	-	-	-	-	≤0.25	-	-	**(10)** Breakpoints are based on a dosage regimen of 1500 mg (single dose) or 1000 mg (two doses) IV administered over 30 minutes followed one week later by 500 mg IV administered over 30 minutes. **(11) Report only on** *S. anginosus* group (includes *S. anginosus*, *S. intermedius*, and *S. constellatus*).
Oritavancin	-	-	-	-	≤0.25	-	-	**(12)** Breakpoints are based on a dosage regimen of 1200 mg IV administered once.
Telavancin	-	-	-	-	≤0.06	-	-	**(13)** Breakpoints are based on a dosage regimen of 10 mg/kg administered every 24 h.
LIPOPEPTIDES								
Daptomycin*	-	-	-	-	≤1	-	-	**(14) Not routinely** reported **on organisms isolated** from the respiratory tract.
MACROLIDES								
(15) Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(16)** Not routinely reported on **organisms isolated** from the urinary tract.								
Erythromycin	15 µg	≥21	16-20	≤15	≤0.25	0.5	≥1	
Azithromycin*	15 µg	≥18	14-17	≤13	≤0.5	1	≥2	
Clarithromycin*	15 µg	≥21	17-20	≤16	≤0.25	0.5	≥1	
Dirithromycin*	15 µg	≥18	14-17	≤13	≤0.5	1	≥2	
TETRACYCLINES								
(17) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, resistance to doxycycline and minocycline cannot be inferred from tetracycline resistance.								
Tetracycline*	30 µg	≥23	19-22	≤18	≤2	4	≥8	
FLUOROQUINOLONES								
Levofloxacin	5 µg	≥17	14-16	≤13	≤2	4	≥8	
Ofloxacin*	5 µg	≥16	13-15	≤12	≤2	4	≥8	
Gatifloxacin*	5 µg	≥21	18-20	≤17	≤1	2	≥4	
Grepafloxacin*	5 µg	≥19	16-18	≤15	≤0.5	1	≥2	
Trovafloxacin*	10 µg	≥19	16-18	≤15	≤1	2	≥4	

Table 2H-2. *Streptococcus* spp. Viridans Group (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm S	I	R	Interpretive Categories and MIC Breakpoints, µg/mL S	I	R	Comments
PHENICOLS								
Chloramphenicol*	30 µg	≥21	18-20	≤17	≤4	8	≥16	See comment (16).
LINCOSAMIDES								
Clindamycin	2 µg	≥19	16-18	≤15	≤0.25	0.5	≥1	See comment (16).
STREPTOGRAMINS								
Quinupristin-dalfopristin*	15 µg	≥19	16-18	≤15	≤1	2	≥4	
OXAZOLIDINONES								
(18) *S. anginosus* group that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that are nonsusceptible to linezolid may be susceptible to tedizolid.								
Linezolid	30 µg	≥21	-	-	≤2	-	-	
Tedizolid	-	-	-	-	≤0.25	-	-	**(19)** Breakpoints are based on a dosage regimen of 200 mg administered every 24 h. See comment (11).

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CSF, cerebrospinal fluid; I, intermediate; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

<u>Footnote</u>

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

Reference for Table 2H-2

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

Table 2I. Zone Diameter and MIC Breakpoints for *Neisseria meningitidis*

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood
Broth microdilution: CAMHB supplemented with LHB (2.5% to 5% v/v) (see M07[1] for preparation of LHB)
Agar dilution: MHA supplemented with sheep blood (5% v/v)

Inoculum: Colony suspension from 20–24 hours growth from chocolate agar incubated at 35°C; 5% CO_2; equivalent to a 0.5 McFarland standard. Colonies grown on sheep blood agar may be used for inoculum preparation. However, the 0.5 McFarland suspension obtained from sheep blood agar will contain approximately 50% fewer CFU/mL. This must be considered when preparing the final dilution before panel inoculation, as guided by colony counts.

Incubation: 35°C ± 2°C; 5% CO_2; 20–24 hours

Routine QC Recommendations (See Tables 4A-1, 4B, 5A-1, and 5B for acceptable QC ranges.)

Streptococcus pneumoniae ATCC®[a] 49619:

Disk diffusion: incubate in 5% CO_2.

Broth microdilution: incubate in ambient air or CO_2 (except azithromycin QC tests that must be incubated in ambient air).

E. coli ATCC® 25922

Disk diffusion, broth microdilution or agar dilution for ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole: incubate in ambient air or CO_2.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

Important: For complete information on safety precautions, see *Biosafety in Microbiological and Biomedical Laboratories*. 6th ed. Washington, DC: US Department of Health and Human Services; 2020. Accessed 10 January 2023. http://www.cdc.gov/biosafety/publications/bmbl5/

(1) **Recommended precautions:** Perform all AST of *N. meningitidis* in a BSC. Manipulating *N. meningitidis* outside a BSC is associated with increased risk for contracting meningococcal disease. Laboratory-acquired meningococcal disease is associated with a case fatality rate of 50%. Exposure to droplets or aerosols of *N. meningitidis* is the most likely risk for laboratory-acquired infection. Rigorous protection from droplets or aerosols is mandated when microbiological procedures (including AST) are performed on all *N. meningitidis* isolates.

(2) If a BSC is unavailable, manipulation of these isolates should be minimized, limited to Gram staining or serogroup identification using phenolized saline solution, while wearing a laboratory coat and gloves and working behind a full face splash shield. Use BSL-3 practices, procedures, and containment equipment for activities with a high potential for droplet or aerosol production and for activities involving production quantities or high concentrations of infectious materials. If BSL-2 or BSL-3 facilities are not available, forward isolates to a referral or public health laboratory with a minimum of BSL-2 facilities.

(3) Laboratorians who are exposed routinely to potential aerosols of *N. meningitidis* should consider vaccination according to the current recommendations of the Centers for Disease Control and Prevention Advisory Committee on Immunization Practices. Accessed 10 January 2023. http://www.cdc.gov/vaccines/acip/index.html

Table 2I. *Neisseria meningitidis* (Continued)

(4) For disk diffusion, test a maximum of 5 disks on a 150-mm plate and 2 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(5) Breakpoints are based on population distributions of MICs of various agents, pharmacokinetics of the agents, previously published literature, and the clinical experience of subcommittee members. Systematically collected clinical data were not available to review with many of the antimicrobial agents in this table.

(6) With azithromycin, breakpoints were developed initially using MICs determined by incubation in ambient air for the pharmacodynamic calculations.

NOTE: Information in black boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Penicillin*		-	-	-	≤0.06	0.12-0.25	≥0.5	**(7)** Breakpoints for ampicillin are based on a dosage regimen of 2 g administered every 4 h.
Ampicillin*		-	-	-	≤0.12	0.25-1	≥2	
CEPHEMS								
Cefotaxime or*	30 µg	≥34	-	-	≤0.12	-	-	
Ceftriaxone*	30 µg	≥34	-	-	≤0.12	-	-	
CARBAPENEMS								
Meropenem*	10 µg	≥30	-	-	≤0.25	-	-	
MACROLIDES								
Azithromycin*	15 µg	≥20	-	-	≤2	-	-	See general comment (6). **(8)** May be appropriate only for prophylaxis of meningococcal case contacts. These breakpoints do not apply to therapy of patients with invasive meningococcal disease.
TETRACYCLINES								
Minocycline*	30 µg	≥26	-	-	≤2	-	-	See comment (8).
FLUOROQUINOLONES								
(9) For surveillance purposes, a nalidixic acid MIC ≥8 µg/mL or a zone ≤25 mm may correlate with diminished fluoroquinolone susceptibility.								
Ciprofloxacin*	5 µg	≥35	33-34	≤32	≤0.03	0.06	≥0.12	See comment (8).
Levofloxacin*	-	-	-	-	≤0.03	0.06	≥0.12	

Table 2I. *Neisseria meningitidis* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
FOLATE PATHWAY ANTAGONISTS								
Sulfisoxazole*	-	-	-	-	≤2	4	≥8	See comment (8).
Trimethoprim-sulfamethoxazole*	1.25/23.75 µg	≥30	26-29	≤25	≤0.12/2.4	0.25/4.75	≥0.5/9.5	**(10)** Trimethoprim-sulfamethoxazole is the preferred disk for detection of sulfonamide resistance. Trimethoprim-sulfamethoxazole testing predicts susceptibility and resistance to trimethoprim-sulfamethoxazole and sulfonamides. Sulfonamides may be appropriate only for prophylaxis of meningococcal case contacts.
PHENICOLS								
Chloramphenicol*	30 µg	≥26	20-25	≤19	≤2	4	≥8	**(11)** Not routinely reported on **organisms isolated** from the urinary tract.
ANSAMYCINS								
Rifampin*	5 µg	≥25	20-24	≤19	≤0.5	1	≥2	See comment (8).

Abbreviations: AST, antimicrobial susceptibility testing; ATCC®, American Type Culture Collection; BSC, biological safety cabinet; BSL-2, biosafety level 2; BSL-3, biosafety level 3; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); I, intermediate; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Symbol: *, designation for "Other" agents not included in Tables 1 but have established clinical breakpoints.

Footnote

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

Reference for Table 2I

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

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Table 2J. MIC Breakpoints for Anaerobes

Testing Conditions

Medium: Agar dilution (for all anaerobes): Brucella agar supplemented with hemin (5 µg/mL), vitamin K_1 (1 µg/mL), and laked sheep blood (5% v/v)
Broth microdilution (for *Bacteroides* spp. and *Parabacteroides* spp. only): Brucella broth supplemented with hemin (5 µg/mL), vitamin K_1 (1 µg/mL), and LHB (5% v/v)

Inoculum: Broth culture method or colony suspension, equivalent to 0.5 McFarland suspension
Agar: 10^5 CFU per spot
Broth: 10^6 CFU/mL

Incubation: 36°C ± 1°C, anaerobically
Broth microdilution: 46–48 hours
Agar dilution: 42–48 hours

Routine QC Recommendations (see Tables 5D and 5E for acceptable QC ranges)

Test one or more of the following organisms. The choice and number of QC strains tested should be based on obtaining on-scale end points for the antimicrobial agent tested.

B. fragilis ATCC®[a] 25285
Bacteroides thetaiotaomicron ATCC® 29741
Clostridioides (formerly *Clostridium*) *difficile* ATCC® 700057
Eggerthella lenta (formerly *Eubacterium lentum*) ATCC® 43055

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Refer to Tables 1O (gram-negative anaerobes) and 1P (gram-positive anaerobes) for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For isolates for which the antimicrobial agent MICs fall within the intermediate category, maximum dosages, along with proper ancillary therapy, should be used to achieve the best possible levels of drug in abscesses and/or poorly perfused tissues. If this approach is taken, organisms for which the antimicrobial agent MICs fall within the susceptible range are generally amenable to therapy. Organisms for which the antimicrobial agent MICs are in the intermediate range may respond, but in such cases, efficacy as measured by patient clinical response should be carefully monitored. Ancillary therapy, such as drainage procedures and debridement, are of great importance for proper management of anaerobic infections.

(3) Refer to Figures 3 and 4 in CLSI document M11[1] for examples of reading end points.

(4) MIC values using either Brucella blood agar or Wilkins Chalgren agar (former reference medium) are considered equivalent.

(5) Broth microdilution is recommended only for testing *Bacteroides* spp. and *Parabacteroides* spp. MIC values for agar or broth microdilution are considered equivalent for those species.

(6) Until additional studies are performed to validate broth microdilution for testing other organisms, it should be used only for testing members of *Bacteroides* spp. and *Parabacteroides* spp.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 2J. Anaerobes (Continued)

Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL S	I	R	Comments
PENICILLINS				
Ampicillin Penicillin	≤0.5 ≤0.5	1 1	≥2 ≥2	**(7)** Ampicillin and penicillin are recommended for primary testing and reporting for gram-positive organisms **(Tier 1; see Table 1P)** because most of them are β-lactamase negative, but not for gram-negative organisms because many are β-lactamase positive **(Tier 4; see Table 1O).** **(8)** *Bacteroides* spp. are intrinsically resistant to penicillin and ampicillin. *Parabacteroides* spp. are presumed to be resistant to penicillin and ampicillin. Other gram-negative and gram-positive anaerobes may be screened for β-lactamase activity with a chromogenic cephalosporin; if β-lactamase positive, report as resistant to penicillin, ampicillin, and amoxicillin. Be aware that β-lactamase-negative isolates may be resistant to β-lactams by other mechanisms. Because higher blood levels are achievable with these antimicrobial agents, infection with non-β-lactamase-producing organisms with higher MICs (2-4 µg/mL) with adequate dosage regimen might be treatable. **(9)** Results of ampicillin testing can be used to predict results for amoxicillin.
β-LACTAM COMBINATION AGENTS				
(10) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.				
Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam	≤4/2 ≤8/4 ≤16/4	8/4 16/8 32/4-64/4	≥16/8 ≥32/16 ≥128/4	
Imipenem-relebactam	≤4/4	8/4	≥16/4	**(11)** Breakpoints are based on a dosage regimen of 1.25 g administered every 6 h.
Ticarcillin-clavulanate*	≤32/2	64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)				
Cefotetan Cefoxitin	≤16 ≤16	32 32	≥64 ≥64	
Ceftizoxime* Ceftriaxone	≤32 ≤16	64 32	≥128 ≥64	
Cefmetazole*	≤16	32	≥64	
Cefoperazone*	≤16	32	≥64	
Cefotaxime*	≤16	32	≥64	

Table 2J. Anaerobes (Continued)

Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL			Comments
	S	I	R	
CARBAPENEMS				
Doripenem*	≤2	4	≥8	
Ertapenem	≤4	8	≥16	
Imipenem	≤4	8	≥16	
Meropenem	≤4	8	≥16	
TETRACYCLINES				
Tetracycline	≤4	8	≥16	
FLUOROQUINOLONES				
Moxifloxacin	≤2	4	≥8	
LINCOSAMIDES				
Clindamycin	≤2	4	≥8	
PHENICOLS				
Chloramphenicol*	≤8	16	≥32	
NITROIMIDAZOLES				
Metronidazole	≤8	16	≥32	**(12)** Many non-spore-forming, gram-positive anaerobic rods are resistant to metronidazole **(see Appendix D).**

Abbreviations: ATCC®, American Type Culture Collection; CFU, colony-forming unit(s); I, intermediate; LHB, lysed horse blood; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Symbol: *, designation for "Other" agents not included in Tables 1 but have established clinical breakpoints.

Footnote

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

Reference for Table 2J

1 CLSI. *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria.* 9th ed. CLSI standard M11. Clinical and Laboratory Standards Institute; 2018.

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Table 3A. Tests for Extended-Spectrum ß-Lactamases in *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, and *Proteus mirabilis*

NOTE 1: Following evaluation of PK/PD properties, limited clinical data, and MIC distributions, revised breakpoints for cefazolin, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, and aztreonam were published in January 2010 (M100-S20) and are listed in Table 2A. Cefuroxime (parenteral) was also evaluated; however, no change in breakpoints was necessary with the dosage. When using the current breakpoints, routine ESBL testing is not necessary before reporting results. **If ESBL testing is performed, the results may be used to guide therapeutic management or for epidemiological or infection prevention purposes.**

Some phenotypic ESBL tests have known limitations that affect sensitivity (eg, false-negative results due to the coproduction of an AmpC ß-lactamase) and specificity (eg, false-positive results due to hyperproduction of non-ESBL ß-lactamases combined with altered permeability). Genotypic methods are limited by the targets included in the assay (eg, most FDA-cleared ESBL assays target only *bla*$_{CTX-M}$). Limitations of phenotypic and genotypic methods must be considered.

Breakpoints for drugs with limited availability in many countries (eg, moxalactam, cefonicid, cefamandole, and cefoperazone) were not evaluated. If considering use of these drugs for *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, or *Proteus mirabilis*, ESBL testing should be performed. If isolates test ESBL positive, the results for moxalactam, cefonicid, cefamandole, and cefoperazone should be reported as resistant.

NOTE 2: Information in black boldface type is new or modified since the previous edition.

Table 3A. (Continued)

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
Medium	MHA	CAMHB	MHA	CAMHB
Antimicrobial concentration	For *K. pneumoniae*, *K. oxytoca*, and *E. coli*: Cefpodoxime 10 µg or Ceftazidime 30 µg or Aztreonam 30 µg or Cefotaxime 30 µg or Ceftriaxone 30 µg For *P. mirabilis*: Cefpodoxime 10 µg or Ceftazidime 30 µg or Cefotaxime 30 µg (Testing more than one antimicrobial agent improves the sensitivity of ESBL detection.)	For *K. pneumoniae*, *K. oxytoca*, and *E. coli*: Cefpodoxime 4 µg/mL or Ceftazidime 1 µg/mL or Aztreonam 1 µg/mL or Cefotaxime 1 µg/mL or Ceftriaxone 1 µg/mL For *P. mirabilis*: Cefpodoxime 1 µg/mL or Ceftazidime 1 µg/mL or Cefotaxime 1 µg/mL (Testing more than one antimicrobial agent improves the sensitivity of ESBL detection.)	Ceftazidime 30 µg Ceftazidime-clavulanate[a] 30/10 µg and Cefotaxime 30 µg Cefotaxime-clavulanate 30/10 µg (Testing necessitates using both cefotaxime and ceftazidime, alone and in combination with clavulanate.)	Ceftazidime 0.25–128 µg/mL Ceftazidime-clavulanate 0.25/4–128/4 µg/mL and Cefotaxime 0.25–64 µg/mL Cefotaxime-clavulanate 0.25/4–64/4 µg/mL (Testing necessitates using both cefotaxime and ceftazidime, alone and in combination with clavulanate.)
Inoculum	Standard disk diffusion procedure	Standard broth dilution procedure	Standard disk diffusion procedure	Standard broth dilution procedure
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	16–18 hours	16–20 hours	16–18 hours	16–20 hours

Table 3A. (Continued)

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
Results	For *K. pneumoniae*, *K. oxytoca*, and *E. coli*:	Growth at or above the concentrations listed may indicate ESBL production (ie, for *E. coli*, *K. pneumoniae*, and *K. oxytoca*, MIC ≥8 µg/mL for cefpodoxime or MIC ≥2 µg/mL for ceftazidime, aztreonam, cefotaxime, or ceftriaxone; and for *P. mirabilis*, MIC ≥2 µg/mL for cefpodoxime, ceftazidime, or cefotaxime).	A ≥5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone = ESBL (eg, ceftazidime zone = 16; ceftazidime-clavulanate zone = 21).	A ≥3 2-fold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone = ESBL (eg, ceftazidime MIC = 8 µg/mL; ceftazidime-clavulanate MIC = 1 µg/mL).
	Cefpodoxime zone ≤17 mm Ceftazidime zone ≤22 mm Aztreonam zone ≤27 mm Cefotaxime zone ≤27 mm Ceftriaxone zone ≤25 mm			
	For *P. mirabilis*:			
	Cefpodoxime zone ≤22 mm Ceftazidime zone ≤22 mm Cefotaxime zone ≤27 mm			
	Zones above may indicate ESBL production.			
Reporting			For all confirmed ESBL-producing strains: If laboratories use current cephalosporin and aztreonam breakpoints, test interpretations for these agents do not need to be changed from susceptible to resistant.	

Table 3A. (Continued)

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
QC recommendations	When testing antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC®[b] 700603 is provided as a supplemental QC strain (eg, for training, competence assessment, or test evaluation). Either strain, *K. pneumoniae* ATCC® 700603 or *E. coli* ATCC® 25922, may then be used for routine QC (eg, weekly or daily).	When testing antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC® 700603 is provided as a supplemental QC strain (eg, for training, competence assessment, or test evaluation). Either strain, *K. pneumoniae* ATCC® 700603 or *E. coli* ATCC® 25922, may then be used for routine QC (eg, weekly or daily).	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be used for routine QC (eg, weekly or daily).	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be tested routinely (eg, weekly or daily).
	E. coli ATCC® 25922 (see acceptable QC ranges in Table 4A-1)	*E. coli* ATCC® 25922 = no growth (see acceptable QC ranges listed in Table 5A-1)	**Acceptable QC:** *E. coli* ATCC® 25922: ≤ 2-mm increase in zone diameter for antimicrobial agent tested in combination with clavulanate vs the zone diameter when tested alone.	**Acceptable QC:** *E. coli* ATCC® 25922: < 3 2-fold concentration decrease in MIC for antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone.
	K. pneumoniae ATCC® 700603: Cefpodoxime zone 9-16 mm Ceftazidime zone 10-18 mm Aztreonam zone 10-16 mm Cefotaxime zone 17-25 mm Ceftriaxone zone 16-24 mm	*K. pneumoniae* ATCC® 700603 = Growth: Cefpodoxime MIC ≥ 8 µg/mL Ceftazidime MIC ≥ 2 µg/mL Aztreonam MIC ≥ 2 µg/mL Cefotaxime MIC ≥ 2 µg/mL Ceftriaxone MIC ≥ 2 µg/mL	*K. pneumoniae* ATCC® 700603: ≥ 5-mm increase in zone diameter of ceftazidime-clavulanate vs ceftazidime alone; ≥ 3-mm increase in zone diameter of cefotaxime-clavulanate vs cefotaxime alone.	*K. pneumoniae* ATCC® 700603: ≥ 3 2-fold concentration decrease in MIC for an antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; ESBL, extended-spectrum β-lactamase; **FDA, US Food and Drug Administration;** MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control.

Footnotes

a. Preparation of ceftazidime-clavulanate (30 µg/10 µg) and cefotaxime-clavulanate (30 µg/10 µg) disks: Using a stock solution of clavulanate at 1000 µg/mL (either freshly prepared or taken from small aliquots that have been frozen at −70°C), add 10 µL of clavulanate to ceftazidime (30 µg) and cefotaxime (30 µg) disks. Use a micropipette to apply the 10 µL of stock solution to the ceftazidime and cefotaxime disks within one hour before they are applied to the plates, allowing about 30 minutes for the clavulanate to absorb and the disks to be dry enough for application. Use disks immediately after preparation or discard; do not store.

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

Introduction to Tables 3B and 3C. Tests for Carbapenemases in Enterobacterales and *Pseudomonas aeruginosa*

Institutional treatment guidelines, infection prevention procedures, or epidemiological investigations may necessitate identification of carbapenemase-producing Enterobacterales and *P. aeruginosa*.[1]

Carbapenemase-producing isolates of Enterobacterales usually test intermediate or resistant to one or more carbapenems using the current breakpoints as listed in Table 2A (**NOTE:** Testing not susceptible to ertapenem is often the most sensitive indicator of carbapenemase production) and usually test resistant to one or more agents in cephalosporin subclass III (eg, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone). However, some isolates that produce carbapenemases such as SME or IMI often test susceptible to these cephalosporins.

NOTE: Information in black boldface type is new or modified since the previous edition.

Introduction to Tables 3B and 3C. (Continued)

	Tests Used for Epidemiological or Infection Prevention-Related Testing			
	CarbaNP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Organisms	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales that are positive by mCIM	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by CarbaNP or mCIM.
Strengths	Rapid	No special reagents or media necessary	No special reagents or media necessary	Determines type of carbapenemase in addition to absence or presence of the enzyme
Limitations	Special reagents are needed, some of which necessitate in-house preparation (and have a short shelf life). Invalid results occur with some isolates. Certain carbapenemase types (eg, OXA-type, chromosomally encoded) are not consistently detected.	Requires overnight incubation	Requires overnight incubation	Special reagents and equipment are needed. Specific to targeted genes; false-negative result if specific carbapenemase gene present is not targeted.

Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; mCIM, modified carbapenem inactivation method, MIC, minimal inhibitory concentration.

Reference for Introduction to Tables 3B and 3C

1 **Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. IDSA Guidance on the treatment of antimicrobial-restant gram-negative infections: version 2.0. Infectious Diseases Society of America; 2022. Accessed 10 January 2023. https://www.idsociety.org/practice-guideline/amr-guidance-2.0/**

Table 3B. CarbaNP Test for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*[1-7]

Test	CarbaNP Test
When to perform this test	For **treatment (per institutional guidelines), infection prevention procedures, or epidemiological investigations.** **NOTE:** No change in the interpretation of carbapenem susceptibility test results is necessary for CarbaNP-positive isolates. Such testing is not currently recommended for routine use.
Test method	Colorimetric microtube assay
Test reagents and materials	• Clinical laboratory reagent water • Imipenem reference standard powder • Commercially available bacterial protein extraction reagent in Tris HCl buffer, pH 7.4 • Zinc sulfate heptahydrate • Phenol red powder • 1 N NaOH solution • 10% HCl solution • Microcentrifuge tubes 1.5 mL, clear • 1-µL inoculation loops • Containers to store prepared solutions Use reagents above to prepare the following solutions (instructions for preparation are provided below this table): • 10 mM zinc sulfate heptahydrate solution • 0.5% phenol red solution • 0.1 N sodium hydroxide solution • CarbaNP Solution A • CarbaNP Solution B (solution A + imipenem)
Test procedure	1. Label two microcentrifuge tubes (one “a” and one “b”) for each patient isolate, QC organism, and uninoculated reagent control. 2. Add 100 µL of bacterial protein extraction reagent to each tube. 3. For each isolate to be tested, emulsify a 1-µL loopful of bacteria from an overnight blood agar plate in both tubes “a” and “b.” Vortex each tube for 5 seconds. (Uninoculated reagent control tubes should contain only bacterial protein extraction reagent, no organism.) **NOTE:** Do not use growth from selective media or plates containing antibiotics or other agents that select for certain bacteria. 4. Add 100 µL of solution A to tube “a.” 5. Add 100 µL of solution B to tube “b.” 6. Vortex tubes well. 7. Incubate at 35°C ± 2°C for up to 2 hours. Isolates that demonstrate positive results before 2 hours can be reported as carbapenemase producers.

Table 3B. (Continued)

Test	CarbaNP Test
Test interpretation	Strategy for reading (see Figure 1, below): 1. Read uninoculated reagent control tubes “a” and “b” (ie, “blanks”). • Both tubes must be red or red-orange. • If either tube is any other color, the test is invalid. 2. Read inoculated tube “a.” • Tube “a” must be red or red-orange. • If tube “a” is any other color, the test is invalid. 3. Read inoculated tube “b.” • Red or red-orange = negative • Light orange, dark yellow, or yellow = positive • Orange = invalid 4. Interpret results as follows:

Results for Patient and QC Tubes

Tube “a”: Solution A (serves as internal control)	Tube “b”: Solution B	Interpretation
Red or red-orange	Red or red-orange	Negative, no carbapenemase detected
Red or red-orange	Light orange, dark yellow, or yellow	Positive, carbapenemase producer
Red or red-orange	Orange	Invalid
Orange, light orange, dark yellow, or yellow	Any color	Invalid

Table 3B. (Continued)

Test	CarbaNP Test
Test interpretation (Continued)	**NOTES:** A slight color change may be observed with the addition of imipenem to solution A. Compare patient tubes to the uninoculated reagent control tubes when interpreting questionable results. For invalid results: • Check reagents for QC strains and uninoculated reagent controls. Reagent deterioration can cause invalid results. An invalid result for an uninoculated reagent control test indicates a problem with solution A and/or solution B. Check the pH of solution A. If pH is < 7.8, prepare fresh solution A and solution B. • Repeat the test, including the uninoculated reagent controls. • If the repeat test is invalid, perform molecular assay.
Reporting	Report positive as “Carbapenemase producer.” Report negative as “No carbapenemase detected.”
QC recommendations	Test positive and negative QC strains and uninoculated reagent control tubes each day of testing. *K. pneumoniae* ATCC®[a] BAA-1705™—carbapenemase positive *K. pneumoniae* ATCC® BAA-1706™—carbapenemase negative Results for uninoculated reagent control tubes “a” and “b” must be negative (ie, red or red-orange). Any other result invalidates all tests performed on that day with the same lot of reagents. The addition of imipenem to tube “b” might cause tube “b” to appear red-orange when tube “a” is red.

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; pH, negative logarithm of hydrogen ion concentration; QC, quality control.

Table 3B. (Continued)

Footnote

a. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

NOTE 1: Test recommendations were largely derived following testing of US isolates of Enterobacterales and *P. aeruginosa* and provide for a high level of sensitivity (> 90%) and specificity (> 90%) in detecting KPC, NDM, VIM, IMP, SPM, and SME-type carbapenemases in these isolates. The sensitivity and specificity of the test for detecting other carbapenemase production can vary. The ability of this test, as listed in the above procedure, to detect OXA-48-like producers is poor.[6,7]

NOTE 2: In CLSI studies, two KPC-positive strains with low carbapenem MICs (one *E. cloacae* susceptible by MIC to all three carbapenems and one *E. coli* that was susceptible to meropenem and intermediate to imipenem and ertapenem) were not detected by this test.

NOTE 3: Additional investigations of CarbaNP with *Acinetobacter* spp. showed poor sensitivity (ie, 21.3% for *A. baumannii*); therefore, the previous recommendation for use of CarbaNP with *Acinetobacter* spp. was removed.

NOTE 4: Information in black boldface type is new or modified since the previous edition.

Table 3B. (Continued)

Instructions for Preparing Test Components

The steps for preparing 10 mM zinc sulfate heptahydrate solution are listed below.

Step	Action	Comment
1	Weigh out 1.4 g of $ZnSO_4 \bullet 7H2O$.	
2	Add the powder to 500 mL clinical laboratory reagent water.	
3	Mix the solution.	
4	Store the solution at room temperature.	Expiration is 1 year or not to exceed expiration of individual components

The steps for preparing 0.5% phenol red solution are listed below.

Step	Action	Comment
1	Weigh out 1.25 g of phenol red powder.	
2	Add the powder to 250 mL clinical laboratory reagent water.	
3	Mix the solution.	
4	Store the solution at room temperature.	Expiration is 1 year or not to exceed expiration of individual components. **NOTE:** This solution does not remain in solution. Mix well before use.

The steps for preparing 0.1 N sodium hydroxide solution are listed below.

Step	Action	Comment
1	Add 20 mL of 1 N NaOH to 180 mL clinical laboratory reagent water.	
2	Store the solution at room temperature.	Expiration is 1 year or not to exceed expiration of individual components

Table 3B. (Continued)

The steps for preparing CarbaNP solution A are listed below.

Step	Action	Comment
1	To a 25- to 50-mL beaker, add 2 mL of 0.5% phenol red solution to 16.6 mL clinical laboratory reagent water.	
2	Add 180 µL of 10 mM zinc sulfate solution.	
3	Adjust the pH to 7.8 ± 0.1 with 0.1 N NaOH solution (or 10% HCl solution if pH is too high).	10% HCl solution can be used if the pH is too high.
4	Store the solution at 4 to 8°C in a small vial or bottle.	Protect the solution from prolonged light exposure. Expiration is 2 weeks or not to exceed expiration of individual components (solution should remain red or red-orange; do not use if solution turns any other color).

The steps for preparing CarbaNP solution B (solution A + 6 mg/mL imipenem) are listed below.

Step	Action	Comment
1	Determine the amount of solution B needed, allowing 100 µL per tube for each patient, QC strain, and uninoculated reagent control.	**Example:** To test 2 patient isolates, positive and negative controls and an uninoculated reagent control, 500 µL of solution B is needed.
2	Weigh out approximately 10–20 mg of imipenem powder.	It is advisable to weigh out at least 10 mg of powder. Divide the actual weight by 6 to determine the amount (in mL) of solution A to add to the powder. **Example:** 18 mg of imipenem / 6 = 3 mL of solution A, which is sufficient for 30 tubes.
3	Store the solution at 4 to 8°C for up to 3 days.	

Table 3B. (Continued)

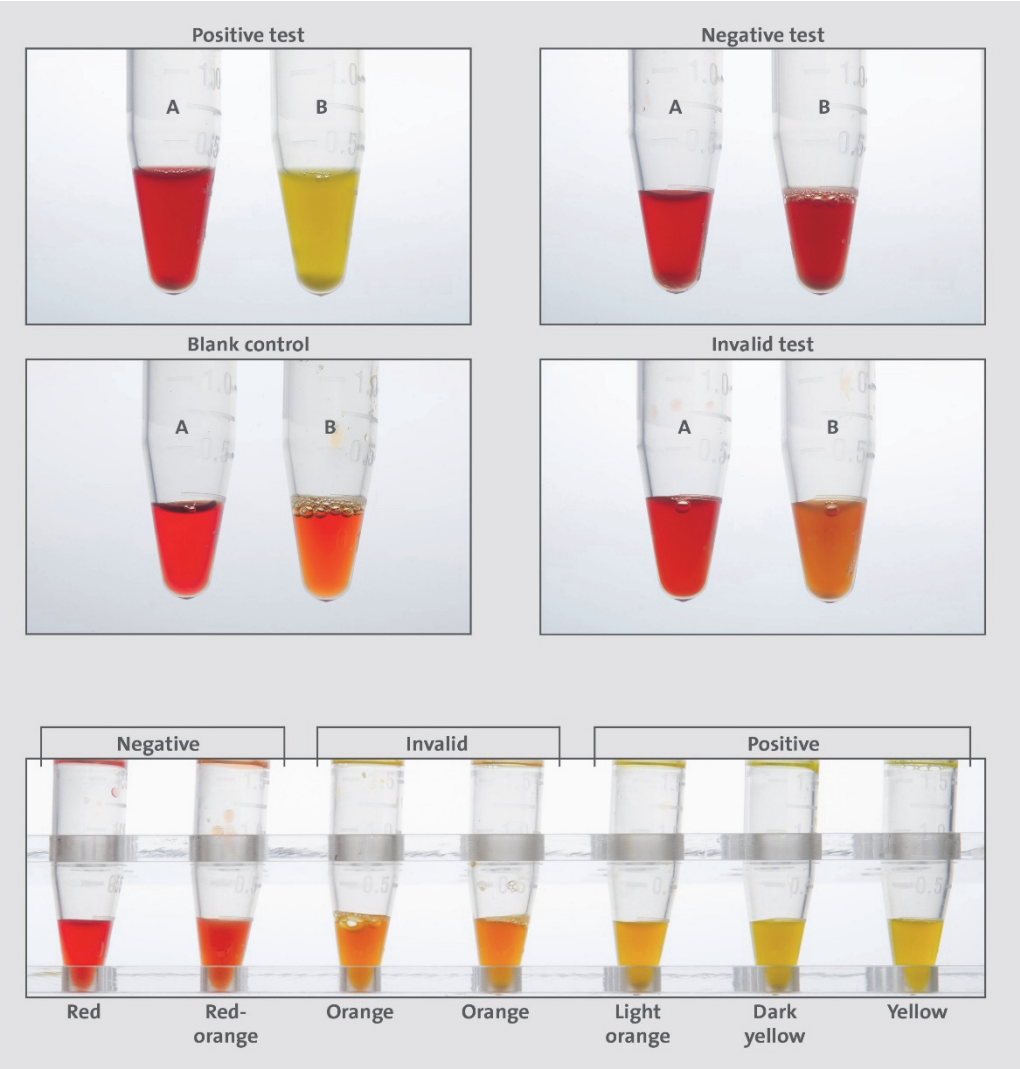


Figure 1. Interpretation of Color Reactions

Table 3B. (Continued)

References for Table 3B

1. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis*. 2012;18(9):1503-1507.

2. Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing *Pseudomonas spp*. *J Clin Microbiol*. 2012;50(11):3773-3776.

3. Dortet L, Poirel L, Nordmann P. Rapid identification of carbapenemase types in Enterobacteriaceae and *Pseudomonas* spp. by using a biochemical test. *Antimicrob Agents Chemother*. 2012;56(12):6437-6440.

4. Cunningham SA, Noorie T, Meunier D, Woodford N, Patel R. Rapid and simultaneous detection of genes encoding *Klebsiella pneumoniae* carbapenemase (bla_{KPC}) and New Delhi metallo-β-lactamase (bla_{NDM}) in Gram-negative bacilli. *J Clin Microbiol*. 2013;51(4):1269-1271.

5. Vasoo S, Cunningham SA, Kohner PC, et al. Comparison of a novel, rapid chromogenic biochemical assay, the Carba NP test, with the modified Hodge test for detection of carbapenemase-producing gram-negative bacilli. *J Clin Microbiol*. 2013;51(9):3097-3101.

6. Lutgring JD, Zhu W, de Man TJB, et al. Phenotypic and genotypic characterization of Enterobacteriaceae producing oxacillinase-48-like carbapenemases, United States. *Emerg Infect Dis*. 2018;24(4):700-709.

7. Cunningham SA, Limbago B, Traczewski M, et al. Multicenter performance assessment of Carba NP test. *J Clin Microbiol*. 2017;55(6):1954-1960.

Table 3C. Modified Carbapenem Inactivation Methods for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*[1-6]

Test	mCIM Only or in Conjunction With eCIM
When to perform this test:	For **treatment (per institutional guidelines), infection prevention procedures, or epidemiological investigations.** **NOTE:** No change in the interpretation of carbapenem susceptibility test results is necessary for mCIM positive and/or eCIM results. mCIM with or without eCIM testing is not currently recommended for routine use. • mCIM is used for detecting carbapenemases in Enterobacterales and *P. aeruginosa* whereas eCIM is used together with mCIM to differentiate metallo-ß-lactamases from serine carbapenemases in Enterobacterales. • mCIM can be performed alone; however, eCIM must be performed together with mCIM. • eCIM is valid only if mCIM is positive.
Test method	Meropenem disk inactivation
Test reagents and materials	• TSB (2 mL aliquots) • Meropenem disks (10 µg) • 1-µL and 10-µL inoculation loops • Nutrient broth (eg, Mueller-Hinton, TSB) or normal saline (3.0–5.0 mL aliquots) • MHA plates (100 mm or 150 mm) • Meropenem-susceptible indicator strain – *E. coli* (ATCC®[a] 25922) • 0.5 M EDTA (only for eCIM)

Table 3C. (Continued)

Test	mCIM Only or in Conjunction With eCIM
Test procedure: mCIM	1. For each isolate to be tested, emulsify a 1-µL loopful of bacteria for Enterobacterales or 10-µL loopful of bacteria for *P. aeruginosa* from an overnight blood agar plate in 2 mL TSB. 2. Vortex for 10–15 seconds. 3. Add a 10-µg meropenem disk to each tube using sterile forceps or a single disk dispenser. Ensure the entire disk is immersed in the suspension. 4. Incubate at 35°C ± 2°C in ambient air for 4 hours ± 15 minutes. 5. Just before or immediately following completion of the TSB-meropenem disk suspension incubation, prepare a 0.5 McFarland suspension (using the colony suspension method) of *E. coli* ATCC® 25922 in nutrient broth or saline. 6. Inoculate an MHA plate with *E. coli* ATCC® 25922 as for the routine disk diffusion procedure (see M02[4]) making sure the inoculum suspension preparation and MHA plate inoculation steps are each completed within 15 minutes. Allow the plates to dry for 3–10 minutes before adding the meropenem disks. 7. Remove the meropenem disk from each TSB-meropenem disk suspension using a 10-µL loop by placing the flat side of the loop against the flat edge of the disk and using surface tension to pull the disk out of the liquid. Carefully drag and press the loop along the inside edge of the tube to expel excess liquid from the disk. Continue using the loop to remove the disk from the tube and then place it on the MHA plate previously inoculated with the meropenem-susceptible *E. coli* ATCC® 25922 indicator strain. Disk capacity: 4 disks on a 100 mm MHA plate; 8 disks on a 150 mm MHA plate (see Figure 1). 8. Invert and incubate the MHA plates at 35°C ± 2°C in ambient air for 18–24 hours. 9. Following incubation, measure the zones of inhibition as for the routine disk diffusion method (see M02[4]).
Test procedure: eCIM for Enterobacterales only; optional	1. For each isolate, label a second 2-mL TSB tube for the eCIM test. 2. Add 20 µL of the 0.5 M EDTA to the 2-mL TSB tube to obtain a final concentration of 5 mM EDTA. 3. Follow steps 1 through 9 above as for mCIM procedure. Process the mCIM and eCIM tubes in parallel. 4. Place the meropenem disks from the mCIM and eCIM tubes on the same MHA plate inoculated with the meropenem-susceptible *E. coli* ATCC® 25922 indicator strain. **NOTE:** Additional QC is needed for the eCIM test (see QC recommendations).

Table 3C. (Continued)

Test	mCIM Only or in Conjunction With eCIM
Test interpretation	For additional explanations, refer to Figures 2A, 2B, and 3A through 3D, as well as the notes section below. mCIM • Carbapenemase positive (see Figures 2A and 2B): – Zone diameter of 6–15 mm or presence of pinpoint colonies within a 16–18 mm zone – If the test isolate produces a carbapenemase, the meropenem in the disk will be hydrolyzed and there will be no inhibition or limited growth inhibition of the meropenem-susceptible *E. coli* ATCC® 25922. • Carbapenemase negative (see Figure 2A): – Zone diameter of ≥ 19 mm (clear zone) – If the test isolate does not produce carbapenemase, the meropenem in the disk will not be hydrolyzed and will inhibit growth of the meropenem-susceptible *E. coli* ATCC® 25922. • Carbapenemase indeterminate: – Zone diameter of 16–18 mm – Zone diameter of ≥ 19 mm and the presence of pinpoint colonies within the zone – The presence or absence of a carbapenemase cannot be confirmed. eCIM – Interpret only when mCIM test is positive • Metallo-ß-lactamase positive: – A ≥ 5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (eg, mCIM = 6 mm; eCIM = 15 mm; zone diameter difference = 9 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figures 3B and 3C). – If the test isolate produces a metallo-ß-lactamase, the activity of the carbapenemase will be inhibited in the presence of EDTA such that the meropenem in the disk will not be hydrolyzed as efficiently as in the tube without EDTA. The result is inhibition of the meropenem-susceptible *E. coli* and an increase in the zone diameter for the eCIM zone diameter compared with the mCIM zone diameter. • Metallo-ß-lactamase negative: – A ≤ 4-mm increase in zone diameter for the eCIM vs zone diameter of mCIM (eg, mCIM = 6 mm; eCIM = 8 mm; zone diameter difference = 2 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figure 3D). – If the test isolate produces a serine carbapenemase, the activity of the carbapenemase will not be affected by the presence of EDTA and there will be no or marginal (≤ 4 mm) increase in zone diameter in the presence of EDTA compared with the mCIM zone diameter.

Table 3C. (Continued)

Test	mCIM Only or in Conjunction With eCIM
Reporting	(see below)

mCIM Only

mCIM Result	eCIM Result	Report
Negative	Not set up	Carbapenemase not detected
Positive	Not set up	Carbapenemase detected
Indeterminate	Not set up	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.[*]

mCIM and eCIM Combination Test

mCIM Result	eCIM Result	Report
Negative	Do not interpret	Carbapenemase not detected
Positive	Negative	Serine carbapenemase detected
Positive	Positive	Metallo-β-lactamase detected
Indeterminate	Do not interpret	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.[a]

[a] If indeterminate results are obtained on repeat testing, consider performing a different phenotypic test for carbapenemase detection (ie, CarbaNP), a test for carbapenemase genes or send isolate to a referral laboratory for further testing.

If both a serine carbapenemase and a metallo-β-lactamase are co-produced by one organism, differentiation between enzymes will not be possible and false-negative eCIM results may occur.

Table 3C. (Continued)

<table>
<tr><th>Test</th><th>mCIM Only or in Conjunction With eCIM</th></tr>
<tr><td>NOTES</td><td>

For mCIM indeterminate results:

Check test isolate and E. coli ATCC® 25922 indicator strain for purity.
Check meropenem disk integrity by confirming acceptable results were obtained when disks were subjected to routine disk diffusion test QC.
Repeat the mCIM and/or eCIM for test isolate and QC strains.

mCIM only: For some tests, pinpoint colonies of the indicator organism (E. coli ATCC® 25922) may be observed within the zone of inhibition. If the colonies are present within a 6- to 18-mm zone of inhibition, the test should be considered carbapenemase positive. If colonies are present within a ≥ 19-mm zone, the test should be considered indeterminant.
eCIM only: Ignore pinpoint colonies within any zone of inhibition. Interpret results strictly based on the difference in zone diameters between the mCIM and eCIM tests.
mCIM negative and eCIM positive results should not occur. If this happens, perform checks as indicated in the first bullet above. If the repeat tests are the same, consider the tests invalid.
CLSI has currently standardized mCIM for Enterobacterales with a 1-µL loopful of bacteria and P. aeruginosa 10-µL loopful of bacteria only.

</td></tr>
<tr><td>QC recommendations</td><td>
Test positive and negative QC strains each day of testing (refer to Figures 2A and 2B for examples of positive and negative QC results).
<table>
<tr><th>QC Strain</th><th>Organism Characteristics</th><th>Expected Results</th></tr>
<tr><td>K. pneumoniae ATCC® BAA-1705™</td><td>KPC positive
Serine carbapenemase producer</td><td>mCIM positive
eCIM negative</td></tr>
<tr><td>K. pneumoniae ATCC® BAA-1706™</td><td>Carbapenemase negative</td><td>mCIM negative</td></tr>
<tr><td>K. pneumoniae ATCC® BAA-2146™[a]</td><td>NDM positive
Metallo-ß-lactamase producer</td><td>mCIM positive
eCIM positive</td></tr>
</table>
[a] eCIM positive control; to be set up only when the eCIM test is performed.

In addition, perform QC of meropenem disks and test media daily or weekly following the routine disk diffusion QC procedure, and handle disks as described in M02.[4] Alternatively, perform QC of meropenem disks with each run by removing a disk from the cartridge of disks used for the run and placing it on the MHA plate inoculated with E. coli ATCC® 25922; incubate as above.
</td></tr>
</table>

Abbreviations: ATCC®, American Type Culture Collection; eCIM, EDTA-modified carbapenem inactivation method; mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; TSB, trypticase soy broth.

Table 3C. (Continued)

Footnotes

a. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

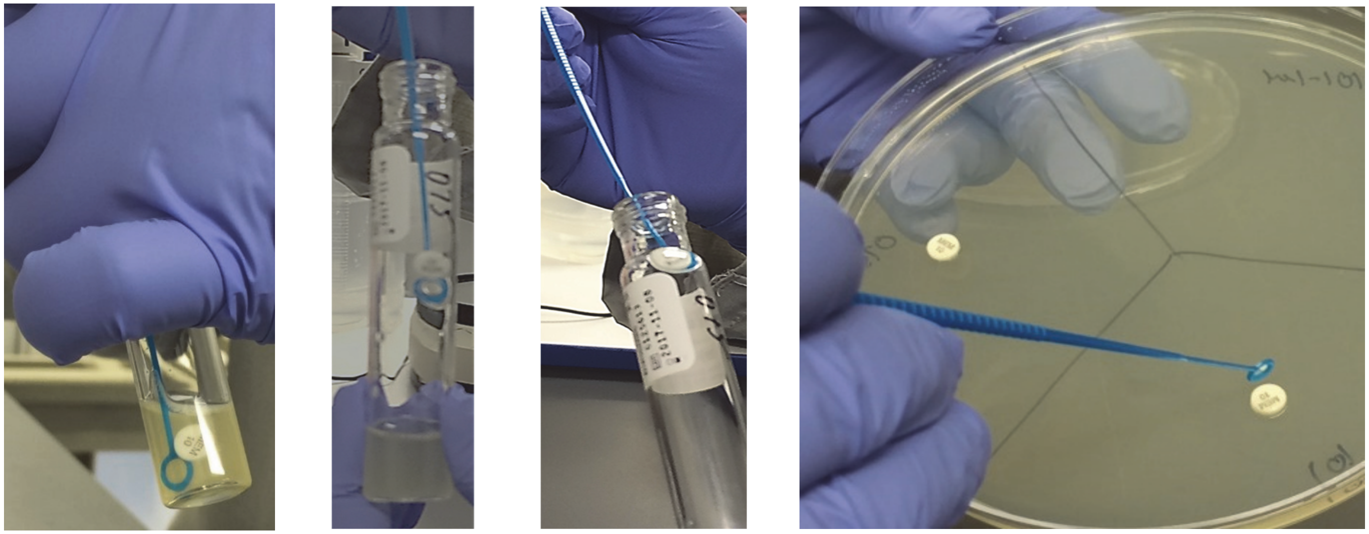
b. The AR Isolate Bank (https://wwwn.cdc.gov/arisolatebank) is a centralized repository of microbial pathogens with well-characterized resistance profiles that are assembled by the Centers for Disease Control and Prevention in collaboration with the US Food and Drug Administration.

NOTE 1: mCIM: This method demonstrated a sensitivity > 99% and specificity > 99% for detection of KPC, NDM, VIM, IMP, IMI, SPM, SME and OXA-type carbapenemases among Enterobacterales isolates investigated by CLSI.[b] This method demonstrated a sensitivity > 97% and specificity 100% for detection of KPC, NDM, VIM, IMP, IMI, SPM and OXA-type carbapenemases among *P. aeruginosa* isolates investigated by CLSI.[b] Performance for other carbapenemases or for testing isolates of non-Enterobacterales other than *P. aeruginosa* has not been established. Investigations of mCIM with *Acinetobacter* spp. showed poor specificity and poor reproducibility between laboratories, and performing mCIM with *Acinetobacter* spp. is not endorsed by CLSI. In CLSI studies, one OXA-232-producing *K. pneumoniae* isolate was negative by this assay at 4 of 9 validation sites.

NOTE 2: eCIM: This method demonstrated a sensitivity > 95% and specificity > 92% for differentiation of metallo-β-lactamases (NDM, VIM, and IMP) from serine carbapenemases (KPC, OXA, and SME) among Enterobacterales isolates investigated by CLSI.[b] In CLSI studies, one *K. pneumoniae* co-producing NDM and OXA-181 yielded a false-negative result at 3 of 4 validation sites.

NOTE 3: Information in black boldface type is new or modified since the previous edition.

Table 3C. (Continued)



A B C D

Figure 1. Procedure for Placing Meropenem Disks for the mCIM. Remove the meropenem disk with a 10-µL loop (A) and drag the loop against the inside edge of the tube to expel any excess liquid (B). Use the same loop to remove the disk from the tube (C) and place it on the MHA plate (D) previously inoculated with the meropenem-susceptible *E. coli* (ATCC® 25922) indicator strain.

Table 3C. (Continued)

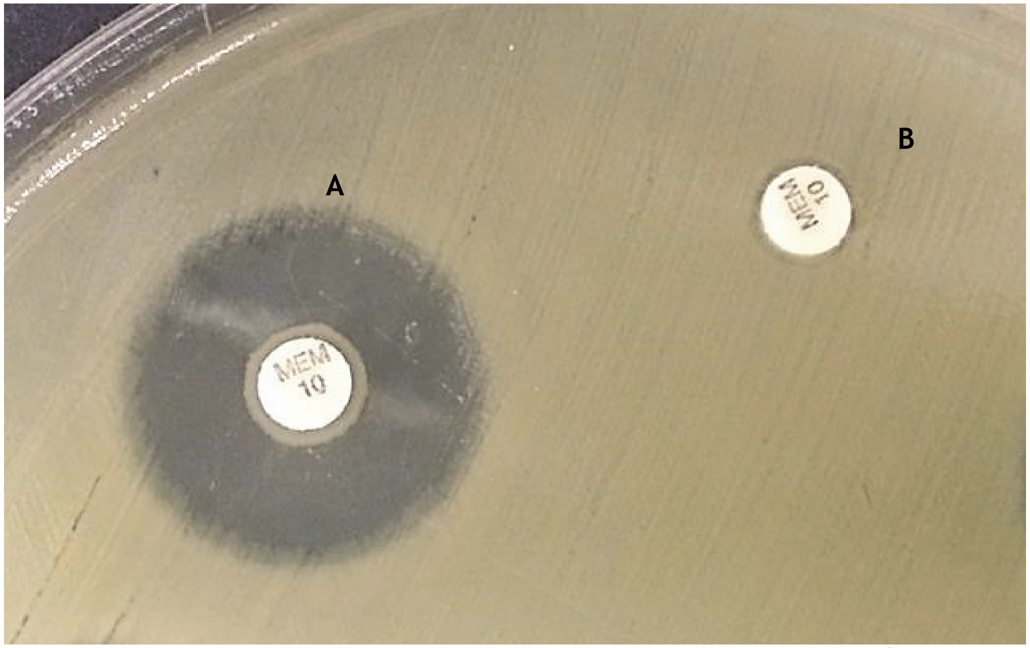


Figure 2A. mCIM Results for QC Strains: Negative Control *K. pneumoniae* ATCC® BAA-1706™ (A) and Positive Control *K. pneumoniae* ATCC® BAA-1705™ (B). NOTE: A narrow ring of growth around the meropenem disk as seen with the negative control (A) results from carryover of the test organism in the TSB and should be ignored.

Table 3C. (Continued)

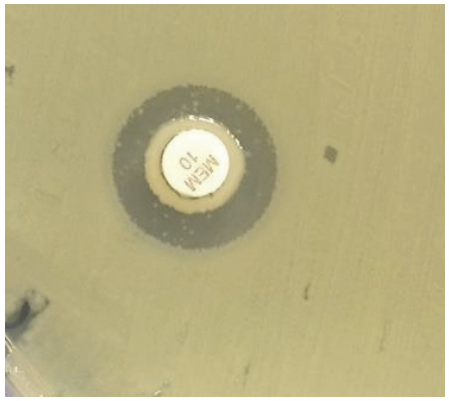


Figure 2B. mCIM Test Interpretation

- Result: positive mCIM
- Report: carbapenemase detected

NOTE: A narrow ring of growth around the meropenem disk results from carryover of the test organism in the TSB and should be ignored.

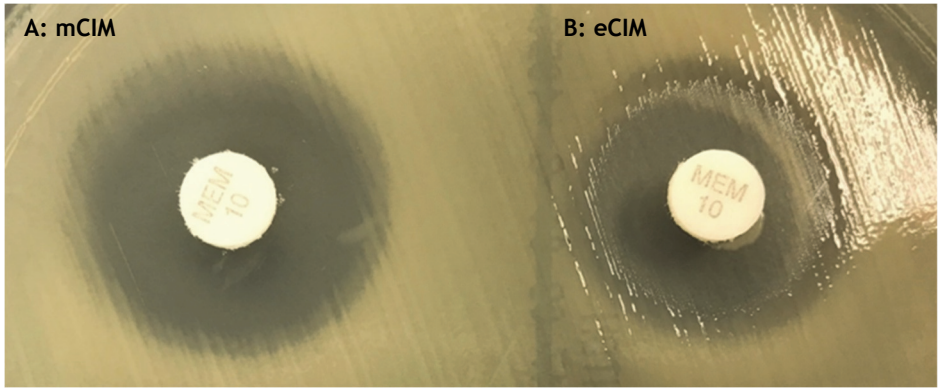


Figure 3A. mCIM and eCIM Test Interpretation: Negative mCIM. "A" shows an mCIM negative result (zone diameter = 20 mm) and "B" shows an eCIM invalid result. Do not interpret the eCIM result when the mCIM is negative as the isolate is negative for carbapenemase production.

- Result: negative for carbapenemase production
- Report: carbapenemase not detected

Table 3C. (Continued)

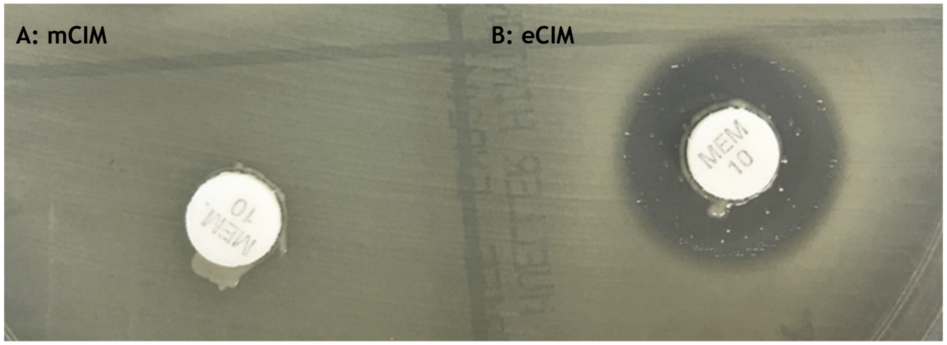


Figure 3B. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM. "A" shows an mCIM positive result (zone diameter of 6 mm) and "B" shows an eCIM positive result (zone diameter = 15 mm with pinpoint colonies throughout the zone of inhibition). **NOTE:** The pinpoint colonies throughout the zone of inhibition are ignored when measuring the zone for the eCIM test. A ≥ 5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (15 mm − 6 mm = 9 mm) demonstrates the inhibition of the metallo-ß-lactamase in the presence of EDTA.

- Result: positive mCIM and eCIM
- Report: metallo-ß-lactamase detected

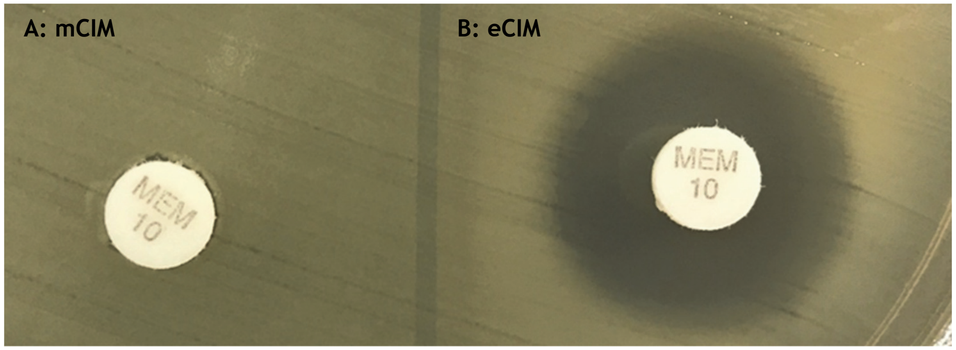


Figure 3C. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM positive result (zone diameter = 19 mm). A ≥ 5-mm increase in zone diameter for eCIM vs diameter for mCIM zone (19 mm − 6 mm = 13 mm) demonstrates the inhibition of the metallo-ß-lactamase in the presence of EDTA.

- Result: positive mCIM and eCIM
- Report: metallo-ß-lactamase detected

Table 3C. (Continued)

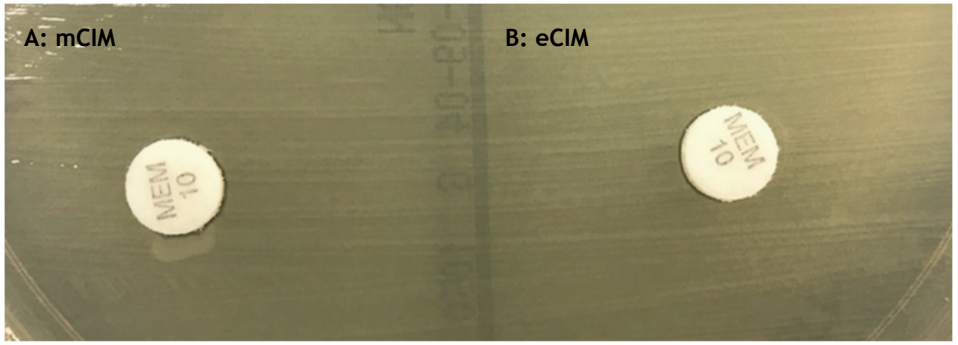


Figure 3D. mCIM and eCIM Test Interpretation: Positive mCIM and Negative eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM negative result (zone diameter = 6 mm). Serine carbapenemases are not inhibited by EDTA and demonstrate a ≤ 4-mm increase in zone diameter for eCIM vs zone diameter for mCIM.

- Result: positive mCIM and negative eCIM
- Report: serine carbapenemase detected

References for Table 3C

1 Tijet N, Patel SN, Melano RG. Detection of carbapenemase activity in Enterobacteriaceae: comparison of the carbapenem inactivation method versus the Carba NP test. *J Antimicrob Chemother*. 2016;71(1):274-276.

2 van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. *PLoS One*. 2015;10(3):e0123690.

3 Pierce VM, Simner PJ, Lonsway DR, et al. Modified carbapenem inactivation method (mCIM) for phenotypic detection of carbapenemase production among Enterobacteriaceae. *J Clin Microbiol*. 2017;55(8): 2321-2333.

4 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

5 Simner PJ, Johnson JK, Brasso WB, et al. Multicenter evaluation of the modified carbapenem inactivation method and the Carba NP for detection of carbapenemase-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *J Clin Microbiol*. 2017;56(1):e01369-17.

6 Sfeir MM, Hayden JA, Fauntleroy KA, et al. EDTA-modified carbapenem inactivation method: a phenotypic method for detecting metallo-ß-lactamase-producing Enterobacteriaceae. *J Clin Microbiol*. 2019;57(5):e01757-18.

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Table 3D. Tests for Colistin Resistance for Enterobacterales and *Pseudomonas aeruginosa*

The polymyxins (colistin and polymyxin B) are antimicrobial agents of last resort for treating multidrug-resistant infections. Clinical and PK/PD data suggest that these agents have limited clinical efficacy. Alternative agents are strongly preferred. If these agents are not available, knowledge of the colistin MIC may be helpful to inform treatment decisions.

For colistin, broth microdilution, broth disk elution and agar dilution MIC methods are acceptable. Broth microdilution is the only approved method for polymyxin B. Disk diffusion and gradient diffusion methods should not be performed.

Colistin and polymyxin B are considered equivalent agents, so MICs obtained from testing colistin predict MICs to polymyxin B and vice versa. At this time, CLSI has not evaluated polymyxin B testing methods, and the procedures below should not be adapted to polymyxin B. The methods below were evaluated for *Acinetobacter* spp. by CLSI and found to yield inaccurate results.

These methods were established with limited disk and/or media manufacturers and are considered provisional until additional data are evaluated by CLSI and shown to meet CLSI document M23[1] guidelines.

Test	Colistin Broth Disk Elution	Colistin Agar Test
Approved organisms	Enterobacterales and *Pseudomonas aeruginosa*	Enterobacterales and *P. aeruginosa*
Strengths	No special reagents or media necessary	Ability to test up to 10 isolates at one time
Limitations	Hands-on time and cost	Requires special media (colistin agar plate)
When to perform this test	Testing multidrug-resistant isolates for clinical or infection prevention purposes	Testing multidrug-resistant isolates for clinical or infection prevention purposes
Test method	Tube dilution using colistin disk as the colistin source	Agar dilution: slight variation of method described in M07[2] (ie, different inoculum and different approach to interpreting results)
Organism group	Enterobacterales and *P. aeruginosa*	Enterobacterales and *P. aeruginosa*
Medium	CAMHB (10-mL tubes)	MHA (20 mL in 100-mm Petri plate)[a]
Antimicrobial concentration	10-µg colistin sulfate disks Final concentration: 0 µg/mL (growth control), 1 µg/mL, 2 µg/mL, and 4 µg/mL colistin	Colistin sulfate Final concentration: 0 µg/mL (growth control), 1 µg/mL, 2 µg/mL, and 4 µg/mL colistin[a]
Inoculum	1. Using a loop or swab, pick 3–5 colonies from a fresh (18–24 hours) nonselective agar plate and transfer to sterile saline (4–5 mL). 2. Adjust turbidity to equivalent of a 0.5 McFarland turbidity standard.	1. Using a loop or swab, pick 3–5 colonies from a fresh (18–24 hours) nonselective agar plate and transfer to sterile saline (4–5 mL). 2. Adjust turbidity to equivalent of a 0.5 McFarland turbidity standard. 3. Dilute the standardized inoculum 1:10 in saline.

Table 3D. (Continued)

Test	Colistin Broth Disk Elution	Colistin Agar Test
Test procedure	1. Let the CAMHB tubes (10 mL) and colistin disks warm to room temperature. 2. Label 4 tubes of CAMHB for each isolate to be tested with 1, 2, and 4 μg/mL and control (see Figure 1). 3. Using aseptic technique, carefully add: • 1 colistin disk to the tube labeled "1 μg/mL" • 2 colistin disks to tube labeled "2 μg/mL" • 4 colistin disks to the tube labeled "4 μg/mL" 4. Gently vortex the tubes with the added disk and let the colistin elute from the disks for at least 30 minutes but no longer than 60 minutes at room temperature. 5. Prepare the standardized inoculum. 6. Add 50 μL standardized inoculum to the control and 1-, 2-, and 4-μg/mL tubes to attain a final inoculum concentration of approximately 7.5 × 10^5 CFU/mL. 7. Using a 10-μL loop, subculture from the original inoculum tube to a blood agar plate as a purity check. 8. Cap the tubes tightly and vortex each inoculated tube on slow speed to mix. Slow speed is suggested to prevent colistin from sticking to the cap and glass surface above the meniscus of liquid. 9. Loosen the caps slightly before incubation. 10. Incubate the tubes and purity plate.	1. Divide each colistin agar plate with increasingly doubled dilutions of colistin in up to 10 parts, with a marker to test up to 10 isolates per plate. Label each part with the appropriate isolate number (see Figure 2). 2. Using a pipette or a 10-μL loop, streak 10 μL of the 1:10 dilution onto the appropriate part of each colistin agar plate. 3. Using a 10-μL loop, subculture from the original inoculum tube to a blood agar plate as a purity check. 4. Incubate the colistin agar plates and purity plate.
Incubation conditions	33 to 35°C; ambient air	33 to 35°C; ambient air
Incubation length	16–20 hours	16–20 hours

Table 3D. (Continued)

Test	Colistin Broth Disk Elution	Colistin Agar Test
Results	1. Examine the purity plate to ensure inoculum was pure. 2. Examine the growth control tube, which must demonstrate obvious turbidity for the test to be valid. NOTE: Some *P. aeruginosa* isolates may grow only near the meniscus. 3. Read the MIC as the lowest concentration that completely inhibits growth of the test isolate. (See Figure 1 for examples.) For Enterobacterales and *P. aeruginosa:* • ≤ 2 µg/mL = intermediate • ≥ 4 µg/mL = resistant	1. Examine the purity plate to ensure inoculum was pure. 2. Examine the growth control plate, which must demonstrate confluent growth for the test to be valid. 3. Examine the colistin plates carefully with transmitted light for colony or light film of growth. 4. Read the MIC as the lowest colistin agar plate concentration that completely inhibits growth of the test isolate (eg, even 1 colony would be considered growth). See Figure 2 for examples. For Enterobacterales and *P. aeruginosa:* • ≤ 2 µg/mL = intermediate • ≥ 4 µg/mL = resistant
Additional testing and reporting	If there is an inconsistent growth pattern (eg, no growth in 2 µg/mL but growth at 1 µg/mL and 4 µg/mL), repeat the test. An inconsistent growth pattern may occur as a result of: • Contamination at higher dilutions • Heteroresistance • Improper concentrations of antimicrobial agent in the tubes • Error inoculating the tubes	If there is an inconsistent growth pattern (eg, no growth in 2 µg/mL but growth at 1 µg/mL and 4 µg/mL), repeat the test. An inconsistent growth pattern may occur as a result of: • Contamination at higher dilutions • Heteroresistance • Improper concentrations of antimicrobial agent in the colistin agar plates • Error inoculating the plates
QC recommendations - routine[b]	*Escherichia coli* ATCC® BAA-3170™ (formerly AR Bank #0349 *mcr-1*) (≤ **1-4** µg/mL, with a **mode** of 2 µg/mL)[c] and *P. aeruginosa* ATCC®[d] 27853 (≤ 1-4 µg/mL)	*E. coli* ATCC® BAA-3170™ (formerly AR Bank #0349 *mcr-1*) (≤ **1-4** µg/mL, with a **mode** of 2 µg/mL)[c] and *P. aeruginosa* ATCC® 27853 (**≤** 1-4 µg/mL)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control.

Table 3D. (Continued)

Footnotes

a. Refer to M07[2] for preparation of media and antimicrobial agents.

b. QC recommendations – routine

Test recommended routine QC strains:

- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[3] and M07[2]) and the individualized QC plan is complete
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

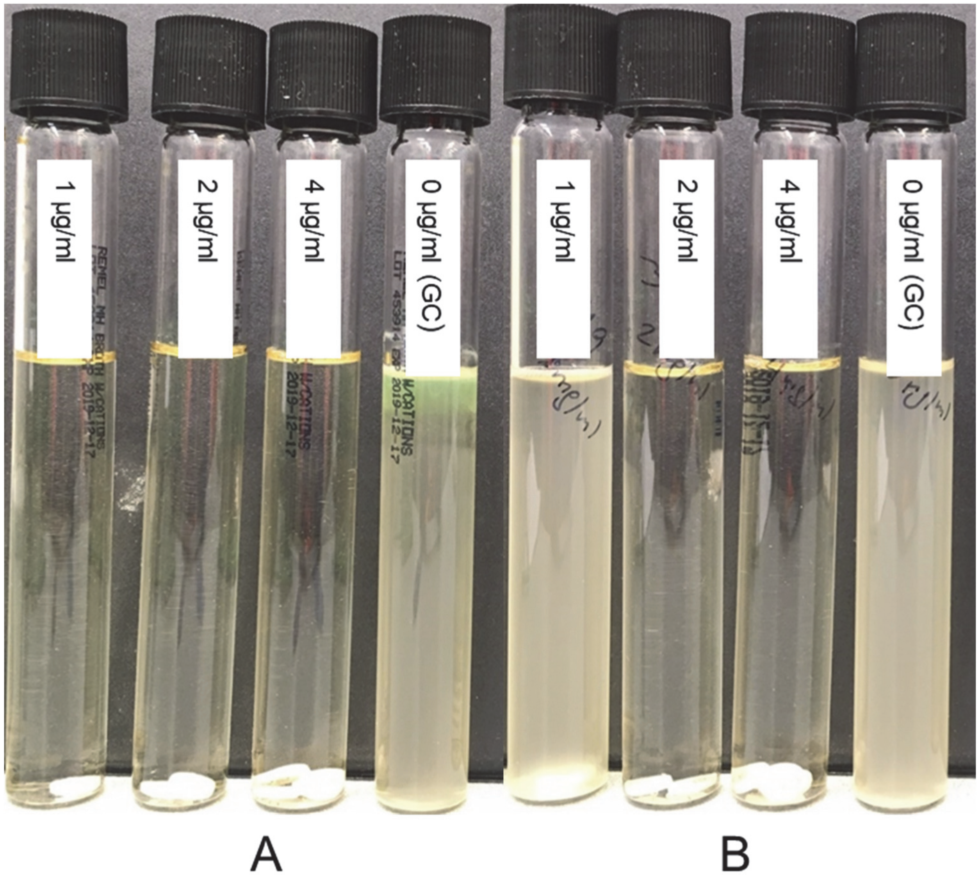
Perform QC of colistin disks and test media daily or weekly following the routine disk diffusion QC procedure and handle disks as described in M02.[3]

c. The QC ranges were established with disks (colistin broth disk elution) and media from a limited number of manufacturers and are considered provisional until additional data are evaluated by CLSI and shown to meet CLSI document M23[1] guidelines.

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

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 3D. (Continued)



Abbreviation: GC, growth control.

Figure 1. Colistin Broth Disk Elution. Results for routine QC strain *P. aeruginosa* ATCC® 27853 with an MIC ≤ 1 µg/mL (A) and supplemental QC strain ***E. coli*** **ATCC® BAA-3170™ (formerly** *E. coli* AR Bank #0349 *mcr-1***)** with an MIC 2 µg/mL (B).

Table 3D. (Continued)

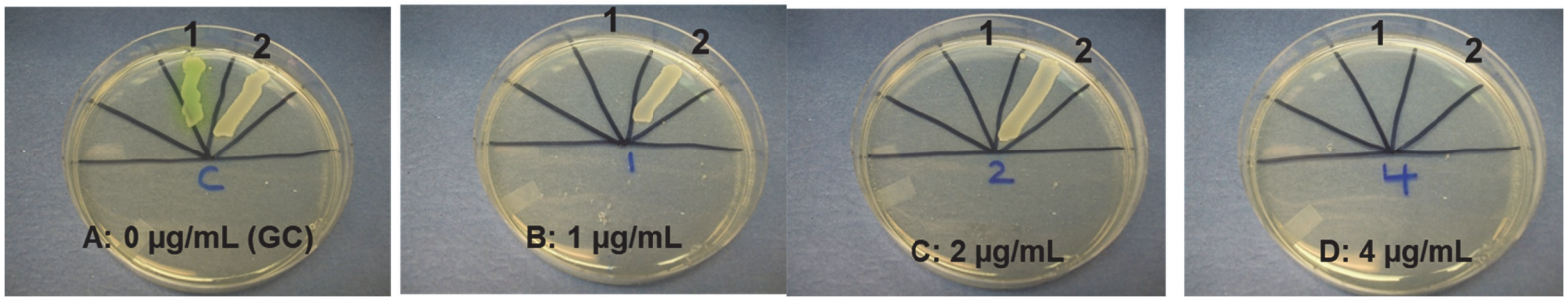


Figure 2. Colistin Agar Test. The plates need to be examined carefully with transmitted light for confluent growth, individual colonies, or light film of growth to determine the MIC. Colistin agar test results for routine QC strain *P. aeruginosa* ATCC® 27853 (position 1) with an MIC ≤ 1 µg/mL and for supplemental QC strain ***E. coli*** **ATCC® BAA-3170™ (formerly** *E. coli* AR Bank #0349 *mcr-1*) (position 2) with an MIC 4 µg/mL. The plates shown contain 0 µg/mL (control) (A), 1 µg/mL (B), 2 µg/mL (C), and 4 µg/mL (D) colistin.

References for Table 3D

1 CLSI. *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*. 5th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

Table 3E-1. Test for Performing Disk Diffusion Directly From Positive Blood Culture Broth

Test	Direct Disk Diffusion
Test method	Disk diffusion using positive blood culture broth
Organism group	Enterobacterales and *Pseudomonas aeruginosa*
Medium	MHA
Antimicrobial concentration	Standard disk contents for the antimicrobials are detailed in Table 3E-2 (Enterobacterales) and Table 3E-3 (*P. aeruginosa*)
Inoculum	Positive blood culture broth with gram-negative bacilli, used within 8 hours of flagging positive by the blood culture system
Test procedure	1. Invert blood culture bottle 5–10 times to thoroughly mix. 2. Sterilize the top of the bottle with an alcohol wipe (allow to dry) and insert 20-gauge venting needle into the blood culture bottle. 3. Dispense 4 drops of blood culture broth onto an MHA plate. As a purity check, use an inoculated blood agar plate streaked for isolation. 4. Spread blood culture broth across the entire surface of the MHA plate using a sterile cotton swab. 5. Repeat this procedure by streaking twice more, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. 6. Leave the lid ajar for 3–5 minutes (ideally) but no more than 15 minutes. 7. Dispense antimicrobial disks onto the surface of the inoculated MHA plate. 8. Press each disk down to ensure complete contact with the agar surface. 9. Invert the plate and place in the incubator within 15 minutes of disks being applied.
Incubation conditions	35°C ± 2°C; ambient air
Incubation length	8-10 hours or 16-18 hours **(refer to Tables 3E-2 and 3E-3 for antimicrobial agent-specific incubation lengths)**
Results	1. Examine the blood agar purity plate to ensure pure growth. 2. Examine the test plate to ensure confluent lawn of growth appropriate to read disk zone tests per M02.[1] 3. Measure the zone diameters according to routine disk diffusion recommendations in M02.[1] 4. Report results using the interpretive categories and zone diameter breakpoints in Table 3E-2 or Table 3E-3 if the gram-negative bacillus tested is confirmed to be an Enterobacterales or *P. aeruginosa*, respectively. If species is identified as another organism, do not interpret or report results.

Table 3E-1. (Continued)

Test	Direct Disk Diffusion
Additional testing and reporting	• If there is an inconsistent growth pattern on the plate (eg, mixed inoculum, nonconfluent growth, growth is too faint to read), do not interpret or report results from the direct disk diffusion test, and perform standard susceptibility testing from pure colony growth. • Antimicrobial agents to which the organism is intrinsically resistant (see Appendix B) should be reported as resistant, regardless of measured zone size. • If two zones of growth inhibition are observed, measure the inner zone diameter. In case of colonies present within zones, or presence of both inner and outer zones, check the purity plate and, if pure, record the inner zone diameter.
QC recommendations	• Perform QC according to the standard disk diffusion QC procedures per M02[1] (eg, daily or weekly). • See Tables 4A-1 and 4A-2 for acceptable QC ranges. • *E. coli* ATCC®[a] 25922, *P. aeruginosa* ATCC® 27853 • Refer to Table 4A-2 to select strains for routine QC of β-lactam combination agents.

Abbreviations: ATCC®, American Type Culture Collection; MHA, Mueller-Hinton agar; QC, quality control.

Footnote

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

NOTE: Information in black boldface type is new or modified since the previous edition.

Reference for Table 3E-1

(3) CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

Table 3E-2. Zone Diameter Disk Diffusion Breakpoints for Enterobacterales Direct From Blood Culture

General Comments

(1) Organism identification must be known before interpreting and reporting results. Fluoroquinolone breakpoints do not apply to *Salmonella* spp.

(2) The dosage regimens shown in the Comments column below are needed to achieve plasma drug exposure (in adults with normal renal and hepatic function) on which breakpoints were based. When new breakpoints are implemented, it is strongly recommended that laboratories share this information with the antimicrobial stewardship team **and other relevant institutional stakeholders.**

(3) For additional testing and reporting recommendations, refer to Table 2A.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 3E-2. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Read Times, hours	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Comments
			S	SDD	I	R	
PENICILLINS							
Ampicillin	10 µg	8-10	**≥16**	-	**12-15**	**≤ 11**	**(4)** Results of ampicillin testing can be used to predict results for amoxicillin.
		16-18	≥ 17	-	14-16	≤ 13	**(5)** Breakpoints are based on an ampicillin dosage regimen of 2 g parenterally administered every 4-6 h or an amoxicillin dosage regimen of 1-2 g parenterally administered every 6 h.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)							
Ceftriaxone	30 µg	8-10	≥ 23	-	20-22	≤ 19	**(6)** Breakpoints are based on a dosage regimen of 1 g administered every 24 h.
		16-18	≥ 23	-	20-22	≤ 19	
Ceftazidime	30 µg	8-10	≥ 21	-	18-20	≤ 17	**(7)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
		16-18	≥ 21	-	18-20	≤ 17	
MONOBACTAMS							
Aztreonam	30 µg	8-10	≥ 21	-	18-20	≤ 17	**(8)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
		16-18	≥ 21	-	18-20	≤ 17	
CARBAPENEMS							
Meropenem	**10 µg**	**8-10**	**≥22**	**-**	**20-21**	**≤ 19**	
		16-18	**≥22**	**-**	**19-21**	**≤ 18**	
AMINOGLYCOSIDES							
Tobramycin	10 µg	8-10	≥ 15	-	13-14	≤ 12	
		16-18	≥ 15	-	13-14	≤ 12	
FLUOROQUINOLONES for Enterobacterales except *Salmonella* spp.							
Ciprofloxacin	**5 µg**	**8-10**	**≥ 21**	**-**	**18-20**	**≤ 17**	
		16-18	**≥ 21**	**-**	**18-20**	**≤ 17**	
FOLATE PATHWAY ANTAGONISTS							
Trimethoprim-sulfamethoxazole	1.25/ 23.75 µg	8-10	-	-	-	-	
		16-18	≥16	-	11-15	≤ 10	

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

Table 3E-2
Zone Diameter Disk Diffusion Breakpoints for Enterobacterales Direct From Blood Culture

Table 3E-3. Zone Diameter Disk Diffusion Breakpoints for *Pseudomonas aeruginosa* Direct From Blood Culture

General Comments

(1) Organism identification must be known before interpreting and reporting results.

(2) The dosage regimens shown in the Comments column below are necessary to achieve plasma drug exposure (in adults with normal renal and hepatic function) on which breakpoints were derived. When new breakpoints are implemented, it is strongly recommended that laboratories share this information with the antimicrobial stewardship team **and other relevant institutional stakeholders.**

(3) For additional testing and reporting recommendations, refer to Table 2B-1.

NOTE: Information in black boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Read Times, hours	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Comments
			S	SDD	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)							
Ceftazidime	30 µg	8-10	-	-	-	-	**(4)** Breakpoints are based on a dosage regimen of 1 g administered every 6 h or 2 g administered every 8 h.
		16-18	≥ 18	-	15-17	≤ 14	
CARBAPENEMS							
Meropenem	10 µg	**8-10**	**≥ 19**	-	**16-18**	**≤ 15**	**(5)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
		16-18	≥ 19	-	16-18	≤ 15	
AMINOGLYCOSIDES							
Tobramycin	10 µg	8-10	≥ 15	-	13-14	≤ 12	
		16-18	≥ 15	-	13-14	≤ 12	
FLUOROQUINOLONES							
Ciprofloxacin	5 µg	8-10	≥ 23	-	18-22	≤ 17	**(6)** Breakpoints are based on a dosage regimen of 400 mg administered parenterally every 8 h.
		16-18	≥ 25	-	19-24	≤ 18	

Abbreviations: I, intermediate; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

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Table 3F. Tests for Detection of ß-Lactamase Production in *Staphylococcus* spp.

Test	ß-Lactamase Production	
Test method	Disk diffusion (penicillin zone-edge test)	Nitrocefin-based test
Organism group	*S. aureus* with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm[a]	*Staphylococcus* spp.[a,b] with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm
Medium	MHA	N/A
Antimicrobial concentration	10 units penicillin disk	N/A
Inoculum	Standard disk diffusion procedure	Induced growth (ie, growth taken from the zone margin surrounding a penicillin or cefoxitin disk test on either MHA or a blood agar plate after 16–18 hours of incubation)
Incubation conditions	35°C ± 2°C; ambient air	Room temperature
Incubation length	16–18 hours	Up to 1 hour for nitrocefin-based test or follow manufacturer's directions
Results	Sharp zone edge ("cliff") = ß-lactamase positive (see Figure 1 below this table) Fuzzy zone edge ("beach") = ß-lactamase negative (see Figure 2 below this table)	Nitrocefin-based test: conversion from yellow to red/pink = ß-lactamase positive.
Additional testing and reporting	ß-lactamase-positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.	Nitrocefin-based tests can be used for *S. aureus*, but negative results should be confirmed with the penicillin zone-edge test before reporting penicillin as susceptible. ß-lactamase-positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.
QC recommendations - routine[c]	*S. aureus* ATCC®[d] 25923 for routine QC of penicillin disk to include examination of zone-edge test (fuzzy edge = "beach")	
QC recommendations - lot/shipment[e]		*S. aureus* ATCC® 29213 – positive *S. aureus* ATCC® 25923 – negative (or see local regulations and manufacturers' recommendations)
QC recommendations - supplemental[f]	*S. aureus* ATCC® 29213 – positive penicillin zone-edge test (sharp edge = "cliff")	

Abbreviations: ATCC®, American Type Culture Collection; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; N/A, not applicable; QC, quality control.

Table 3F. (Continued)

Footnotes

a. The penicillin disk diffusion zone-edge test was shown to be more sensitive than nitrocefin-based tests for detection of β-lactamase production in *S. aureus*. The penicillin zone-edge test is recommended if only one test is used for β-lactamase detection. However, some laboratories may choose to perform a nitrocefin-based test first and, if this test is positive, report the results as positive for β-lactamase (or penicillin resistant). If the nitrocefin test is negative, the penicillin zone-edge test should be performed before reporting the isolate as penicillin susceptible in cases in which penicillin may be used for therapy (eg, endocarditis).[1,2]

b. For *S. lugdunensis*, tests for β-lactamase detection are not necessary because isolates producing a β-lactamase will test penicillin resistant (MIC > 0.12 µg/mL and zone diameters < 29 mm). If a laboratory is using a method other than the CLSI disk diffusion or MIC reference methods and is unsure if the method can reliably detect penicillin resistance with contemporary isolates of *S. lugdunensis*, the laboratory should perform an induced nitrocefin assay or other CLSI reference method on isolates that test penicillin susceptible before reporting the isolate as penicillin susceptible.

c. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[3] and M07[4])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

e. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

f. QC recommendations – supplemental

- Supplemental QC strains can be used to assess a new test, for training personnel, and for competence assessment. It is not necessary to include supplemental QC strains in routine daily or weekly antimicrobial susceptibility testing QC programs. See Appendix C, which describes use of QC strains.

Table 3F. (Continued)

Figure 1. Positive Penicillin Disk Zone-Edge Test for ß-Lactamase Detection. The zone edge is sharp or like a "cliff" indicating ß-lactamase production.

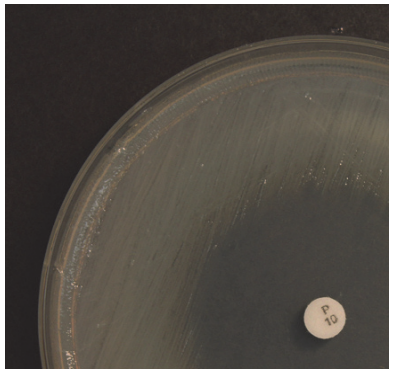

Figure 2. Negative Penicillin Disk Zone-Edge Test for ß-Lactamase Detection. The zone edge is fuzzy or like a "beach," indicating no ß-lactamase production.

Table 3F. (Continued)

References for Table 3F

[1] Kaase M, Lenga S, Friedrich S, et al. Comparison of phenotypic methods for penicillinase detection in *Staphylococcus aureus*. *Clin Microbiol Infect*. 2008;14(6):614-616.

[2] Gill VJ, Manning CB, Ingalls CM. Correlation of penicillin minimum inhibitory concentrations and penicillin zone edge appearance with staphylococcal beta-lactamase production. *J Clin Microbiol*. 1981;14(4):437-440.

[3] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

[4] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

Table 3G-1. Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus aureus*[a] and *Staphylococcus lugdunensis*

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin[b]		Detecting *mecA*-Mediated Resistance Using Oxacillin	Detecting *mecA*-mediated Resistance Using Oxacillin Salt Agar for *S. aureus* Only
Test method	Disk diffusion	Broth microdilution	Broth microdilution and agar dilution	Agar dilution for *S. aureus*
Medium	MHA	CAMHB	CAMHB with 2% NaCl (broth microdilution) MHA with 2% NaCl (agar dilution)	MHA with 4% NaCl
Antimicrobial concentration	30-µg cefoxitin disk	4 µg/mL cefoxitin	2 µg/mL oxacillin	6 µg/mL oxacillin
Inoculum	Standard disk diffusion procedure	Standard broth microdilution procedure	Standard broth microdilution procedure or standard agar dilution procedure	Colony suspension to obtain 0.5 McFarland turbidity Using a 1-µL loop that was dipped in the suspension, spot an area 10–15 mm in diameter. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot a similar area or streak an entire quadrant.
Incubation conditions	33 to 35°C; ambient air[c]			
Incubation length	16–18 hours	16–20 hours	24 hours (may be reported after 18 hours, if resistant)	24 hours; read with transmitted light
Results	≤ 21 mm = positive for *mecA*-mediated resistance ≥ 22 mm = negative for *mecA*-mediated resistance	≥ 8 µg/mL = positive for *mecA*-mediated resistance ≤ 4 µg/mL = negative for *mecA*-mediated resistance	≥4 µg/mL = positive for *mecA*-mediated resistance ≤ 2 µg/mL = negative for *mecA*-mediated resistance	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = positive for *mecA*-mediated resistance
Additional testing and reporting	Isolates that test positive for *mecA*-mediated resistance should be reported as methicillin (oxacillin) (not cefoxitin) resistant; other β-lactam agents, except ceftaroline, should be reported as resistant or should not be reported.[d]			
QC recommendations - routine[e,f]	*S. aureus* ATCC®[g] 25923 – *mecA* negative (zone 23–29 mm)	*S. aureus* ATCC® 29213 – *mecA* negative (MIC 1–4 µg/mL)	*S. aureus* ATCC® 29213 – *mecA* negative (MIC 0.12–0.5 µg/mL)	*S. aureus* ATCC®[c] 29213 – susceptible (≤ 1 colony; with each test day)
QC recommendations - lot/shipment[h]	N/A	*S. aureus* ATCC® 43300 – *mecA* positive (MIC ≥ 8 µg/mL)	*S. aureus* ATCC® 43300 – *mecA* positive (MIC ≥ 8 µg/mL)	*S. aureus* ATCC® 43300 – *mecA* positive (>1 colony)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant *Staphylococcus* spp.; N/A, not applicable.

Table 3G-1. (Continued)

Footnotes

a. Including members of the *S. aureus* complex (see Table 2C, comment [3]).

b. Cefoxitin is used as a surrogate test for detecting *mecA*-mediated methicillin (oxacillin) resistance.

c. Testing at temperatures above 35°C may not detect MRS.

d. Testing of other β-lactam agents, except ceftaroline, is not advised.

e. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[1] and M07[2])

f. Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

h. QC Recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

References for Table 3G-1

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

Table 3G-2. Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp. Except *Staphylococcus aureus*[a] and *Staphylococcus lugdunensis*

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin[b]	Detecting *mecA*-Mediated Resistance Using Oxacillin	
Test method	Disk diffusion	Disk diffusion	Broth microdilution and agar dilution
Organism group	*Staphylococcus* spp. except: *S. aureus* (refer to Table 3G-1) *S. lugdunensis* (refer to Table 3G-1) *S. pseudintermedius* (not recommended) *S. schleiferi* (not recommended)	Testing is only indicated for the species listed below: *S. epidermidis* *S. pseudintermedius* *S. schleiferi*	*Staphylococcus* spp. except: *S. aureus* (refer to Table 3G-1) *S. lugdunensis* (refer to Table 3G-1)
Medium	MHA	MHA	CAMHB with 2% NaCl (broth microdilution) MHA with 2% NaCl (agar dilution)
Antimicrobial concentration	30 µg cefoxitin disk	1-µg oxacillin disk	0.5 µg/mL oxacillin
Inoculum	Standard disk diffusion procedure	Standard disk diffusion procedure	Standard broth microdilution procedure or standard agar dilution procedure
Incubation conditions	33 to 35°C; ambient air[c]		
Incubation length	24 hours (may be reported after 18 hours, if resistant)	16–18 hours	24 hours (may be reported after 18 hours, if resistant)
Results	≤ 24 mm = positive for *mecA*-mediated resistance ≥ 25 mm = negative for *mecA*-mediated resistance	≤ 17 mm = positive for *mecA*-mediated resistance ≥ 18 mm = negative for *mecA*-mediated resistance	≥ 1 µg/mL = positive for *mecA*-mediated resistance ≤ 0.5 µg/mL = negative for *mecA*-mediated resistance
Additional testing and reporting	Isolates that test positive for *mecA*-mediated resistance should be reported as methicillin (oxacillin) (not cefoxitin) resistant; other ß-lactam agents, except ceftaroline, should be reported as resistant or should not be reported.[d]		
			For *Staphylococcus* spp., excluding *S. aureus*, *S. lugdunensis*, *S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi*, oxacillin MIC breakpoints may overcall resistance, and some isolates for which the oxacillin MICs are 1–2 µg/mL may be *mecA* negative. Isolates from serious infections for which oxacillin MICs are 1–2 µg/mL may be tested for *mecA* or for PBP2a. Isolates that test *mecA* or PBP2a negative should be reported as methicillin (oxacillin) susceptible.
QC recommendations - routine[e]	*S. aureus* ATCC®[f] 25923 – *mecA* negative (zone 23–29 mm)	*S. aureus* ATCC® 25923 – *mecA* negative (zone 18–24 mm)	*S. aureus* ATCC® 29213 – *mecA* negative (MIC 0.12–0.5 µg/mL)
QC recommendations - lot/shipment[g]	N/A	*S. aureus* ATCC® 43300 – *mecA* positive (zone ≤ 24 mm)	*S. aureus* ATCC® 43300 – *mecA* positive (MIC ≥ 8 µg/mL)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant *Staphylococcus* spp.; N/A, not applicable.

Table 3G-2. (Continued)

Footnotes

a. Including members of the *S. aureus* complex (see Table 2C, general comment [3]).

b. Cefoxitin is tested as a surrogate for detecting *mecA*-mediated methicillin (oxacillin) resistance; however, recent data suggest that the cefoxitin disk diffusion test may not perform reliably for all species (eg, *S. haemolyticus*).[1]

c. Testing at temperatures above 35°C may not detect MRS.

d. Testing of other ß-lactam agents, except ceftaroline, is not advised.

e. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[2] and M07[3])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

g. QC Recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

References for Table 3G-2

1 Humphries RM, Magnano P, Burnham CA, et al. Evaluation of surrogate tests for the presence of *mecA*-mediated methicillin resistance in *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis* and *Staphylococcus warneri*. *J Clin Microbiol*. 2020;59(1):e02290-20.

2 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

Table 3H. Vancomycin Agar Screen for *Staphylococcus aureus* and *Enterococcus* spp.

Screen Test	Vancomycin MIC ≥ 8 µg/mL	
Test method	Agar dilution	Agar dilution
Organism group	*S. aureus*	*Enterococcus* spp.
Medium	BHI agar	BHI[a] agar
Antimicrobial concentration	6 µg/mL vancomycin	6 µg/mL vancomycin
Inoculum	Colony suspension to obtain 0.5 McFarland turbidity Preferably, using a micropipette, spot a 10-µL drop onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10–15 mm in diameter or streak a portion of the plate.	1–10 µL of a 0.5 McFarland suspension spotted onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10–15 mm in diameter or streak a portion of the plate.
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	24 hours	24 hours
Results	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = presumptive reduced susceptibility to vancomycin	> 1 colony = presumptive vancomycin resistance
Additional testing and reporting	Perform a vancomycin MIC using a validated MIC method to determine vancomycin MICs on *S. aureus* that grow on BHI-vancomycin screening agar. Testing on BHI-vancomycin screening agar does not reliably detect all vancomycin-intermediate *S. aureus* strains. Some strains for which the vancomycin MICs are 4 µg/mL will fail to grow.	Perform vancomycin MIC on *Enterococcus* spp. that grow on BHI-vancomycin screening agar and test for motility and pigment production to distinguish species with acquired resistance (eg, *vanA* and *vanB*) from those with intrinsic, intermediate-level resistance to vancomycin (eg, *vanC*), such as *Enterococcus gallinarum* and *Enterococcus casseliflavus*, which often grow on the vancomycin screen plate. In contrast to other enterococci, *E. casseliflavus* and *E. gallinarum* with vancomycin MICs of 8–16 µg/mL (intermediate) differ from vancomycin-resistant enterococci for infection prevention purposes.
QC recommendations - routine[b]	*E. faecalis* ATCC®[c] 29212 – susceptible	*E. faecalis* ATCC® 29212 – susceptible
QC recommendations - lot/shipment[d]	*E. faecalis* ATCC® 51299 – resistant	*E. faecalis* ATCC® 51299 – resistant

Abbreviations: ATCC®, American Type Culture Collection; BHI, brain heart infusion; MIC, minimal inhibitory concentration; QC, quality control.

Table 3H. (Continued)

Footnotes

a. Even though not as widely available, dextrose phosphate agar and broth have been shown in limited testing to perform comparably with BHI media.

b. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[1] and M07[2])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

d. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

References for Table 3H

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

Table 3I. Tests for Detecting Inducible Clindamycin Resistance in *Staphylococcus* spp., *Streptococcus pneumoniae*, and *Streptococcus* spp. ß-Hemolytic Group[a,b]

Test	ICR			
Test method	**Disk Diffusion (D-zone test)**		**Broth Microdilution**	
Organism group (applies only to organisms resistant to erythromycin and susceptible or intermediate to clindamycin)	All *Staphylococcus* spp.	*S. pneumoniae* and ß-hemolytic *Streptococcus* spp.	All *Staphylococcus* spp.[c]	*S. pneumoniae* and ß-hemolytic *Streptococcus* spp.
Medium	MHA or blood agar purity plate used with MIC tests	MHA supplemented with sheep blood (5% v/v) or TSA supplemented with sheep blood (5% v/v)	CAMHB	CAMHB with LHB (2.5% to 5% v/v)
Antimicrobial concentration	15-µg erythromycin and 2-µg clindamycin disks spaced 15–26 mm apart	15-µg erythromycin and 2-µg clindamycin disks spaced 12 mm apart	4 µg/mL erythromycin and 0.5 µg/mL clindamycin in same well	1 µg/mL erythromycin and 0.5 µg/mL clindamycin in same well
Inoculum	Standard disk diffusion procedure or heavily inoculated area of purity plate	Standard disk diffusion procedure	Standard broth microdilution procedure	
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; 5% CO_2	35°C ± 2°C; ambient air	
Incubation length	16–18 hours	20–24 hours	18–24 hours	20–24 hours
Results	Flattening of the zone of inhibition adjacent to the erythromycin disk (referred to as a D-zone) = ICR. Hazy growth within the zone of inhibition around clindamycin = clindamycin resistance, even if no D-zone is apparent.		Any growth = ICR. No growth = no ICR.	

Table 3I. (Continued)

Test	ICR			
Test method	Disk Diffusion (D-zone test)		Broth Microdilution	
Organism group (applies only to organisms resistant to erythromycin and susceptible or intermediate to clindamycin)	All *Staphylococcus* spp.	*S. pneumoniae* and ß-hemolytic *Streptococcus* spp.	All *Staphylococcus* spp.[c]	*S. pneumoniae* and ß-hemolytic *Streptococcus* spp.
Additional testing and reporting	Report isolates with ICR as "clindamycin resistant." The following comment may be included with the report: "This isolate is presumed to be resistant based on detection of ICR, as determined by testing clindamycin in combination with erythromycin."			
QC recommendations - routine[c]	*S. aureus* ATCC®[d] 25923 for routine QC of erythromycin and clindamycin disks	*S. pneumoniae* ATCC® 49619 for routine QC of erythromycin and clindamycin disks	*S. aureus* ATCC® BAA-976™ or *S. aureus* ATCC® 29213 - no growth	*S. pneumoniae* ATCC® 49619 or *S. aureus* ATCC® BAA-976™ - no growth
QC recommendations - lot/shipment[e]	**Perform QC according to standard disk diffusion QC procedures per M02[1] (eg, daily or weekly)**		*S. aureus* ATCC® BAA-977™ - growth	
QC recommendations - supplemental[f]	*S. aureus* ATCC® BAA-976™ (D-zone test negative) *S. aureus* ATCC® BAA-977™ (D-zone test positive) Use of unsupplemented MHA is acceptable for these strains.		*S. aureus* ATCC® BAA-976™ (no growth) *S. aureus* ATCC® BAA-977™ (growth)	

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; ICR, inducible clindamycin resistance; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; TSA, tryptic soy agar.

Footnotes

a. Antimicrobial susceptibility testing of ß-hemolytic streptococci does not need to be performed routinely (see general comment [5] in Table 2H-1). When susceptibility testing is clinically indicated, test for ICR in strains that are erythromycin resistant and clindamycin susceptible or intermediate.

b. In accordance with 2010 guidance from the Centers for Disease Control and Prevention, colonizing isolates of group B streptococci from penicillin-allergic pregnant women should be tested for clindamycin (including ICR) (see comment [16] in Table 2H-1).[2] For isolates that test susceptible to clindamycin (with erythromycin induction), consider adding the following comment to the patient's report: "This group B *Streptococcus* does not demonstrate inducible clindamycin resistance as determined by testing clindamycin in combination with erythromycin."

Table 3I. (Continued)

c. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[1] and M07[3])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

d. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

e. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

f. QC recommendations – supplemental

- Supplemental QC strains can be used to assess a new test, for training personnel, and for competence assessment. It is not necessary to include supplemental QC strains in routine daily or weekly AST QC programs. See Appendix C, which describes use of QC strains.

NOTE: Information in black boldface type is new or modified since the previous edition.

References for Table 3I

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

2 Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC). Prevention of perinatal group B streptococcal disease – revised guidelines from CDC, 2010. *MMWR Recomm Rep*. 2010;59(RR-10):1-36.

3 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

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Table 3J. Test for Detecting High-Level Mupirocin Resistance in *Staphylococcus aureus*

Test	High-Level Mupirocin Resistance[a,1-3]	
Test method	Disk diffusion	Broth microdilution
Organism group	*S. aureus*	
Medium	MHA	CAMHB
Antimicrobial concentration	200-µg mupirocin disk	Single mupirocin 256-µg/mL well
Inoculum	Standard disk diffusion procedure	Standard broth microdilution procedure
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	24 hours; read with transmitted light	24 hours
Results	Examine carefully with transmitted light for light growth within the zone of inhibition. No zone = high-level mupirocin resistance. Any zone = the absence of high-level mupirocin resistance.	For single 256-µg/mL well: Growth = high-level mupirocin resistance. No growth = the absence of high-level mupirocin resistance.
Additional testing and reporting	Report isolates with no zone as high-level mupirocin resistant. Report any zone of inhibition as the absence of high-level resistance.	Report growth in the 256-µg/mL well as high-level mupirocin resistant. Report no growth in the 256-µg/mL well as the absence of high-level resistance.
QC recommendations - routine[b]	*S. aureus* ATCC®[c] 25923 (200-µg disk) – *mupA* negative (zone 29–38 mm)	*S. aureus* ATCC® 29213 – *mupA* negative (MIC 0.06–0.5 µg/mL) or *E. faecalis* ATCC® 29212 – *mupA* negative (MIC 16–128 µg/mL)
QC recommendations - lot/shipment[d]	*S. aureus* ATCC® BAA-1708™ – *mupA* positive (no zone)	*S. aureus* ATCC® BAA-1708™ – *mupA* positive (growth in 256-µg/mL well)

Abbreviations. ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control.

Table 3J. (Continued)

Footnotes

a. Although not formally validated by CLSI document M23[1]-based analyses, some studies have linked a lack of response to mupirocin-based decolonization regimens with isolates for which the mupirocin MICs are ≥ 512 µg/mL.[2-4] Although this document does not provide guidance on breakpoints for mupirocin, disk-based testing and the MIC test described here identify isolates for which the mupirocin MICs are ≥ 512 µg/mL.

b. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[5] and M07[6])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

c. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after “BAA” in each catalog number, in conjunction with the registered ATCC® name.

d. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

References for Table 3J

1 CLSI. *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*. 5th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute; 2018.

2 Simor AE, Phillips E, McGeer A, et al. Randomized controlled trial of chlorhexidine gluconate for washing, intranasal mupirocin, and rifampin and doxycycline versus no treatment for the eradication of methicillin-resistant *Staphylococcus aureus* colonization. *Clin Infect Dis*. 2007;44(2):178-185.

3 Harbarth S, Dharan S, Liassine N, Herrault P, Auckenthaler R, Pittet D. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 1999;43(6):1412-1416.

4 Walker ES, Vasquez JE, Dula R, Bullock H, Sarubbi FA. Mupirocin-resistant, methicillin-resistant *Staphylococcus aureus;* does mupirocin remain effective? *Infect Control Hosp Epidemiol*. 2003;24(5):342-346.

5 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

6 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

Table 3K. Test for Detecting High-Level Aminoglycoside Resistance in *Enterococcus* spp.[a] (includes disk diffusion)

Test	Gentamicin HLAR			Streptomycin HLAR		
Test method	Disk diffusion	Broth microdilution	Agar dilution	Disk diffusion	Broth microdilution	Agar dilution
Medium	MHA	BHI[b] broth	BHI[b] agar	MHA	BHI[b] broth	BHI[b] agar
Antimicrobial concentration	120-µg gentamicin disk	Gentamicin, 500 µg/mL	Gentamicin, 500 µg/mL	300-µg streptomycin disk	Streptomycin, 1000 µg/mL	Streptomycin, 2000 µg/mL
Inoculum	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	16-18 hours	24 hours	24 hours	16-18 hours	24-48 hours (if susceptible at 24 hours, reincubate)	24-48 hours (if susceptible at 24 hours, reincubate)
Results	6 mm = resistant 7-9 mm = inconclusive ≥ 10 mm = susceptible MIC correlates: R = > 500 µg/mL S = ≤ 500 µg/mL	Any growth = resistant	> 1 colony = resistant	6 mm = resistant 7-9 mm = inconclusive ≥ 10 mm = susceptible MIC correlates: R = > 1000 µg/mL (broth) and > 2000 µg/mL (agar) S = ≤ 1000 µg/mL (broth) and ≤ 2000 µg/mL (agar)	Any growth = resistant	> 1 colony = resistant

Table 3K. (Continued)

Test	Gentamicin HLAR			Streptomycin HLAR		
Additional testing and reporting	Resistant: is not synergistic with cell wall–active agent (eg, ampicillin, penicillin, and vancomycin). Susceptible: is synergistic with cell wall–active agent (eg, ampicillin, penicillin, and vancomycin) that is also susceptible. If disk diffusion result is inconclusive: perform an agar dilution or broth dilution MIC test to confirm. Strains of enterococci with ampicillin and penicillin MICs ≥ 16 µg/mL are categorized as resistant. However, enterococci with penicillin MICs ≤ 64 µg/mL or ampicillin MICs ≤ 32 µg/mL may be susceptible to synergistic killing by these penicillins in combination with gentamicin or streptomycin (in the absence of high-level resistance to gentamicin or streptomycin, see Subchapter 3.12.2.3 in M07[1]) if high doses of penicillin or ampicillin are used. Enterococci possessing higher levels of penicillin (MICs ≥ 128 µg/mL) or ampicillin (MICs ≥ 64 µg/mL) resistance may not be susceptible to the synergistic effect.[2,3] Physicians' requests to determine the actual MIC of penicillin or ampicillin for blood and CSF isolates of enterococci should be considered.					
QC recommendations – routine[c]	*E. faecalis* ATCC®[d] 29212: 16–23 mm	*E. faecalis* ATCC® 29212 – susceptible	*E. faecalis* ATCC® 29212 – susceptible	*E. faecalis* ATCC® 29212: 14–20 mm	*E. faecalis* ATCC® 29212 – susceptible	*E. faecalis* ATCC® 29212 – susceptible
QC recommendations – lot/shipment[e]		*E. faecalis* ATCC® 51299 – resistant	*E. faecalis* ATCC® 51299 – resistant		*E. faecalis* ATCC® 51299 – resistant	*E. faecalis* ATCC® 51299 – resistant

Abbreviations: ATCC®, American Type Culture Collection; BHI, brain heart infusion; CSF, cerebrospinal fluid; HLAR, high-level aminoglycoside resistance; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control.

Footnotes

a. Other aminoglycosides do not need to be tested, because their activities against enterococci are not superior to gentamicin and streptomycin.

b. Even though not as widely available, dextrose phosphate agar and broth have been shown in limited testing to perform comparably with BHI media.

c. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[4] and M07[1])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

Table 3K. (Continued)

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

e. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

References for Table 3K

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

2 Torres C, Tenorio C, Lantero M, Gastañares MJ, Baquero F. High-level penicillin resistance and penicillin-gentamicin synergy in *Enterococcus faecium*. *Antimicrob Agents Chemother*. 1993;37(11):2427-2431.

3 Murray BE. Vancomycin-resistant enterococci. *Am J Med*. 1997;102(3):284-293.

4 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

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Table 4A-1. Disk Diffusion QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding ß-Lactam Combination Agents[a]

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
Amikacin	30 µg	19-26	20-26	20-26
Ampicillin	10 µg	15-22	-	27-35
Azithromycin	15 µg	-	-	21-26
Azlocillin	75 µg	-	24-30	-
Aztreonam	30 µg	28-36	23-29	-
Carbenicillin	100 µg	23-29	18-24	-
Cefaclor	30 µg	23-27	-	27-31
Cefamandole	30 µg	26-32	-	26-34
Cefazolin	30 µg	21-27	-	29-35
Cefdinir	5 µg	24-28	-	25-32
Cefditoren	5 µg	22-28	-	20-28
Cefepime	30 µg	31-37	25-31	23-29
Cefetamet	10 µg	24-29	-	-
Cefiderocol	30 µg	25-31	22-31	-
Cefixime	5 µg	20-26	-	-
Cefmetazole	30 µg	26-32	-	25-34
Cefonicid	30 µg	25-29	-	22-28
Cefoperazone	75 µg	28-34	23-29	24-33
Cefotaxime	30 µg	29-35	18-22	25-31
Cefotetan	30 µg	28-34	-	17-23
Cefoxitin	30 µg	23-29	-	23-29
Cefpodoxime	10 µg	23-28	-	19-25
Cefprozil	30 µg	21-27	-	27-33
Ceftaroline	30 µg	26-34	-	26-35
Ceftazidime	30 µg	25-32	22-29	16-20
Ceftibuten	30 µg	27-35	-	-
Ceftizoxime	30 µg	30-36	12-17	27-35
Ceftobiprole	5 µg	25-31	-	20-27
Ceftriaxone	30 µg	29-35	17-23	22-28
Cefuroxime	30 µg	20-26	-	27-35
Cephalothin	30 µg	15-21	-	29-37
Chloramphenicol	30 µg	21-27	-	19-26
Cinoxacin	100 µg	26-32	-	-

Table 4A-1. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
Ciprofloxacin	5 µg	29-38	25-33	22-30
Clarithromycin	15 µg	-	-	26-32
Clinafloxacin	5 µg	31-40	27-35	28-37
Clindamycin[c]	2 µg	-	-	24-30
Colistin	10 µg	11-17	11-17	-
Delafloxacin[d]	5 µg	28–35	23–29	32–40
Dirithromycin	15 µg	-	-	18-26
Doripenem	10 µg	27-35	28-35	33-42
Doxycycline	30 µg	18-24	-	23-29
Enoxacin	10 µg	28-36	22-28	22-28
Eravacycline	20 µg	17-24	-	19-26
Ertapenem	10 µg	29-36	13-21	24-31
Erythromycin[c]	15 µg	-	-	22-30
Faropenem	5 µg	20-26	-	27-34
Fleroxacin	5 µg	28-34	12-20	21-27
Fosfomycin[e]	200 µg	22-30	-	25-33
Fusidic acid	10 µg	-	-	24-32
Garenoxacin	5 µg	28-35	19-25	30-36
Gatifloxacin	5 µg	30-37	20-28	27-33
Gemifloxacin	5 µg	29-36	19-25	27-33
Gentamicin[f]	10 µg	19-26	17-23	19-27
Gepotidacin	10 µg	18-26	-	23-29
Grepafloxacin	5 µg	28-36	20-27	26-31
Iclaprim	5 µg	14-22	-	25-33
Imipenem[g]	10 µg	26-32	20-28	-
Kanamycin	30 µg	17-25	-	19-26
Lefamulin	20 µg	-	-	26-32
Levofloxacin	5 µg	29-37	19-26	25-30
Levonadifloxacin	10 µg	27-33[d]	17-23[d]	32-39[d]
Linezolid	30 µg	-	-	25-32[h]
Lomefloxacin	10 µg	27-33	22-28	23-29
Loracarbef	30 µg	23-29	-	23-31
Mecillinam	10 µg	24-30	-	-
Meropenem	10 µg	28-35	27-33	29-37
Minocycline	30 µg	19-25	-	25-30
Moxalactam	30 µg	28-35	17-25	18-24

Table 4A-1. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
Moxifloxacin	5 µg	28-35	17-25	28-35
Nafcillin	1 µg	-	-	16-22
Nafithromycin	15 µg	-	-	25-31[d]
Nalidixic acid	30 µg	22-28	-	-
Netilmicin	30 µg	22-30	17-23	22-31
Nitrofurantoin	300 µg	20-25	-	18-22
Norfloxacin	10 µg	28-35	22-29	17-28
Ofloxacin	5 µg	29-33	17-21	24-28
Omadacycline	30 µg	22-28	-	22-30
Oxacillin	1 µg	-	-	18-24
Pefloxacin	5 µg	25-33	-	-
Penicillin	10 units	-	-	26-37
Piperacillin	100 µg	24-30	25-33	-
Plazomicin	30 µg	21-27	15-21	19-25
Polymyxin B	300 units	13-19	14-18	-
Quinupristin-dalfopristin	15 µg	-	-	21-28
Razupenem	10 µg	21-26	-	-[i]
Rifampin	5 µg	8-10	-	26-34
Solithromycin	15 µg	-	-	22-30
Sparfloxacin	5 µg	30-38	21-29	27-33
Streptomycin[f]	10 µg	12-20	-	14-22
Sulfisoxazole[j]	250 µg or 300 µg	15-23	-	24-34
Sulopenem	2 µg	24-30[d]	-	-
Tebipenem[g]	10 µg	30-37	20-26	-
Tedizolid[k]	2 µg	-	-	18-24[h]
Teicoplanin	30 µg	-	-	15-21
Telithromycin	15 µg	-	-	24-30
Tetracycline	30 µg	18-25	-	24-30
Ticarcillin	75 µg	24-30	21-27	-
Tigecycline	15 µg	20-27	9-13	20-25
Tobramycin	10 µg	18-26	20-26	19-29
Trimethoprim[j]	5 µg	21-28	-	19-26
Trimethoprim-sulfamethoxazole[j]	1.25/23.75 µg	23-29	-	24-32
Trospectomycin	30 µg	10-16	-	15-20
Trovafloxacin	10 µg	29-36	21-27	29-35
Ulifloxacin (prulifloxacin)[l]	5 µg	32-38	27-33	20-26
Vancomycin	30 µg	-	-	17-21

Abbreviations: ATCC®, American Type Culture Collection, ICR, inducible clindamycin resistance; QC, quality control.

Table 4A-1. (Continued)

Footnotes

a. Refer to Table 4A-2 for QC of β-lactam combination agents.

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

c. When disk approximation tests are performed with erythromycin and clindamycin, *S. aureus* ATCC® BAA-977™ (containing inducible *erm*A-mediated resistance) and *S. aureus* ATCC® BAA-976™ (containing *msr*A-mediated macrolide-only efflux) are recommended as supplemental QC strains (eg, for training, competence assessment, or test evaluation). *S. aureus* ATCC® BAA-977™ should demonstrate ICR (ie, a positive D-zone test), whereas *S. aureus* ATCC® BAA-976™ should not demonstrate ICR. *S. aureus* ATCC® 25923 should be used for routine QC (eg, weekly or daily) of erythromycin and clindamycin disks using standard Mueller-Hinton agar.

d. QC ranges were established using data from only one disk manufacturer. Disks from other manufacturers were not available at the time of testing.

e. The 200-µg fosfomycin disk contains 50 µg of glucose-6-phosphate.

f. For control ranges of gentamicin 120-µg and streptomycin 300-µg disks, use *E. faecalis* ATCC® 29212 (gentamicin: 16–23 mm; streptomycin: 14–20 mm).

g. *Klebsiella pneumoniae* ATCC® 700603 is a supplemental QC strain for testing QC of imipenem (25-33 mm) and tebipenem (26–32 mm).

h. Zones of inhibition for linezolid and tedizolid with *S. aureus* ATCC® 25923 should be read using transmitted light.

i. Razupenem tested with *S. aureus* ATCC® 25923 can often produce the double or target zone phenomenon. For accurate QC results, use *S. aureus* ATCC® 29213 (no double zones) with acceptable range 33–39 mm.

j. These agents can be affected by excess levels of thymidine and thymine. See M02,[1] Subchapter 3.1.1.2 for guidance, should a problem with QC occur.

k. *E. faecalis* ATCC® 29212 is a supplemental QC strain for testing QC of tedizolid (14–21 mm) to assist with reading.

l. Ulifloxacin is the active metabolite of the prodrug prulifloxacin. Only ulifloxacin should be used for antimicrobial susceptibility testing.

Reference for Table 4A-1

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

Table 4A-2. Disk Diffusion QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents[a]

Antimicrobial Agent	Disk Content	QC Organisms and Characteristics								
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
		β-lactamase negative	Inducible AmpC	β-lactamase negative, *mecA* negative	TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37 TEM-1	CTX-M-15 OXA-1	KPC-2 SHV	KPC-3 SHV-11 TEM-1	OXA-27
		Zone Diameter QC Ranges, mm								
Amoxicillin-clavulanate (2:1)	20/10 µg	18-24	-	28-36	17-22	-	-	-	-	-
Ampicillin	10 µg	15-22	-	27-35	6	-	-	-	-	-
Ampicillin-sulbactam (2:1)	10/10 µg	19-24	-	29-37	13-19	-	-	-	-	-
Aztreonam	30 µg	28-36	23-29	-	31-38	10-16	-	-	-	-
Aztreonam-avibactam	30/20 µg	32-38	24-30	-	31-38	26-32[f]	-	-	-	-
Cefepime	30 µg	31-37	25-31	23-29	31-37	23-29	6-15[g]	-	-	6-16[g]
Cefepime-enmetazobactam[f]	30/20 µg	32-38	26-32	-	32-38	26-32	27-33	-	-	-
Cefepime-taniborbactam	30/20 µg	31-37	25-31	-	31-37	24-31	24-30	22-27	-	-
Cefepime-tazobactam	30/20 µg	32-37	27-31	24-30	-	25-30[f]	27-31	-	-	-
Cefepime-zidebactam	30/30 µg	33-40	29-35	-	-	28-34	29-35	-	-	19-25
Cefotaxime	30 µg	29-35	18-22	25-31	-	17-25	-	-	-	-
Cefpodoxime	10 µg	23-28	-	19-25	-	9-16	-	-	-	-
Ceftaroline	30 µg	26-34	-	26-35	-	-	-	-	-	-
Ceftaroline-avibactam	30/15 µg	27-34	17-26	25-34	27-35	21-27[f]	-	-	-	-
Ceftazidime	30 µg	25-32	22-29	16-20	-	10-18	-	-	-	-
Ceftazidime-avibactam	30/20 µg	27-35	25-31	16-22	28-35	21-27[f]	-	-	-	-
Ceftibuten	**30 µg**	-	-	-	-	-	**15-23**	-	-	-
Ceftibuten-ledaborbactam	**5/2.5 µg**	-	-	-	-	-	**24-29**	-	-	-
Ceftolozane-tazobactam	30/10 µg	24-32	25-31	10-18	25-31	17-25	-	-	-	-
Ceftriaxone	30 µg	29-35	17-23	22-28	-	16-24	-	-	-	-
Imipenem	10 µg	26-32	20-28	-	-	25-33	-	11-22	6-14	-
Imipenem-relebactam[f,h]	10/25 µg	27-33	26-31	-	-	26-32	-	23-29	22-28	-
Meropenem[g]	10 µg	28-35	27-33	29-37	-	-	-	11-18[f]	6[f]	-

Table 4A-2. (Continued)

Antimicrobial Agent	Disk Content	QC Organisms and Characteristics								
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
		ß-lactamase negative	Inducible AmpC	ß-lactamase negative, *mecA* negative	TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37 TEM-1	CTX-M-15 OXA-1	KPC-2 SHV	KPC-3 SHV-11 TEM-1	OXA-27
		Zone Diameter QC Ranges, mm								
Meropenem-vaborbactam[h]	20/10 µg	31-37	29-35	32-38	-	29-35	-	21-27	16-20	-
Piperacillin	100 µg	24-30	25-33	-	12-18	-	-	-	-	-
Piperacillin-tazobactam	100/10 µg	24-30	25-33	27-36	24-30	-	-	-	-	-
Sulbactam-durlobactam	10/10 µg	26-32	-	-	-	-	-	-	-	24-30
Ticarcillin	75 µg	24-30	21-27	-	6	-	-	-	-	-
Ticarcillin-clavulanate	75/10 µg	24-30	20-28	29-37	21-25	-	-	-	-	-

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; N/A, not applicable; NCTC, National Collection of Type Cultures; QC, quality control.

QC strain selection codes: QC strain is recommended for routine QC.
Test one of these agents by a disk diffusion or MIC method to confirm the integrity of the respective QC strain.[c,d]

Footnotes

a. Unsupplemented Mueller-Hinton medium. See Table 4A-1 for QC ranges for combination agents from other drug classes.

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

c. Careful attention to organism maintenance (eg, minimal subcultures) and storage (eg, −60°C or below) is especially important for these QC strains because spontaneous loss of the plasmid encoding the ß-lactamase has been documented. If stored at temperatures above −60°C or if repeatedly subcultured, these strains may lose their resistance characteristics and QC results may be outside the acceptable ranges.

d. To confirm the integrity of the QC strain, test one of the single ß-lactam agents highlighted in orange by either a disk diffusion or MIC test method when the strain is first subcultured from a frozen or lyophilized stock culture. In some cases, only MIC ranges are available to accomplish this confirmation (see Table 5A-2). In-range results for the single agent indicate the QC strain is reliable for QC of ß-lactam combination agents. It is not necessary to check the QC strain again with a single agent until a new frozen or lyophilized stock culture is put into use, providing recommendations for handling QC strains as described in M02[1] and M07[2] are followed.

Table 4A-2
Nonfastidious Disk Diffusion QC for ß-Lactam Combination Agents
M02

Table 4A-2. (Continued)

e. Strain may demonstrate two colony morphologies: 1) opaque and cream colored and 2) translucent. Both colony morphologies can be used.

f. QC ranges were established using data from only one disk manufacturer. Disks from other manufacturers were not available at the time of testing.

g. If discrete colonies or a haze of growth are present inside the zone of inhibition, measure the colony-free inner zone.

h. Either strain highlighted in green may be used for routine QC of this antimicrobial agent.

NOTE: Information in black boldface type is new or modified since the previous edition.

References for Table 4A-2

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

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Table 4B. Disk Diffusion QC Ranges for Fastidious Organisms

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm			
		Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619[b]
Amoxicillin-clavulanate[c]	20/10 µg	15-23	-	-	-
Ampicillin	10 µg	13-21	-	-	30-36
Ampicillin-sulbactam	10/10 µg	14-22	-	-	-
Azithromycin	15 µg	13-21	-	30-38	19-25
Aztreonam	30 µg	30-38	-	-	-
Cefaclor	30 µg	-	25-31	-	24-32
Cefdinir	5 µg	-	24-31	40-49	26-31
Cefditoren	5 µg	25-34	-	-	27-35
Cefepime	30 µg	25-31	-	37-46	28-35
Cefetamet	10 µg	23-28	-	35-43	-
Cefixime	5 µg	25-33	-	37-45	16-23
Cefmetazole	30 µg	16-21	-	31-36	-
Cefonicid	30 µg	-	30-38	-	-
Cefotaxime	30 µg	31-39	-	38-48	31-39
Cefotetan	30 µg	-	-	30-36	-
Cefoxitin	30 µg	-	-	33-41	-
Cefpodoxime	10 µg	25-31	-	35-43	28-34
Cefprozil	30 µg	-	20-27	-	25-32
Ceftaroline	30 µg	29-39	-	-	31-41
Ceftaroline-avibactam[d]	30/15 µg	30-38	-	-	-
Ceftazidime	30 µg	27-35	-	35-43	-
Ceftazidime-avibactam[d]	30/20 µg	28-34	-	-	23-31
Ceftibuten	30 µg	29-36	-	-	-
Ceftizoxime	30 µg	29-39	-	42-51	28-34
Ceftobiprole[e]	30 µg	28-36	30-38	-	33-39
Ceftolozane-tazobactam[d]	30/10 µg	23-29	-	-	21-29
Ceftriaxone	30 µg	31-39	-	39-51	30-35
Cefuroxime	30 µg	-	28-36	33-41	-
Cephalothin	30 µg	-	-	-	26-32
Chloramphenicol	30 µg	31-40[f]	-	-	23-27
Ciprofloxacin	5 µg	34-42	-	48-58	-
Clarithromycin	15 µg	11-17[g]	-	-	25-31
Clinafloxacin	5 µg	34-43	-	-	27-34
Clindamycin	2 µg	-	-	-	19-25
Delafloxacin	5 µg	40–51	-	-	28-36[g]
Dirithromycin	15 µg	-	-	-	18-25
Doripenem	10 µg	21-31	-	-	30-38
Doxycycline	30 µg	-	-	-	25-34
Enoxacin	10 µg	-	-	43-51	-
Eravacycline	20 µg	-	-	-	23-30
Ertapenem[e]	10 µg	20-28	27-33	-	28-35

Table 4B. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm			
		Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619[b]
Erythromycin	15 µg	-	-	-	25-30
Faropenem	5 µg	15-22	-	-	27-35
Fleroxacin	5 µg	30-38	-	43-51	-
Fusidic acid	10 µg	-	-	-	9-16
Garenoxacin	5 µg	33-41	-	-	26-33
Gatifloxacin	5 µg	33-41	-	45-56	24-31
Gemifloxacin	5 µg	30-37	-	-	28-34
Gentamicin	**10 µg**	-	-	**15-20**	-
Gepotidacin	10 µg	-	-	32-40	22-28
Grepafloxacin	5 µg	32-39	-	44-52	21-28
Iclaprim	5 µg	24-33	-	-	21-29
Imipenem	10 µg	21-29	-	-	-
Lefamulin	20 µg	22-28	-	-	19-27
Levofloxacin	5 µg	32-40	-	-	20-25
Levonadifloxacin	10 µg	33-41[g]	-	-	24-31[g]
Linezolid	30 µg	-	-	-	25-34
Lomefloxacin	10 µg	33-41	-	45-54	-
Loracarbef	30 µg	-	26-32	-	22-28
Meropenem	10 µg	20-28	-	-	28-35
Moxifloxacin	5 µg	31-39	-	-	25-31
Nafithromycin	15 µg	16-20[g]	-	-	25-31[g]
Nitrofurantoin	300 µg	-	-	-	23-29
Norfloxacin	10 µg	-	-	-	15-21
Ofloxacin	5 µg	31-40	-	43-51	16-21
Omadacycline	30 µg	21-29	-	-	24-32
Oxacillin	1 µg	-	-	-	≤ 12[h]
Penicillin	10 units	-	-	26-34	24-30
Piperacillin-tazobactam	100/10 µg	33-38	-	-	-
Quinupristin-dalfopristin	15 µg	15-21	-	-	19-24
Razupenem	10 µg	24-30	-	-	29-36
Rifampin	5 µg	22-30	-	-	25-30
Solithromycin	15 µg	16-23	-	33-43	25-33
Sparfloxacin	5 µg	32-40	-	43-51	21-27
Spectinomycin	100 µg	-	-	23-29	-
Tedizolid	2 µg	-	-	-	18-25
Telithromycin	15 µg	17-23	-	-	27-33
Tetracycline	30 µg	14-22	-	30-42	27-31
Tigecycline	15 µg	23-31	-	30-40	23-29
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	24-32	-	-	20-28
Trospectomycin	30 µg	22-29	-	28-35	-
Trovafloxacin	10 µg	32-39	-	42-55	25-32
Vancomycin	30 µg	-	-	-	20-27

Table 4B. (Continued)

Disk Diffusion Testing Conditions for Clinical Isolates and Performance of QC

Organism	*H. influenzae*	*N. gonorrhoeae*	Streptococci and *N. meningitidis*
Medium	HTM **MH-F agar**	GC agar base and 1% defined growth supplement. The use of a cysteine-free growth supplement is not required for disk diffusion testing.	MHA supplemented with 5% defibrinated sheep blood MH-F agar for *S. pneumoniae* only
Inoculum	Colony suspension	Colony suspension	Colony suspension
Incubation conditions	5% CO_2; 16–18 hours; 35°C **± 2°C**	5% CO_2; 20–24 hours; **36°C ± 1°C (do not exceed 37°C)**	5% CO_2; 20–24 hours; 35°C**± 2°C**

Abbreviations: ATCC®, American Type Culture Collection; HTM, *Haemophilus* test medium; MHA, Mueller-Hinton agar; MH-F agar, Mueller-Hinton fastidious agar; QC, quality control.

Footnotes

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

b. Despite the lack of reliable disk diffusion breakpoints for *S. pneumoniae* with certain β-lactams, *S. pneumoniae* ATCC® 49619 is the strain designated for QC of all disk diffusion tests with all *Streptococcus* spp.

c. When testing on HTM incubated in ambient air, the acceptable QC limits for *E. coli* ATCC® 35218 are 17–22 mm for amoxicillin-clavulanate.

d. QC limits for *E. coli* ATCC® 35218 in HTM: ceftaroline-avibactam 26–34 mm; ceftazidime-avibactam 27–34 mm; ceftolozane-tazobactam 25–31 mm.

e. Either *H. influenzae* ATCC® 49247 or 49766 may be used for routine QC testing **with HTM; *H. influenzae* ATCC® 49247 should be used for routine QC testing with MH-F.**

f. QC limits for *H. influenzae* ATCC® 49247 in MH-F agar: chloramphenicol 28-36 mm.

g. QC ranges for delafloxacin, levonadifloxacin, and nafithromycin, **as well as for clarithromycin with MH-F agar,** were established using data from only one disk manufacturer. Disks from other manufacturers were not available at the time of testing.

h. Deterioration in oxacillin disk content is best assessed with QC organism *S. aureus* ATCC® 25923, with an acceptable zone diameter of 18–24 mm.

NOTE: Information in black boldface type is new or modified since the previous edition.

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Table 4C. Disk Diffusion Reference Guide to QC Frequency

This table summarizes the suggested QC frequency when modifications are made to antimicrobial susceptibility test systems (refer to CLSI document EP23™[1]). It applies only to antimicrobial agents for which satisfactory results have been obtained with either the 15-replicate (3- × 5-day) plan or 20 or 30 consecutive test day plan. Otherwise QC is required each test day.

Test Modification	Recommended QC Frequency			Comments
	1 Day	5 Days	15-Replicate Plan or 20- or 30-Day Plan	
Disks				
Use new shipment or lot number.	X			
Use new manufacturer.	X			
Addition of new antimicrobial agent to existing system.			X	In addition, perform in-house verification studies.
Media (prepared agar plates)				
Use new shipment or lot number.	X			
Use new manufacturer.		X		
Inoculum preparation				
Convert inoculum preparation/ standardization to use of a device that has its own QC protocol.		X		**Example:** Convert from visual adjustment of turbidity to use of a photometric device for which a QC procedure is provided.
Convert inoculum preparation/ standardization to a method that depends on user technique.			X	**Example:** Convert from visual adjustment of turbidity to another method that is not based on a photometric device.
Measuring zones				
Change method of measuring zones.			X	**Example:** Convert from manual zone measurements to automated zone reader. In addition, perform in-house verification studies.
Instrument/software (eg, automated zone reader)				
Software update that affects AST results		X		Monitor all drugs, not just those implicated in software modification.
Repair of instrument that affects AST results	X			Depending on extent of repair (eg, critical component such as the photographic device), additional testing may be appropriate (eg, 5 days).

Abbreviations: AST, antimicrobial susceptibility testing; QC, quality control.

Table 4C. (Continued)

NOTE 1: QC can be performed before or concurrent with testing patient isolates. Patient results can be reported for that day if QC results are within the acceptable limits.

NOTE 2: Manufacturers of commercial or in-house-prepared tests should follow their own internal procedures and applicable regulations.

NOTE 3: For troubleshooting out-of-range results, refer to M02,[2] Subchapter 4.8 and M100 Table 4D. Additional information is available in Appendix C (eg, QC organism characteristics, QC testing recommendations).

NOTE 4: Broth, saline, and/or water used to prepare an inoculum does not need routine QC.

References for Table 4C

1 CLSI. *Laboratory Quality Control Based on Risk Management; Approved Guideline*. CLSI document EP23-A™. Clinical and Laboratory Standards Institute; 2011.

2 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

Table 4D. Disk Diffusion Troubleshooting Guide

This table provides guidance for troubleshooting and corrective action for out-of-range QC, primarily using antimicrobial susceptibility tests with MHA. Refer to M02,[1] Chapter 4, for additional information. Out-of-range QC tests are often the result of contamination or the use of an incorrect QC strain; corrective action should first include repeating the test with a pure culture of a freshly subcultured QC strain. If the issue is unresolved, this troubleshooting guide should be consulted regarding additional suggestions for troubleshooting out-of-range QC results and unusual clinical isolate results. In addition, see general corrective action outlined in M02[1] and notify manufacturers of potential product problems.

General Comment

(1) QC organism maintenance: Avoid repeated subcultures. Retrieve new QC strain from stock (refer to M02,[1] Subchapter 4.4). If using lyophilized strains, follow the maintenance recommendations of the manufacturer.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS				
β-lactam combination agents	*A. baumannii* ATCC®[a] 13304 *E. coli* ATCC® 35218 *E. coli* ATCC® 13353 *K. pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™	Zone too large or susceptible for single β-lactam agent; in range for combination β-lactam agent	Spontaneous loss of the plasmid encoding the β-lactamase	Obtain new frozen or lyophilized stock culture. Use other routine QC strains (if available). These strains should be stored at −60°C or below, and frequent subcultures should be avoided. **NOTE:** *K. pneumoniae* BAA-2814™ is stable and does not require QC integrity check.
β-lactam combination agents	*A. baumannii* ATCC® 13304 *E. coli* ATCC® 35218 *E. coli* ATCC® 13353 *K. pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™ *K. pneumoniae* ATCC® BAA-2814™	Zone too small or resistant for both the single β-lactam agent and the combination β-lactam agent	Antimicrobial agent is degrading.	Use alternative lot of test materials. Check storage and package integrity. Imipenem and clavulanate are especially labile.
Carbenicillin	*P. aeruginosa* ATCC® 27853	Zone too small	QC strain develops resistance after repeated subculture.	See general comment (1) on QC strain maintenance.
Cefepime	*A. baumannii* NCTC 13304 *E. coli* NCTC 13353	QC strain integrity test	Discrete colonies may grow within the zone of inhibition when this organism is tested with cefepime 30-µg disk.	If this occurs, measure the colony-free inner zone.
Imipenem	*K. pneumoniae* ATCC® BAA-1705™ *K. pneumoniae* ATCC® BAA-2814™	QC strain integrity test	Discrete colonies may grow within the zone of inhibition when this organism is tested with cefepime. 30-µg disk.	If this occurs, measure the colony-free inner zone.
Penicillins	Any	Zone too large	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Penicillins	Any	Zone too small	pH of media too high	Acceptable pH range = 7.2–7.4

Table 4D. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
NON-β-LACTAMS				
β-lactam group	Any	Zone initially acceptable, but decreases to possibly be out of range over time	Imipenem, clavulanate, and cefaclor are especially labile. Disks have lost potency.	Use alternative lot of disks. Check storage conditions and package integrity.
Aminoglycosides Quinolones	Any	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4
Aminoglycosides	*P. aeruginosa* ATCC® 27853	Zone too small	Ca++ and/or Mg++ content too high	Use alternative lot of media.
Aminoglycosides	*P. aeruginosa* ATCC® 27853	Zone too large	Ca++ and/or Mg++ content too low	Use alternative lot of media.
Clindamycin Macrolides	*S. aureus* ATCC® 25923	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
	S. aureus ATCC® 25923	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4
Quinolones	Any	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Quinolones	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4
Tedizolid	*E. faecalis* ATCC® 29212	Zone with *Enterococcus* spp. is difficult to read	Light growth on MHA	*E. faecalis* ATCC® 29212 is provided as supplemental QC to assist in personnel training and assessment of proper reading. Measure zone edge where there is a significant decrease in density of growth when using transmitted light as illustrated in the photographs.[b]
Tetracyclines	Any	Zone too large	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Tetracyclines	Any	Zone too small	pH of media too high	Acceptable pH range = 7.2–7.4
Tetracyclines	Any	Zone too small	Ca++ and/or Mg++ content too high	Use alternative lot of media.
Tetracyclines	Any	Zone too large	Ca++ and/or Mg++ content too low	Use alternative lot of media.
Sulfonamides Trimethoprim Trimethoprim-sulfamethoxazole	*E. faecalis* ATCC® 29212	Zone ≤ 20 mm	Media too high in thymidine content	Use alternative lot of media.

Table 4D. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
ALL AGENTS				
Various	*S. pneumoniae* ATCC® 49619	Zones too large Lawn of growth scanty	Inoculum source plate too old and contains too many nonviable cells. Plate used to prepare inoculum should be 18–20 hours.	Subculture QC strain and repeat QC test or retrieve new QC strain from stock.
Various	Various	Zone too small	Contamination Use of magnification to read zones	Measure zone edge with visible growth detected with unaided eye. Subculture to determine purity and repeat if necessary.
Various	Any	Many zones too small	Inoculum too heavy Error in inoculum preparation Media depth too thick	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Use agar with depth approximately 4 mm. Recheck alternate lots of MHA.
Various	Any	One or more zones too small or too large	Measurement error Transcription error Random defective disk Disk not pressed firmly against agar	Recheck readings for measurement or transcription errors. Retest. If retest results are out of range and no errors are detected, initiate corrective action.
Various	Various	Zone too large	Did not include lighter growth in zone measurement (eg, double zone, fuzzy zone edge)	Measure zone edge with visible growth detected with unaided eye.
Various	Any	QC results from one strain are out of range, but results from other QC strain(s) is in range with the same antimicrobial agent.	One QC strain may be a better indicator of a QC problem.	Retest this strain to confirm reproducibility of acceptable results. Evaluate with alternative strains with known MICs. Initiate corrective action with problem QC strain/antimicrobial agent(s).
Various	Any	QC results from two strains are out of range with the same antimicrobial agent.	A problem with the disk	Use alternative lot of disks. Check storage conditions and package integrity.
Various	Any	Zones overlap.	Too many disks per plate	Place no more than 12 disks on a 150-mm plate and 5 disks on a 100-mm plate; for some fastidious bacteria that produce large zones, use fewer.

Abbreviations: ATCC®, American Type Culture Collection; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; pH, negative logarithm of hydrogen ion concentration; QC, quality control.

Table 4D. (Continued)

Footnotes

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

b. Figure 1 shows examples of tedizolid disk diffusion results for *E. faecalis*.

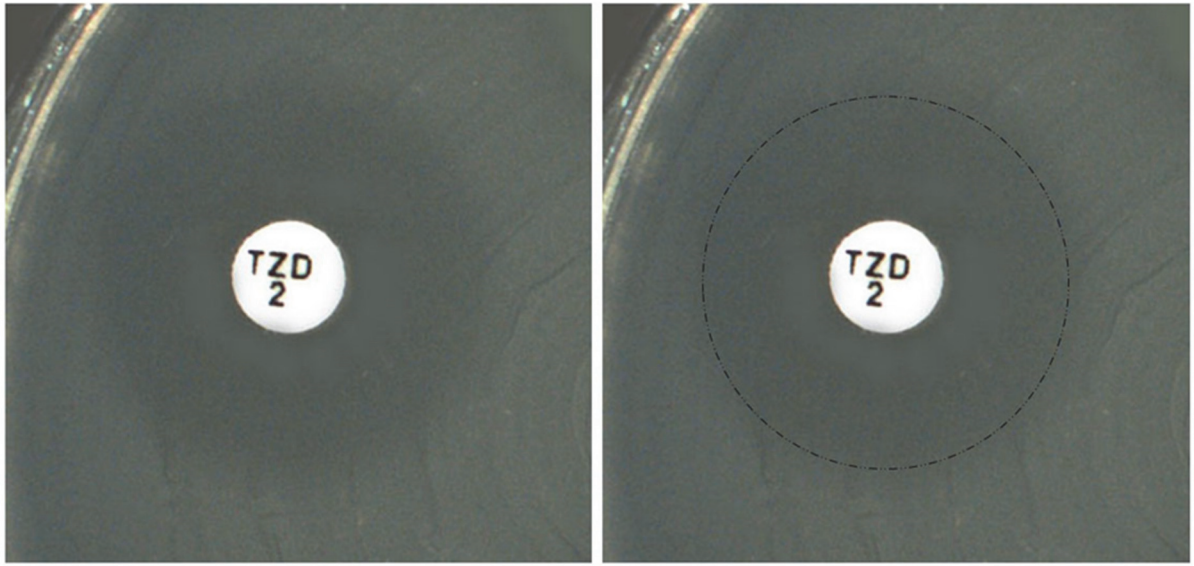

Figure 1. Measuring the Tedizolid Zone for *E. faecalis* ATCC® 29212 When Light Growth Is Observed

Reference for Table 4D

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

Table 5A-1. MIC QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-Lactam Combination Agents[a]

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212
Amikacin	0.5-4	1-4	1-4	64-256
Amikacin-fosfomycin (5:2)[c]	0.25/0.1–2/0.8	1/0.4–8/3.2	0.5/0.2–4/1.6	32/12.8–128/51.2
Amoxicillin	-	-	-	-
Ampicillin	2-8	-	0.5-2	0.5-2
Azithromycin	-	-	0.5-2	-
Azlocillin	8-32	2-8	2-8	1-4
Aztreonam	0.06-0.25	2-8	-	-
Besifloxacin	0.06-0.25	1-4	0.016-0.06	0.06-0.25
Biapenem	0.03-0.12	0.5-2	0.03-0.12	-
Cadazolid	-	-	0.06–0.5	0.06–0.25
Carbenicillin	4-16	16-64	2-8	16-64
Cefaclor	1-4	-	1-4	-
Cefamandole	0.25-1	-	0.25-1	-
Cefazolin	1-4	-	0.25-1	-
Cefdinir	0.12-0.5	-	0.12-0.5	-
Cefditoren	0.12-1	-	0.25-2	-
Cefepime	0.016-0.12	0.5-4	1-4	-
Cefetamet	0.25-1	-	-	-
Cefiderocol[d]	0.06-0.5	0.06-0.5	-	-
Cefixime	0.25-1	-	8-32	-
Cefmetazole	0.25-1	> 32	0.5-2	-
Cefonicid	0.25-1	-	1-4	-
Cefoperazone	0.12-0.5	2-8	1-4	-
Cefotaxime	0.03-0.12	8-32	1-4	-
Cefotetan	0.06-0.25	-	4-16	-
Cefoxitin	2-8	-	1-4	-
Cefpodoxime	0.25-1	-	1-8	-
Cefprozil	1-4	-	0.25-1	-
Ceftaroline	0.03-0.12	-	0.12-0.5	0.25-2[e]
Ceftazidime	0.06-0.5	1-4	4-16	-
Ceftibuten[f]	**0.12-1**	-	-	-
Ceftizoxime	0.03-0.12	16-64	2-8	-
Ceftobiprole	0.03-0.12	1-4	0.12-1	0.06-0.5
Ceftriaxone	0.03-0.12	8-64	1-8	-
Cefuroxime	2-8	-	0.5-2	-
Cephalothin	4-16	-	0.12-0.5	-

Table 5A-1. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212
Chloramphenicol	2-8	-	2-16	4-16
Cinoxacin	2-8	-	-	-
Ciprofloxacin[g]	0.004-0.016	0.12-1	0.12-0.5	0.25-2
Clarithromycin	-	-	0.12-0.5	-
Clinafloxacin	0.002-0.016	0.06-0.5	0.008-0.06	0.03-0.25
Clindamycin[h]	-	-	0.06-0.25	4-16
Colistin[i,j]	0.25-2	0.5-4	-	-
Dalbavancin[k]	-	-	0.03-0.12	0.03-0.12
Daptomycin[l]	-	-	0.12-1	1-4
Delafloxacin	0.008–0.03	0.12–0.5	0.001–0.008	0.016–0.12
Dirithromycin	-	-	1-4	-
Doripenem	0.016-0.06	0.12-0.5	0.016-0.06	1-4
Doxycycline	0.5-2	-	0.12-0.5	2-8
Enoxacin	0.06-0.25	2-8	0.5-2	2-16
Eravacycline	0.016-0.12	2-16	0.016-0.12	0.016-0.06
Ertapenem	0.004-0.016	2-8	0.06-0.25	4-16
Erythromycin[h]	-	-	0.25-1	1-4
Exebacase[m]	-	-	0.25-2	8-64
Faropenem	0.25-1	-	0.03-0.12	-
Fidaxomicin	-	-	2-16	1-4
Finafloxacin	0.004-0.03	1-8	0.03-0.25	0.25-1
Fleroxacin	0.03-0.12	1-4	0.25-1	2-8
Fosfomycin[n]	0.5-2	2-8	0.5-4	32-128
Fusidic acid	-	-	0.06-0.25	-
Garenoxacin	0.004-0.03	0.5-2	0.004-0.03	0.03-0.25
Gatifloxacin	0.008-0.03	0.5-2	0.03-0.12	0.12-1.0
Gemifloxacin	0.004-0.016	0.25-1	0.008-0.03	0.016-0.12
Gentamicin[o]	0.25-1	0.5-2	0.12-1	4-16
Gepotidacin	1-4	-	0.12-1	1-4
Grepafloxacin	0.004-0.03	0.25-2.0	0.03-0.12	0.12-0.5
Iclaprim	1-4	-	0.06-0.25	0.004-0.03
Imipenem	0.06-0.5	1-4	0.016-0.06	0.5-2
Kanamycin	1-4	-	1-4	16-64
Lefamulin	–	–	0.06-0.25	–
Levofloxacin	0.008-0.06	0.5-4	0.06-0.5	0.25-2
Levonadifloxacin	0.03–0.25	0.5–4	0.008–0.03	-
Linezolid[p]	-	-	1-4	1-4
Lomefloxacin	0.03-0.12	1-4	0.25-2	2-8
Loracarbef	0.5-2	> 8	0.5-2	-

Table 5A-1. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212
Mecillinam	0.03-0.25[q]	-	-	-
Meropenem	0.008-0.06	0.12-1	0.03-0.12	2-8
Minocycline[g]	0.25-1	-	0.06-0.5	1-4
Moxalactam	0.12-0.5	8-32	4-16	-
Moxifloxacin	0.008-0.06	1-8	0.016-0.12	0.06-0.5
Nafcillin	-	-	0.12-0.5	2-8
Nafithromycin	-	-	0.06-0.25	0.016-0.12
Nalidixic acid[g]	1-4	-	-	-
Netilmicin	≤0.5-1	0.5-8	≤0.25	4-16
Nitrofurantoin	4-16	-	8-32	4-16
Norfloxacin	0.03-0.12	1-4	0.5-2	2-8
Ofloxacin	0.016-0.12	1-8	0.12-1	1-4
Omadacycline[r]	0.25-2	-	0.12-1	0.06-0.5
Oritavancin[k]	-	-	0.016-0.12	0.008-0.03
Oxacillin	-	-	0.12-0.5	8-32
Ozenoxacin	-	-	0.001-0.004	0.016-0.06
Penicillin	-	-	0.25-2	1-4
Pexiganan	2-8	2-16	8-32	16-64
Piperacillin	1-4	1-8	1-4	1-4
Plazomicin	0.25-2	1-4	0.25-2	-
Polymyxin B	0.25-2	0.5-2	-	-
Quinupristin-dalfopristin	-	-	0.25-1	2-8
Razupenem	0.06-0.5	-	0.008-0.03	0.25-1
Rifampin	4-16	16-64	0.004-0.016	0.5-4
Solithromycin	-	-	0.03-0.12	0.016-0.06
Sparfloxacin	0.004-0.016	0.5-2	0.03-0.12	0.12-0.5
Sulfisoxazole[g,s]	8-32	-	32-128	32-128
Sulopenem	0.016-0.06	-	0.016-0.12	2-8
Tebipenem[f]	0.008-0.03	1-8	0.016-0.06	0.25-1
Tedizolid[t]	-	-	0.12-1	0.25-1
Teicoplanin	-	-	0.25-1	0.25-1
Telavancin[k]	-	-	0.03-0.12	0.03-0.12
Telithromycin	-	-	0.06-0.25	0.016-0.12
Tetracycline	0.5-2	8-32	0.12-1	8-32
Ticarcillin	4-16	8-32	2-8	16-64
Tigecycline[r]	0.03-0.25	-	0.03-0.25	0.03-0.12
Tobramycin	0.25-1	0.25-1	0.12-1	8-32

Table 5A-1. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Escherichia coli ATCC®[a,b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212
Trimethoprim[s]	0.5-2	> 64	1-4	0.12-0.5
Trimethoprim-sulfamethoxazole[s] (1:19)	≤0.5/9.5	8/152-32/608	≤0.5/9.5	≤0.5/9.5
Trospectomycin	8-32	-	2-16	2-8
Trovafloxacin	0.004-0.016	0.25-2	0.008-0.03	0.06-0.25
Ulifloxacin (prulifloxacin)[u]	0.004-0.016	0.12-0.5	-	-
Vancomycin[v]	-	-	0.5-2	1-4
Zidebactam	0.06-0.25	1-8	-	-
Zoliflodacin	1-4	-	0.12-0.5	0.25-2

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; ICR, inducible clindamycin resistance; MHB, Mueller-Hinton broth; MIC, minimal inhibitory concentration; QC, quality control.

Footnotes

a. Refer to Table 5A-2 for QC of β-lactam combination agents.

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

c. QC ranges reflect MICs obtained when medium is supplemented with 25 µg/mL of glucose-6-phosphate.

d. QC ranges reflect MICs obtained when CAMHB is iron depleted. Chelation is used for iron depletion, which also removes other cations (ie, calcium, magnesium, and zinc). Following this process, cations are added back to concentrations of calcium 20-25 mg/L, magnesium 10-12.5 mg/L, and zinc 0.5-1.0 mg/L.

e. Testing this strain with this antimicrobial agent is considered supplemental QC only and is not required as routine user QC testing.

f. MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available.

g. QC limits for *E. coli* ATCC® 25922 with ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole when tested in CAMHB with 2.5% to 5% lysed horse blood incubated either in ambient air or 5% CO_2 (when testing *N. meningitidis*) are the same as those listed in Table 5A-1.

h. When the erythromycin/clindamycin combination well for detecting ICR is used, *S. aureus* ATCC® BAA-977™ (containing inducible *erm*A-mediated resistance) and *S. aureus* ATCC® 29213 or *S. aureus* ATCC® BAA-976™ (containing *msr*A-mediated macrolide-only efflux) are recommended for QC purposes. *S. aureus* ATCC® BAA-977™ should demonstrate ICR (ie, growth in the well), whereas *S. aureus* ATCC® 29213 and *S. aureus* ATCC® BAA-976™ should not demonstrate ICR (ie, no growth in the well).

Table 5A-1. (Continued)

i. ***P. aeruginosa*** **ATCC® 27853 is recommended for routine QC. *E. coli* ATCC® 25922 is provided as a supplemental QC strain. Additional ranges for colistin are also provided as supplemental QC (eg, confirm quality of production lots, validation studies). These supplemental** QC strains and ranges for colistin include *E. coli* NCTC 13846 **(1-8 µg/mL, bimodal 2-4)** and *E. coli* ATCC® BAA-3170™ (formerly AR Bank #0349 *mcr-1*) (1-4 µg/mL, mode 2). **Results of 1 µg/mL or 8 µg/mL were infrequent (< 5%) during Tier 2 studies to establish colistin QC ranges. Determine whether MIC results trend at the low or high end of the range (1 µg/mL or 8 µg/mL) (for troubleshooting, see Table 5G).**

j. Colistin results are significantly affected by preparation and handling of testing materials, including stock solutions and test medium, as well as by the composition of the testing tube and/or plate (eg, glass, polystyrene, polypropylene). QC results may fall outside the established CLSI QC ranges if methods other than CLSI reference methods described in M07[1] and M100 are used.

k. QC ranges reflect MICs obtained when CAMHB is supplemented with 0.002% polysorbate-80.

l. QC ranges reflect MICs obtained when MHB is supplemented with calcium to a final concentration of 50 µg/mL. Agar dilution has not been validated for daptomycin.

m. QC ranges reflect MICs obtained when CAMHB is supplemented with **horse serum (25% v/v) and** 0.5 mM DL-dithiothreitol (pH 7.2-7.4) **(CAMHB-HSD). This medium is used for testing exebacase against *S. aureus* and β-hemolytic streptococci. CAMHB-HSD does not require addition of lysed horse blood when β-hemolytic streptococci is tested. *S. aureus* ATCC® 29213 is the recommended QC strain for testing both *S. aureus* and β-hemolytic streptococci. *E. faecalis* ATCC® 29212 is also recommended for testing *S. aureus*. Agar dilution is not recommended for exebacase testing. Most end points will be clear (see Figure 1). In some cases, there may be a faint haze or tiny buttons of growth where the MIC should be read as the first well where growth is significantly reduced (see Figure 2).**

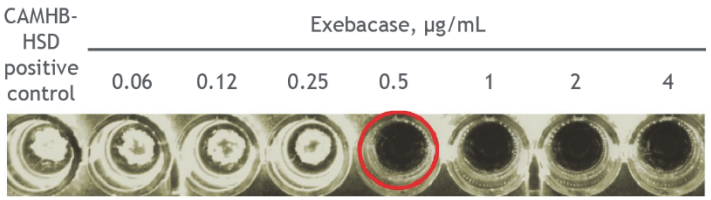

Figure 1. Exebacase MIC Test With Complete Inhibition of Growth Compared With Growth Control. The exebacase MIC (0.5 µg/mL) is shown in the red circle.

Table 5A-1. (Continued)

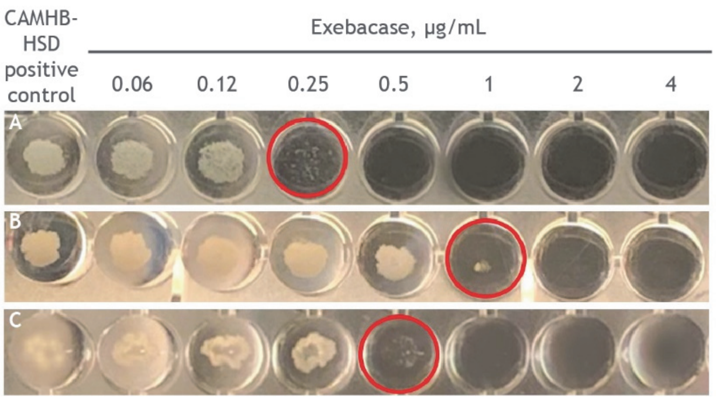

Figure 2. Exebacase MIC Test With Marked Reduction in Growth Compared With Growth Control. The exebacase MIC is shown in the red circles (A = 0.25 µg/mL, B = 1 µg/mL, and C = 0.5 µg/mL).

n. The approved MIC susceptibility testing method is agar dilution. Agar media should be supplemented with 25 µg/mL of glucose-6-phosphate. Broth dilution should not be performed.

o. For control organisms for gentamicin and streptomycin high-level aminoglycoside tests for enterococci, see Table 3K.

p. QC range for *S. aureus* ATCC® 25923 with linezolid is 1–4 µg/mL; this strain exhibits less trailing, and MIC end points are easier to interpret. *S. aureus* ATCC® 25923 is considered a supplemental QC strain and is not required for routine QC of linezolid MIC tests.

q. This test should be performed by agar dilution only.

r. For broth microdilution testing of omadacycline and tigecycline, when MIC panels are prepared, the medium must be prepared fresh on the day of use. The medium must be no more than 12 hours old at the time the panels are made; however, the panels may then be frozen for later use.

s. Very medium-dependent, especially with enterococci.

t. QC range for *S. aureus* ATCC® 25923 with tedizolid is 0.12-0.5 µg/mL; this strain exhibits less trailing, and MIC end points are easier to interpret. *S. aureus* ATCC® 25923 is considered a supplemental QC strain and is not required for routine QC of tedizolid MIC tests.

u. Ulifloxacin is the active metabolite of the prodrug prulifloxacin. Only ulifloxacin should be used for antimicrobial susceptibility testing.

v. For QC organisms for vancomycin screen test for enterococci, see Table 3H.

Table 5A-1. (Continued)

NOTE 1: These MICs were obtained in several referral laboratories by dilution methods. If four or fewer concentrations are tested, QC may be more difficult.

NOTE 2: MIC ranges apply to both broth microdilution and agar dilution unless otherwise specified.

NOTE 3: Information in black boldface type is new or modified since the previous edition.

Reference for Table 5A-1

[1] **CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.**

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Table 5A-2. MIC QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents[a]

Antimicrobial Agent	QC Organisms and Characteristics									
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
	β-lactamase negative	Inducible Amp C	Weak β-lactamase *mecA* negative		TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37	CTX-M-15 OXA-1	KPC-2 TEM SHV	KPC-3 SHV-11 TEM-1	OXA-27
	MIC QC Ranges, µg/mL									
Amoxicillin	-	-	-	-	-	> 128	-	-	-	-
Amoxicillin-clavulanate (2:1)[f]	2/1-8/4	-	0.12/0.06-0.5/0.25	0.25/0.12-1.0/0.5	4/2-16/8	4/2-16/8	-	-	-	-
Ampicillin	2-8	-	0.5-2	0.5-2	> 32	> 128	-	-	-	-
Ampicillin-sulbactam (2:1)[f]	2/1-8/4	-	-	-	8/4-32/16	8/4-32/16	-	-	-	-
Aztreonam	0.06-0.25	2-8	-	-	0.03-0.12	8-64	-	-	> 128	-
Aztreonam-avibactam	0.03/4-0.12/4	2/4-8/4	-	-	0.016/4-0.06/4	0.06/4-0.5/4	-	-	-	-
Aztreonam-nacubactam (1:1)[f]	0.06/0.06-0.25/0.25	2/2-8/8	-	-	-	0.5/0.5-2/2	-	-	0.5/0.5-2/2	-
Cefepime	0.016-0.12	0.5-4	1-4	-	0.008-0.06	0.5-2	≥ 64	-	> 32	16-128
Cefepime-enmetazobactam	0.03/8-0.12/8	0.5/8-2/8	-	-	0.008/8-0.06/8	0.12/8-0.5/8	0.03/8-0.12/8	-	-	-
Cefepime-nacubactam (1:1)	0.016/0.016-0.12/0.12	0.5/0.5-2/2	-	-	-	0.12/0.12-0.5/0.5	-	-	0.5/0.5-2/2	-
Cefepime-taniborbactam	0.03/4-0.12/4	0.5/4-4/4	-	-	0.016/4-0.06/4	0.12/4-0.5/4	0.12/4-1/4	0.12/4-0.5/4	-	-
Cefepime-tazobactam	0.03/8–0.12/8	0.5/8–4/8	1/8–4/8	–	-	0.12/8-0.5/8	0.06/8-0.25/8	-	-	-
Cefepime-zidebactam (1:1)	0.016-0.06	0.5-2	-	-	-	0.06-0.25	0.06-0.5	-	-	4-16
Zidebactam[g]	0.06-0.25	1-8	-	-	-	-	0.06-0.5	-	-	≥ 128
Cefotaxime	0.03-0.12	8-32	1-4	-	-	-	-	-	-	-
Cefpodoxime	0.25-1	-	1-8	–	0.12-0.5	4-32	32-128	-	-	-
Ceftaroline	0.03-0.12	-	0.12-0.5	0.25-2	-	2-8	-	-	-	-
Ceftaroline-avibactam	0.03/4-0.12/4	-	0.12/4-0.5/4	-	0.016/4-0.06/4	0.25/4-1/4	-	-	-	-
Ceftazidime	0.06-0.5	1-4	4-16	-	-	16-64	-	-	-	-
Ceftazidime-avibactam[h]	0.06/4-0.5/4	0.5/4-4/4	4/4-16/4	-	0.03/4-0.12/4	0.25/4-2/4	**0.12/4-0.5/4**	**0.25/4-2/4**	**1/4-4/4**	-

Table 5A-2. (Continued)

Antimicrobial Agent	QC Organisms and Characteristics									
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*A. baumannii* NCTC 13304[c,d]
	β-lactamase negative	Inducible Amp C	Weak β-lactamase *mecA* negative		TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37	CTX-M-15 OXA-1	KPC-2 TEM SHV	KPC-3 SHV-11 TEM-1	OXA-27
	MIC QC Ranges, μg/mL									
Ceftibuten[h]	0.12-**1**	-	-	-	-	**0.25-1**	16-64	4-32	8-32	-
Ceftibuten-avibactam[h]	**0.016/4-0.12/4**	**-**	**-**	**-**	**-**	**0.06/4-0.25/4**	**0.03/4-0.12/4**	**0.03/4-0.25/4**	**0.12/4-0.5/4**	**-**
Ceftibuten-ledaborbactam		-	-	-	-	-	**0.03/4-0.25/4**	**0.12/4-0.5/4**	**0.5/4-2/4**	-
Ceftolozane-tazobactam	0.12/4-0.5/4	0.25/4-1/4	16/4-64/4	-	0.06/4-0.25/4	0.5/4-2/4	-	-	-	-
Ceftriaxone	0.03-0.12	8-64	1-8	-	-	-	-	-	-	-
Durlobactam	0.12-0.5	-	-	-	-	-	-	-	-	32-128
Imipenem	0.06-0.5	1-4	0.016-0.06	0.5-2	-	0.06-0.5	-	4-16	16-64	-
Imipenem-relebactam[f]	0.06/4-0.5/4	0.25/4-1/4	0.008/4-0.03/4	0.5/4-2/4	0.06/4-0.25/4	0.06/4-0.5/4	-	0.03/4-0.25/4	0.06/4-0.5/4	-
Meropenem[i]	0.008-0.06	0.12-1	0.03-0.12	2-8	0.008-0.06	-	0.016-0.06	8-64	32-256	32-128
Meropenem-nacubactam (1:1)	0.016/0.016-0.06/0.06	0.12/0.12-1/1	-	-	-	-	-	-	0.5/0.5-2/2	-
Meropenem-vaborbactam[e]	0.008/8-0.06/8	0.12/8-1/8	0.03/8-0.12/8	-	0.008/8-0.06/8	0.016/8-0.06/8	-	0.008/8-0.06/8	0.12/8-0.5/8	-
Meropenem-xeruborbactam[j]	-	**0.06/8-0.5/8**	-	-	-	-	-	-	**0.015/8-0.06/8**	-
Nacubactam[g]	0.5-4	64-256	-	-	-	-	-	-	0.5-4	-
Piperacillin	1-4	1-8	1-4	1-4	> 64	-	-	-	-	-
Piperacillin-tazobactam[f]	1/4-**8**/4	1/4-8/4	0.25/4-2/4	1/4-4/4	0.5/4-2/4	8/4-32/4	-	-	-	-
Sulbactam	16-64	-	-	-	-	32-128	-	-	-	16-64
Sulbactam-durlobactam	-	-	-	-	-	-	-	-	-	0.5/**4**-2/**4**
Ticarcillin	4-16	8-32	2-8	16-64	> 128	> 256	-	-	-	-
Ticarcillin-clavulanate[f]	4/2-16/2	8/2-32/2	0.5/2-2/2	16/2-64/2	8/2-32/2	32/2-128/2	-	-	-	-

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; QC, quality control; R, resistant; S, susceptible.

QC strain selection codes:

QC strain is recommended for routine QC.

Test one of these agents by a disk diffusion or MIC method to confirm the integrity of the respective QC strain.[c,d]

Table 5A-2. (Continued)

Footnotes

a. Unsupplemented Mueller-Hinton medium (cation-adjusted if broth). See Table 5A-1 for QC ranges for combination agents from other drug classes.

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

c. Careful attention to organism maintenance (eg, minimal subcultures) and storage (eg, −60°C or below) is especially important for these QC strains because spontaneous loss of the plasmid encoding the β-lactamase has been documented. If stored at temperatures above −60°C or if repeatedly subcultured, these strains may lose their resistance characteristics and QC results may be outside the acceptable ranges.

d. To confirm the integrity of the QC strain, test one of the single β-lactam agents highlighted in orange by either a disk diffusion or MIC test method when the strain is first subcultured from a frozen or lyophilized stock culture. In-range results for the single agent indicate the QC strain is reliable for QC of β-lactam combination agents. It is not necessary to check the QC strain again with a single agent until a new frozen or lyophilized stock culture is put into use, providing recommendations for handling QC strains as described in M02[1] and M07[2] are followed. If the highest concentration tested on a panel is lower than the QC range listed for the particular antimicrobial agent and the MIC result obtained for the QC strain is interpreted as resistant, the QC strain can be considered reliable for QC of β-lactam combination agents (eg, ampicillin panel concentrations 1–16 µg/mL; ampicillin Enterobacterales breakpoints [µg/mL]: ≤8 [S], 16 [I], ≥32 [R]; MIC of >16 µg/ml [R] would be acceptable for *K. pneumoniae* ATCC® 700603).

e. Strain may demonstrate two colony morphologies: 1) opaque and cream colored and 2) translucent. Both colony morphologies can be used.

f. Either strain highlighted in green may be used for routine QC of this antimicrobial agent.

g. Not tested as a single agent routinely.

h. MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available.

i. Additional QC strain and range for meropenem include *P. aeruginosa* ATCC BAA-3197™ (formerly *P. aeruginosa* PA5257) (128-1024 µg/mL) to be used as integrity check strain.

j. Additional QC strain and range for *P. aeruginosa* ATCC BAA-3197™ (formerly *P. aeruginosa* PA5257) (1/8-4/8 µg/mL) provided as supplemental QC strain.

NOTE 1: MIC ranges apply to both broth microdilution and agar dilution unless otherwise specified.

NOTE 2: Information in black boldface type is new or modified since the previous edition.

References for Table 5A-2

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

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Table 5B. MIC QC Ranges for Fastidious Organisms (Broth Dilution Methods)

Antimicrobial Agent	MIC QC Ranges, µg/mL		
	Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Streptococcus pneumoniae* ATCC® 49619
Amikacin-fosfomycin (5:2)[b]	0.5/0.2-4/1.6	-	8/3.2-64/25.6
Amoxicillin	-	-	0.03-0.12
Amoxicillin-clavulanate (2:1)[c]	2/1-16/8	-	0.03/0.016-0.12/0.06
Ampicillin	2-8	-	0.06-0.25
Ampicillin-sulbactam (2:1)	2/1-8/4	-	-
Azithromycin	1-4	-	0.06-0.25
Aztreonam	0.12-0.5	-	-
Besifloxacin	0.016-0.06	-	0.03-0.12
Cefaclor	-	1-4	1-4
Cefamandole	-	0.25-1	-
Cefdinir	-	0.12-0.5	0.03-0.25
Cefditoren	0.06-0.25	-	0.016-0.12
Cefepime	0.5-2	-	0.03-0.25
Cefepime-tazobactam	0.5/8-2/8	-	0.03/8-0.12/8
Cefetamet	0.5-2	-	0.5-2
Cefixime	0.12-1	-	-
Cefmetazole	2-16	-	-
Cefonicid	-	0.06-0.25	-
Cefotaxime	0.12-0.5	-	0.03-0.12
Cefotetan	-	-	-
Cefoxitin	-	-	-
Cefpirome	0.25-1	-	-
Cefpodoxime	0.25-1	-	0.03-0.12
Cefprozil	-	1-4	0.25-1
Ceftaroline	0.03-0.12	-	0.008-0.03
Ceftaroline-avibactam	0.016/4-0.12/4	-	-
Ceftazidime	0.12-1	-	-
Ceftazidime-avibactam[d,e]	0.06/4-0.5/4	0.016/4-0.06/4	0.25/4-2/4
Ceftibuten[d]	0.25-1	-	-
Ceftizoxime	0.06-0.5	-	0.12-0.5
Ceftobiprole[f]	0.12-1	0.016-0.06	0.004-0.03
Ceftolozane-tazobactam	0.5/4-2/4	-	0.25/4-1/4
Ceftriaxone	0.06-0.25	-	0.03-0.12
Cefuroxime	-	0.25-1	0.25-1
Cephalothin	-	-	0.5-2
Chloramphenicol	0.25-1	-	2-8
Ciprofloxacin[g]	0.004-0.03	-	-
Clarithromycin	4-16	-	0.03-0.12
Clinafloxacin	0.001-0.008	-	0.03-0.12
Clindamycin	-	-	0.03-0.12
Dalbavancin[h]	-	-	0.008-0.03

Table 5B. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL		
	Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Streptococcus pneumoniae* ATCC® 49619
Daptomycin[i]	-	-	0.06-0.5
Delafloxacin	0.00025-0.001	-	0.004-0.016
Dirithromycin	8-32	-	0.06-0.25
Doripenem	-	0.06-0.25	0.03-0.12
Doxycycline	-	-	0.016-0.12
Enoxacin	-	-	-
Eravacycline	0.06-0.5	-	0.004-0.03
Ertapenem	-	0.016-0.06	0.03-0.25
Erythromycin	-	-	0.03-0.12
Faropenem	-	0.12-0.5	0.03-0.25
Finafloxacin	-	0.002-0.008	0.25-1
Fleroxacin	0.03-0.12	-	-
Fusidic acid	-	-	4-32
Garenoxacin	0.002-0.008	-	0.016-0.06
Gatifloxacin	0.004-0.03	-	0.12-0.5
Gemifloxacin	0.002-0.008	-	0.008-0.03
Gentamicin	-	-	-
Gepotidacin	0.25-1	-	0.06-0.25
Grepafloxacin	0.002-0.016	-	0.06-0.5
Iclaprim	0.12-1	-	0.03-0.12
Imipenem	-	0.25-1	0.03-0.12
Imipenem-relebactam	-	0.25/4-1/4	0.016/4-0.12/4
Lefamulin	0.5-2	-	0.06-0.5
Levofloxacin	0.008-0.03	-	0.5-2
Levonadifloxacin	0.008-0.06	-	0.12-0.5
Linezolid	-	-	0.25-2
Lomefloxacin	0.03-0.12	-	-
Loracarbef	-	0.5-2	2-8
Meropenem	-	0.03-0.12	0.03-0.25
Metronidazole	-	-	-
Minocycline[g]	-	-	-
Moxifloxacin	0.008-0.03	-	0.06-0.25
Nafithromycin	2-8	-	0.008-0.03
Nalidixic acid[g]	-	-	-
Nitrofurantoin	-	-	4-16
Norfloxacin	-	-	2-8
Ofloxacin	0.016-0.06	-	1-4
Omadacycline[j]	0.5-2	-	0.016-0.12

Table 5B. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL		
	Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Streptococcus pneumoniae* ATCC® 49619
Oritavancin[h]	-	-	0.001-0.004
Ozenoxacin	-	-	0.008-0.06
Penicillin	-	-	0.25-1
Pexiganan	8-32	-	16-64
Piperacillin-tazobactam	0.06/4-0.5/4	-	-
Quinupristin-dalfopristin	2-8	-	0.25-1
Razupenem	-	0.008-0.03	0.008-0.06
Rifampin	0.25-1	-	0.016-0.06
Solithromycin	1-4	-	0.004-0.016
Sparfloxacin	0.004-0.016	-	0.12-0.5
Spectinomycin	-	-	-
Sulfisoxazole[g]	-	-	-
Sulopenem	-	0.06-0.25	0.03-0.12
Tebipenem[d]	**-**	**0.06-0.25[k]**	**0.004-0.03**
Tedizolid	-	-	0.12-0.5
Telavancin[h]	-	-	0.004-0.016
Telithromycin	1-4	-	0.004-0.03
Tetracycline	4-32	-	0.06-0.5
Tigecycline[j]	0.06-0.5	-	0.016-0.12
Trimethoprim-sulfamethoxazole (1:19)	0.03/0.59-0.25/4.75	-	0.12/2.4-1/19
Trospectomycin	0.5-2	-	1-4
Trovafloxacin	0.004-0.016	-	0.06-0.25
Vancomycin	-	-	0.12-0.5
Zoliflodacin	0.12-1	-	0.12-0.5

MIC Testing Conditions for Clinical Isolates and Performance of QC

Organism	*Haemophilus influenzae*	*Streptococcus pneumoniae* and streptococci	*Neisseria meningitidis*
Medium	Broth dilution: HTM broth **or MH-F broth**	Broth dilution: CAMHB with LHB (2.5% to 5% v/v)	Broth dilution: CAMHB with LHB (2.5% to 5% v/v)
Inoculum	Colony suspension	Colony suspension	Colony suspension
Incubation conditions	Ambient air; 20-24 hours; 35°C **± 2°C**	Ambient air; 20-24 hours; 35°C **± 2°C**	5% CO_2; 20-24 hours; 35°C **± 2°C** (for QC with *S. pneumoniae* ATCC® 49619, 5% CO_2 or ambient air, except for azithromycin, ambient air only)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; HTM, *Haemophilus* test medium; LHB, lysed horse blood; MIC, minimal inhibitory concentration; QC, quality control.

Table 5B. (Continued)

Footnotes

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

b. QC ranges reflect MICs obtained when medium is supplemented with 25 µg/mL of glucose-6-phosphate.

c. QC limits for *E. coli* ATCC® 35218 when tested on HTM are 4/2–16/8 µg/mL for amoxicillin-clavulanate and ≥ 256 µg/mL for amoxicillin; testing amoxicillin may help to determine if the isolate has maintained its ability to produce ß-lactamase.

d. MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available.

e. QC limits for *K. pneumoniae* ATCC® 700603 with ceftazidime-avibactam when testing in HTM are 0.25/4–1/4 µg/mL. *K. pneumoniae* ATCC® 700603 should be tested against ceftazidime-avibactam and ceftazidime alone to confirm the activity of avibactam in the combination and to ensure that the plasmid encoding the ß-lactamase has not been lost in this strain. The acceptable range for ceftazidime alone is > 16 µg/mL.

f. Either *H. influenzae* ATCC® 49247 or 49766 may be used for routine QC testing.

g. QC limits for *E. coli* ATCC® 25922 with ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole when tested in CAMHB with 2.5% to 5% LHB incubated either in ambient air or 5% CO_2 (when testing *N. meningitidis*) are the same as those listed in Table 5A-1.

h. QC ranges reflect MICs obtained when CAMHB is supplemented with 0.002% polysorbate-80.

i. QC ranges reflect MICs obtained when Mueller-Hinton broth is supplemented with calcium to a final concentration of 50 µg/mL. Agar dilution has not been validated for daptomycin.

j. For broth microdilution testing of omadacycline and tigecycline, when MIC panels are prepared, the medium must be prepared fresh on the day of use. The medium must be no more than 12 hours old at the time the panels are made; however, the panels may then be frozen for later use.

k. QC ranges were established with a limited number of media manufacturers.

NOTE 1: For four-dilution ranges, results at the extremes of the acceptable ranges should be suspect. Verify validity with data from other QC strains.

NOTE 2: MIC ranges apply to both broth microdilution and agar dilution unless otherwise specified.

NOTE 3: Information in black boldface type is new or modified since the previous edition.

Table 5C. MIC QC Ranges for *Neisseria gonorrhoeae* (Agar Dilution Method)

Antimicrobial Agent	MIC QC Ranges, µg/mL
	Neisseria gonorrhoeae ATCC®[a] 49226
Azithromycin	0.25-1
Cefdinir	0.008-0.03
Cefepime	0.016-0.06
Cefetamet	0.016-0.25
Cefixime	0.004-0.03
Cefmetazole	0.5-2
Cefotaxime	0.016-0.06
Cefotetan	0.5-2
Cefoxitin	0.5-2
Cefpodoxime	0.03-0.12
Ceftazidime	0.03-0.12
Ceftizoxime	0.008-0.03
Ceftriaxone	0.004-0.016
Cefuroxime	0.25-1
Ciprofloxacin	0.001-0.008
Enoxacin	0.016-0.06
Fleroxacin	0.008-0.03
Gatifloxacin	0.002-0.016
Gentamicin	4-16
Gepotidacin	0.25-1
Grepafloxacin	0.004-0.03
Lomefloxacin	0.008-0.03
Moxifloxacin	0.008-0.03
Ofloxacin	0.004-0.016
Penicillin	0.25-1
Solithromycin	0.03–0.25
Sparfloxacin	0.004-0.016
Spectinomycin	8-32
Tetracycline	0.25-1
Trospectomycin	1-4
Trovafloxacin	0.004-0.016
Zoliflodacin	0.06-0.5

Table 5C. (Continued)

Testing Conditions for Clinical Isolates and Performance of QC

Organism	*Neisseria gonorrhoeae*
Medium	Agar dilution: GC agar base and 1% defined growth supplement. The use of a cysteine-free supplement is necessary for agar dilution tests with carbapenems and clavulanate. Cysteine-containing defined growth supplements do not significantly alter dilution test results with other drugs.
Inoculum	Colony suspension, equivalent to a 0.5 McFarland standard
Incubation conditions	36°C ± 1°C (do not exceed 37°C); 5% CO_2; 20-24 hours

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC; quality control.

Footnote

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

Table 5D. MIC QC Ranges for Anaerobes (Agar Dilution Method)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Bacteroides fragilis ATCC®[a] 25285	*Bacteroides thetaiotaomicron* ATCC® 29741	*Clostridioides* (formerly *Clostridium*) *difficile* ATCC® 700057	*Eggerthella lenta* (formerly *Eubacterium lentum*) ATCC® 43055[b]
Amoxicillin-clavulanate (2:1)	0.25/0.125-1/0.5	0.5/0.25-2/1	0.25/0.125-1/0.5	-
Ampicillin	16-64	16-64	1-4	-
Ampicillin-sulbactam (2:1)	0.5/0.25-2/1	0.5/0.25-2/1	0.5/0.25-4/2	0.25/0.125-2/1
Cadazolid	-	-	0.12-0.5	-
Cefmetazole	8-32	32-128	-	4-16
Cefoperazone	32-128	32-128	-	32-128
Cefotaxime	8-32	16-64	-	64-256
Cefotetan	4-16	32-128	-	32-128
Cefoxitin	4-16	8-32	-	4-16
Ceftaroline	4-32	16-128	2-16	8-32
Ceftaroline-avibactam	0.12/4-0.5/4	4/4-16/4	0.5/4-4/4	4/4-16/4
Ceftizoxime	-	4-16	-	16-64
Ceftolozane-tazobactam	0.12/4-1/4	16/4-128/4	-	-
Ceftriaxone	32-128	64-256	-	-
Chloramphenicol	2-8	4-16	-	-
Clinafloxacin	0.03-0.125	0.06–0.5	-	0.03-0.125
Clindamycin	0.5-2	2–8	2-8	0.06-0.25
Doripenem	-	-	0.5-4	-
Eravacycline	0.06-0.25	0.12-1	0.06-0.25	-
Ertapenem	0.06-0.25	0.25-1	-	0.5-2
Faropenem	0.03-0.25	0.12-1	-	1-4
Fidaxomicin	-	-	0.03-0.25	-
Finafloxacin	0.12-0.5	1-4	1-4	0.12-0.5
Garenoxacin	0.06-0.5	0.25-1	0.5-2	1-4
Imipenem	0.03-0.125	0.125-0.5	-	0.125-0.5
Imipenem-relebactam	0.03/4-0.25/4	0.06/4-0.5/4	-	0.12/4-1/4
Linezolid	2-8	2-8	1-4	0.5-2
Meropenem	0.03-0.25	0.125-0.5	0.5-4	0.125-1
Metronidazole	0.25-1	0.5-2	0.125-0.5	-
Moxifloxacin	0.125-0.5	1-4	1-4	0.125-0.5
Nitazoxanide	-	-	0.06-0.5	-
Omadacycline	0.25-2	0.5-4	0.25-2	0.25-2
Penicillin	8-32	8-32	1–4	-
Piperacillin	2-8	8-32	4-16	8-32
Piperacillin-tazobactam	0.125/4-0.5/4	4/4-16/4	4/4-16/4	4/4-16/4

Table 5D. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Bacteroides fragilis ATCC®[a] 25285	*Bacteroides thetaiotaomicron* ATCC® 29741	*Clostridioides* (formerly *Clostridium*) *difficile* ATCC® 700057	*Eggerthella lenta* (formerly *Eubacterium lentum*) ATCC® 43055[b]
Ramoplanin	-	-	0.125-0.5	-
Razupenem	0.016-0.12	0.06-0.25	0.06-0.25	0.06-0.5
Ridinilazole	-	-	0.06-0.25	-
Rifaximin	-	-	0.004-0.016	-
Secnidazole	0.25-1	0.5-2	0.06-0.5	0.25-2
Sulopenem	-	0.06-0.5	1-4	0.5-2
Surotomycin[c]	-	-	0.12-1	2-8
Tebipenem	0.03-0.25	0.12-0.5	0.5-2	0.06-0.25
Tetracycline	0.125-0.5	8-32	-	-
Ticarcillin	16-64	16-64	16-64	16-64
Ticarcillin-clavulanate	-	0.5/2-2/2	16/2-64/2	16/2-64/2
Tigecycline	0.12-1	0.5-2	0.125-1	0.06-0.5
Tinidazole	-	-	0.125-0.5	-
Tizoxanide	-	-	0.06-0.5	-
Vancomycin	-	-	0.5-4	-

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.

Footnotes

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

b. MIC variability with some agents has been reported with *Eggerthella lenta* (formerly *E. lentum*) ATCC® 43055; therefore, QC ranges have not been established for all antimicrobial agents with this organism.

c. QC ranges reflect MICs obtained when media are supplemented with calcium to a final concentration of 50 µg/mL.

Table 5E. MIC QC Ranges for Anaerobes (Broth Microdilution Method)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Bacteroides fragilis ATCC®[a] 25285	*Bacteroides thetaiotaomicron* ATCC® 29741	*Clostridioides* (formerly *Clostridium*) *difficile* ATCC® 700057	*Eggerthella lenta* (formerly *Eubacterium lentum*) ATCC® 43055[b]
Amoxicillin-clavulanate (2:1)	0.25/0.125-1/0.5	0.25/0.125-1/0.5	-	-
Ampicillin-sulbactam (2:1)	0.5/0.25-2/1	0.5/0.25-2/1	-	0.5/0.25-2/1
Cadazolid	-	-	0.06-0.25	-
Cefotetan	1-8	16-128	-	16-64
Cefoxitin	2-8	8-64	-	2-16
Ceftaroline	2-16	8-64	0.5-4	-
Ceftaroline-avibactam	0.06/4-0.5/4	2/4-8/4	0.25/4-1/4	4/4-16/4
Ceftizoxime	-	-	-	8-32
Ceftolozane-tazobactam	0.12/4-1/4	16/4-64/4	-	-
Chloramphenicol	4-16	8-32	-	4-16
Clindamycin	0.5-2	2-8	-	0.06-0.25
Doripenem	0.12-0.5	0.12-1	-	-
Doxycycline	-	2-8	-	2-16
Eravacycline	0.016-0.12	0.06-0.25	0.016-0.06	-
Ertapenem	0.06-0.5	0.5-2	-	0.5-4
Faropenem	0.016-0.06	0.12-1	-	0.5-2
Garenoxacin	0.06-0.25	0.25-2	-	0.5-2
Imipenem	0.03-0.25	0.25-1	-	0.25-2
Imipenem-relebactam	0.03/4-0.125/4	-	-	-
Linezolid	2-8	2-8	-	0.5-2
Meropenem	0.03-0.25	0.06-0.5	-	0.125-1
Metronidazole	0.25-2	0.5-4	-	0.125-0.5
Moxifloxacin	0.12-0.5	1.0-8	-	0.12-0.5
Omadacycline[c]	0.12-1	0.25-1	0.06-0.25	0.06-5
Penicillin	8-32	8-32	-	-
Piperacillin	4-16	8-64	-	8-32
Piperacillin-tazobactam	0.03/4-0.25/4	2/4-16/4	-	8/4-32/4
Razupenem	0.03-0.25	0.12-0.5	0.06-0.5	0.12-0.5
Ridinilazole	-	-	0.12-0.5	-
Sulopenem	-	0.03-0.25	0.5-2	0.25-1
Surotomycin[d]	-	-	0.12-1	1-4
Ticarcillin-clavulanate	0.06/2-0.5/2	0.5/2-2/2	-	8/2-32/2
Tigecycline[c]	0.06-0.5	0.25-1	0.03-0.12	-

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.

Table 5E. (Continued)

Footnotes

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

b. MIC variability with some agents has been reported with *Eggerthella lenta* (formerly *E. lentum*) ATCC® 43055; therefore, QC ranges have not been established for all antimicrobial agents with this organism.

c. For broth microdilution testing of omadacycline and tigecycline, when MIC panels are prepared, the medium must be prepared fresh on the day of use. The medium must be no greater than 12 hours old at the time the panels are made; however, the panels may then be frozen for later use.

d. QC ranges reflect MICs obtained when broth is supplemented with calcium to a final concentration of 50 µg/mL.

NOTE: For four-dilution ranges, results at the extremes of the acceptable range(s) should be suspect. Verify validity with data from other QC strains.

Table 5F. MIC Reference Guide to QC Frequency

This table summarizes the suggested QC frequency when modifications are made to antimicrobial susceptibility test systems (refer to CLSI documents EP23[1] and M52[2]). It applies only to antimicrobial agents for which satisfactory results have been obtained with either the 15-replicate (3- × 5-day) plan or 20 or 30 consecutive test day plan. Otherwise QC is required each test day.

Test Modification	Recommended QC Frequency			Comments
	1 Day	5 Days	15-Replicate Plan or 20- or 30-Day Plan	
MIC test(s)				
Use new shipment or lot number.	X			
Expand dilution range.	X			**Example:** Convert from breakpoint to expanded range MIC panels.
Reduce dilution range.	X			**Example:** Convert from expanded dilution range to breakpoint panels.
Use new method (same company).			X	**Examples:** Convert from overnight to rapid MIC test. In addition, perform in-house verification studies.
Use new manufacturer of MIC test.			X	In addition, perform in-house verification studies.
Use new manufacturer of broth or agar.		X		
Addition of new antimicrobial agent to existing system			X	In addition, perform in-house verification studies.
Inoculum preparation				
Convert inoculum preparation/standardization to use of a device that has its own QC protocol.		X		**Example:** Convert from visual adjustment of turbidity to use of a photometric device for which a QC procedure is provided.
Convert inoculum preparation/standardization to a method that depends on user technique.			X	**Example:** Convert from visual adjustment of turbidity to another method that is not based on a photometric device.
Instrument/software				
Software update that affects AST results		X		Monitor all drugs, not just those implicated in software modification.
Repair of instrument that affects AST results	X			Depending on extent of repair (eg, critical component such as the photographic device), additional testing may be appropriate (eg, 5 days).

Abbreviations: AST, antimicrobial susceptibility testing; MIC, minimal inhibitory concentration; QC, quality control.

Table 5F. (Continued)

NOTE 1: QC can be performed before or concurrent with testing patient isolates. Patient results can be reported for that day if QC results are within the acceptable limits.

NOTE 2: Manufacturers of commercial or in-house-prepared tests should follow their own internal procedures and applicable regulations.

NOTE 3: Acceptable MIC QC limits for US Food and Drug Administration–cleared antimicrobial susceptibility tests may differ slightly from acceptable CLSI QC limits. Users of each device should use the manufacturer's procedures and QC limits as indicated in the instructions for use.

NOTE 4: For troubleshooting out-of-range results, refer to M07,[3] Subchapter 4.8 and M100 Table 5G. Additional information is available in Appendix C (eg, organism characteristics, QC testing recommendations).

NOTE 5: Broth, saline, and/or water used to prepare an inoculum does not need routine QC.

References for Table 5F

1 CLSI. *Laboratory Quality Control Based on Risk Management; Approved Guideline*. CLSI document EP23-A™. Clinical and Laboratory Standards Institute; 2011.

2 CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.

3 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

Table 5G. MIC Troubleshooting Guide

This table provides guidance for troubleshooting and corrective action for out-of-range QC, primarily using CAMHB for broth microdilution. Refer to M07,[1] Chapter 4, for additional information. Out-of-range QC tests are often the result of contamination or the use of an incorrect QC strain; corrective action should first include repeating the test with a pure culture of a freshly subcultured QC strain. If the issue is unresolved, this troubleshooting guide should be consulted regarding additional suggestions for troubleshooting out-of-range QC results and unusual clinical isolate results. In addition, see general corrective action outlined in M07[1] and notify manufacturers of potential product problems.

General Comment

(1) QC organism maintenance: Avoid repeated subcultures. Retrieve new QC strain from stock (refer to M07,[1] Subchapter 4.4). If using lyophilized strains, follow the maintenance recommendations of the manufacturer.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS				
β-lactam combination agents	*A. baumannii* ATCC®[a] 13304 *E. coli* ATCC® 35218 *E. coli* ATCC® 13353 *K. pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™	MIC too low or susceptible for single β-lactam agent; in range for combination β-lactam agent	Spontaneous loss of the plasmid encoding the β-lactamase	Obtain new frozen or lyophilized stock culture. Use other routine QC strain (if available). These strains should be stored at −60°C or below, and frequent subcultures should be avoided. NOTE: *K. pneumoniae* ATCC® BAA-2814™ is stable and does not require QC integrity check.
β-lactam combination agents	*A. baumannii* ATCC® 13304 *E. coli* ATCC® 35218 *E. coli* ATCC® 13353 *K. pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™ *K. pneumoniae* ATCC® BAA-2814™	MIC too high or resistant for both the single β-lactam agent and the combination β-lactam agent	Antimicrobial agent is degrading.	Use alternative lot of test materials. Check storage and package integrity. Imipenem and clavulanate are especially labile.
Carbenicillin	*P. aeruginosa* ATCC® 27853	MIC too high	QC strain develops resistance after repeated subculture.	See general comment (1) on QC organism maintenance. **Prepare new subculture from the frozen or freeze-dried stock every 2 weeks to prevent loss of viability.**
Cefotaxime-clavulanate Ceftazidime-clavulanate	*K. pneumoniae* ATCC® 700603	Negative ESBL test	Spontaneous loss of the plasmid encoding the β-lactamase	See general comment (1) on QC organism maintenance.
Carbapenems	*P. aeruginosa* ATCC® 27853	MIC too high	Zn++ concentration in media is too high.	Use alternative lot.
Carbapenems	*P. aeruginosa* ATCC® 27853	MIC too high	Antimicrobial agent is degrading.	Use alternative lot. Check storage conditions and package integrity. Repeated imipenem QC results at the upper end of QC range with *P. aeruginosa* ATCC® 27853 may indicate deterioration of the drug.
Penicillin	*S. aureus* ATCC® 29213	MIC too high	QC strain is a β-lactamase producer; overinoculation may yield increased MICs.	Repeat with a carefully adjusted inoculum.

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS (Continued)				
Penicillins	Any	MIC too low	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Penicillins	Any	MIC too high	pH of media too high	Acceptable pH range = 7.2–7.4
β-lactam group	Any	MIC initially acceptable, but increases to possibly be out of range over time	Imipenem, cefaclor, and clavulanate are especially labile. Antimicrobial agents are degrading.	Use alternative lot. Check storage and package integrity.
NON-β-LACTAMS				
Aminoglycosides Quinolones	Any	MIC too high	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Aminoglycosides Quinolones	Any	MIC too low	pH of media too high	Acceptable pH range = 7.2–7.4
Aminoglycosides	*P. aeruginosa* ATCC® 27853	MIC too low	Ca++ and/or Mg++ content too low	Acceptable range = Ca++ 20–25 mg/L Mg++ 10–12.5 mg/L
Aminoglycosides	*P. aeruginosa* ATCC® 27853	MIC too high	Ca++ and/or Mg++ content too high	Acceptable range = Ca++ 20–25 mg/L Mg++ 10–12.5 mg/L
Ceftriaxone	***P. aeruginosa* ATCC® 27853**	**MIC too high**	**QC strain develops resistance after repeated subculture.**	**See general comment (1) on QC organism maintenance. Prepare new subculture from the frozen or freeze-dried stock every 2 weeks to prevent loss of viability.**
Colistin[b]	***E. coli* ATCC® 25922** ***P. aeruginosa* ATCC® 27853** ***E. coli* NCTC 13846** ***E. coli* ATCC® BAA-3170™**	**MIC too high**	**Inadequate concentration of drug available in test medium due to drug adherence to surfaces (eg, tubes, plates)**	**Check composition of containers (eg, tubes, plates) used for production of test reagents and performance of MIC tests. Use tubes/plates made of untreated polystyrene.** **Prepare colistin stock solution on the day of use in production of tubes or panels for MIC testing. Use only the sulphate salts of polymyxins; the methanesulfonate derivative of colistin must not be used (it is an inactive prodrug that breaks down slowly in solution).**
Colistin[b]	***E. coli* ATCC® 25922** ***P. aeruginosa* ATCC® 27853** ***E. coli* NCTC 13846** ***E. coli* ATCC® BAA-3170™**	**MIC too low**	**Surfactant added to test broth or inoculum diluent**	**Check to ensure surfactant (eg, polysorbate-80) was not added to test medium or inoculum diluent.**
Dalbavancin Oritavancin[1] Telavancin	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too high	Lack of polysorbate-80 in the media	Add polysorbate-80 to CAMHB to final concentration of 0.002% (v/v). See M07,[1] Subchapter 3.5.1 and Appendix A.
Chloramphenicol Clindamycin Erythromycin Linezolid Tedizolid Tetracycline	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212 *S. pneumoniae* ATCC® 49619	MIC too high	Trailing end point	Read at first well where the trailing begins; tiny buttons of growth should be ignored. See general comment (3) in Table 2G.

Table 5G
MIC QC Troubleshooting
M07

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
NON-β-LACTAMS (Continued)				
Linezolid Tedizolid	*S. aureus* ATCC® 29213	MIC too high	Trailing end point	*S. aureus* ATCC®25923 may be used as a supplemental QC strain for these drugs. This strain exhibits less trailing and MIC end points are easier to interpret.
Oritavancin[1]	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too high	Lack of polysorbate-80 in the solvent and diluent	Dissolve antimicrobial powder and prepare dilutions in water containing a final concentration of 0.002% polysorbate-80 (v/v).
Oritavancin	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too high	Use of tissue-culture treated microdilution trays	Use only untreated microdilution trays for this antimicrobial agent.[2]
Clindamycin Macrolides Ketolides	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too high	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Clindamycin Macrolides Ketolides	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too low	pH of media too high	Acceptable pH range = 7.2–7.4
Daptomycin	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MICs too high MICs too low	Ca++ content too low Ca++ content too high	Acceptable Ca++ content 50 µg/mL in CAMHB
Tetracyclines	Any	MIC too low	pH of media too low	Acceptable pH range = 7.2–7.4
Tetracyclines	Any	MIC too high	pH of media too high	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Tetracyclines	Any	MIC too high	Ca++ and/or Mg++ content too high	Acceptable range = Ca++ 20–25 mg/L Mg++ 10–12.5 mg/L
Tetracyclines	Any	MIC too low	Ca++ and/or Mg++ content too low	Acceptable range = Ca++ 20–25 mg/L Mg++ 10–12.5 mg/L
Omadacycline Tigecycline	Any	MIC too high	CAMHB has not been freshly prepared.	Reference panels must be used or frozen within 12 hours of CAMHB preparation.
ALL AGENTS				
Various	*S. pneumoniae* ATCC® 49619	MICs too low Light growth	Inoculum source plate too old and contains too many nonviable cells.	**See general comment (1) on QC organism maintenance. Prepare new subculture from the frozen or freeze-dried stock every 2 weeks to prevent loss of viability.** Subculture QC strain and repeat QC test or retrieve new QC strain from stock. **Plate used to prepare inoculum should be incubated 18-20 hours.**
Various	*E. coli* ATCC® 35218 *K. pneumoniae* ATCC® 700603	MIC too low	Spontaneous loss of the plasmid encoding the β-lactamase	See general comment (1) on QC organism maintenance.
Various	***E. faecalis* ATCC® 51299**	**MIC too low**	**QC strain loses resistance after repeated subculture.**	**See general comment (1) on QC organism maintenance. Prepare new subculture from the frozen or freeze-dried stock every 2 weeks to prevent loss of viability.**

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
ALL AGENTS (Continued)				
Various	Any	One QC result is out of range, but the antimicrobial agent is not an agent reported for patient results (eg, not on hospital formulary).	N/A	If antimicrobial agent is not normally reported, no repeat is necessary if adequate controls are in place to prevent reporting of the out-of-range antimicrobial agent.
Various	Any	Many MICs too low	Inoculum too light; error in inoculum preparation	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Check steps in inoculum preparation and inoculation procedure. Perform colony count check of growth control well immediately after inoculation and before incubation (*E. coli* ATCC® 25922 closely approximates 5×10^5 CFU/mL; see M07,[1] Subchapter 3.8).
Various	Any	Many MICs too high or too low	CAMHB not optimal	Use alternative lot.
Various	Any	Many MICs too high or too low	Possible reading/transcription error	Recheck readings. Use alternative lot.
Various	Any	Many MICs too high	Inoculum too heavy	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Check steps in inoculum preparation and inoculation procedure. Perform colony count check of growth control well immediately after inoculation and before incubation (*E. coli* ATCC® 25922 closely approximates 5×10^5 CFU/mL; see M07,[1] Subchapter 3.8).
Various	Any	Skipped wells	Contamination. Improper inoculation of panel or inadequate mixing of inoculum. Actual concentration of drug in wells inaccurate. Volume of broth in wells inaccurate.	Repeat QC test. Use alternative lot.

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
ALL AGENTS (Continued)				
Various	Any	QC results from one strain are out of range, but other QC strains are in range with the same antimicrobial agent.	One QC organism may be a better indicator of a QC problem (eg, *P. aeruginosa* ATCC® 27853 is a better indicator of imipenem deterioration than *E. coli* ATCC® 25922).	Determine whether the in-range QC strain has an on-scale end point for the agent in question. Retest this strain to confirm reproducibility of acceptable results. Evaluate with alternative strains with known MICs. Initiate corrective action with problem QC strain/antimicrobial agent(s).
Various	Any	QC results from two strains are out of range with the same antimicrobial agent.	Indicates a problem with the antimicrobial agent. May be a systemic problem.	Initiate corrective action.
Various	Any	QC results from one strain are out of range, but the antimicrobial agent is not an agent reported for patient results (eg, not on hospital formulary).		If antimicrobial agent is not normally reported, no repeat is necessary if adequate controls are in place to prevent reporting of the out-of-range antimicrobial agent. Carefully check antimicrobial agents of the same class for similar trend toward out-of-control results. If the antimicrobial agent in question is consistently out of control, contact the manufacturer.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); ESBL, extended-spectrum β-lactamase; MIC, minimal inhibitory concentration; N/A, not applicable; pH, negative logarithm of hydrogen ion concentration; QC, quality control.

Footnotes

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

b. Colistin results are significantly affected by preparation and handling of reagents and/or testing materials, including stock solutions, test medium, composition of testing tube and/or plate (eg, glass, polystyrene, polypropylene). QC results may fall outside the established CLSI QC ranges if methods other than CLSI reference methods described in M07[1] and M100 are used.

NOTE: Information in black boldface type is new or modified since the previous edition.

References for Table 5G

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

2 Arhin FF, Sarmiento I, Belley A, et al. Effect of polysorbate 80 on oritavancin binding to plastic surfaces: implications for susceptibility testing. *Antimicrob Agents Chemother*. 2008;52(5):1597-1603.

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Table 6A. Solvents and Diluents for Preparing Stock Solutions of Antimicrobial Agents[a]

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Amikacin	Water	Water
Amoxicillin	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Ampicillin	Phosphate buffer, pH 8, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Avibactam	Water	Water
Azithromycin	95% ethanol or glacial acetic acid[a,c]	Broth media
Azlocillin	Water	Water
Aztreonam	Saturated solution sodium bicarbonate	Water
Besifloxacin	Methanol	Water
Biapenem	Saline[d]	Saline[d]
Cadazolid	DMSO[a]	Water or broth
Carbenicillin	Water	Water
Cefaclor	Water	Water
Cefadroxil	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefamandole	Water	Water
Cefazolin	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Cefdinir	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefditoren	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefepime	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L or water
Cefetamet	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefiderocol	Saline[d]	Saline[d]
Cefixime	Phosphate buffer, pH 7, 0.1 mol/L	Phosphate buffer, pH 7, 0.1 mol/L
Cefmetazole	Water	Water
Cefonicid	Water	Water
Cefoperazone	Water	Water
Cefotaxime	Water	Water
Cefotetan	DMSO[a]	Water
Cefoxitin	Water	Water
Cefpodoxime	0.10% (11.9 mmol/L) aqueous sodium bicarbonate	Water
Cefprozil	Water	Water
Ceftaroline	DMSO[a] to 30% of total volume	Saline[d]
Ceftazidime	Sodium carbonate[e]	Water
Ceftibuten	**Phosphate buffer, pH 8, 0.1 mol/L**	Water **or phosphate buffer, pH 8, 0.1 mol/L**
Ceftizoxime	Water	Water
Ceftobiprole	DMSO plus glacial acetic acid[a,f]	Water, vortex vigorously

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Ceftolozane	Water or saline[d]	Water or saline[d]
Ceftriaxone	Water	Water
Cefuroxime	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Cephalexin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephalothin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephapirin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephradine	Phosphate buffer, pH 6, 0.1 mol/L	Water
Chloramphenicol	95% ethanol	Water
Cinoxacin	1/2 volume of water, then add 1 mol/L NaOH dropwise to dissolve	Water
Ciprofloxacin	Water	Water
Clarithromycin	Methanol[a] or glacial acetic acid[a,c]	Phosphate buffer, pH 6.5, 0.1 mol/L
Clavulanate	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Clinafloxacin	Water	Water
Clindamycin	Water	Water
Colistin[g]	Water	Water
Dalbavancin	DMSO[a]	DMSO[a,h]
Daptomycin	Water	Water
Delafloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Dirithromycin	Glacial acetic acid[c]	Water
Doripenem	Saline[d]	Saline[d]
Doxycycline	Water	Water
Durlobactam	Water	Water
Enoxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Enmetazobactam	Water	Water
Eravacycline	Water	Water
Ertapenem	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L
Erythromycin	95% ethanol or glacial acetic acid[a,c]	Water
Exebacase	Supplied as a frozen stock in a buffer containing 20 mM L-histidine and 5% D-sorbitol, pH 7[i]	CAMHB with horse serum **(25% v/v) and** 0.5 mM DL-dithiothreitol **(DTT)** (pH 7.2-7.4) **(CAMHB-HSD)**[j]
Faropenem	Water	Water
Fidaxomicin	DMSO[a]	Water
Finafloxacin	Water	Water
Fleroxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Fosfomycin	Water	Water
Fusidic acid	Water	Water
Garenoxacin	Water (with stirring)	Water

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Gatifloxacin	Water (with stirring)	Water
Gemifloxacin	Water	Water
Gentamicin	Water	Water
Gepotidacin	DMSO[a]	Water
Iclaprim	DMSO[a]	Water
Imipenem	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L
Kanamycin	Water	Water
Ledaborbactam	**Water**	**Water**
Lefamulin	Water	Water
Levofloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Levonadifloxacin	27.5 µg/mL solution of L-arginine in water	Water
Linezolid	Water	Water
Lomefloxacin	Water	Water
Loracarbef	Water	Water
Mecillinam	Water	Water
Meropenem	Water	Water
Metronidazole	DMSO[a]	Water
Minocycline	Water	Water
Moxalactam (diammonium salt)[k]	0.04 mol/L HCl (let sit for 1.5 to 2 hours)	Phosphate buffer, pH 6, 0.1 mol/L
Moxifloxacin	Water	Water
Mupirocin	Water	Water
Nacubactam	Water	Water
Nafcillin	Water	Water
Nafithromycin	½ volume of water, then glacial acetic acid dropwise to dissolve (acetic acid not to exceed 2.5 µL/mL)	Water
Nalidixic acid	1/2 volume of water, then add 1 mol/L NaOH dropwise to dissolve	
Netilmicin	Water	Water
Nitazoxanide	DMSO[a,l]	DMSO[a,l]
Nitrofurantoin[m]	Phosphate buffer, pH 8, 0.1 mol/L	Phosphate buffer, pH 8, 0.1 mol/L
Norfloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Ofloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Omadacycline	Water	Water
Oritavancin	0.002% polysorbate-80 in water[n]	0.002% polysorbate-80 in water[n]

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Oxacillin	Water	Water
Ozenoxacin	10% volume of water, then 1M NaOH (8% of final volume)	Water
Penicillin	Water	Water
Pexiganan	Water	Water
Piperacillin	Water	Water
Plazomicin	Water	Water
Polymyxin B	Water	Water
Quinupristin-dalfopristin	Water	Water
Ramoplanin	Water	Water
Razupenem	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L
Relebactam	Water	Water
Ridinilazole	DMSO[a]	DMSO[a]
Rifampin	Methanol[a] (maximum concentration = 640 µg/mL)	Water (with stirring)
Rifaximin	Methanol[a]	0.1 M phosphate buffer, pH 7.4 + 0.45% sodium dodecyl sulfate
Secnidazole	DMSO[a]	Water
Solithromycin	Glacial acetic acid[c]	Water
Sparfloxacin	Water	Water
Spectinomycin	Water	Water
Streptomycin	Water	Water
Sulbactam	Water	Water
Sulfonamides	1/2 volume hot water and minimal amount of 2.5 mol/L NaOH to dissolve	Water
Sulopenem[o]	0.01 M phosphate buffer, pH 7.2, vortex to dissolve	0.01 M phosphate buffer, pH 7.2
Surotomycin	Water	Water
Taniborbactam	Water	Water
Tazobactam	Water	Water
Tebipenem	Water	Water
Tedizolid	DMSO[a]	DMSO[a,p]
Teicoplanin	Water	Water
Telavancin	DMSO[a]	DMSO[a,h]
Telithromycin	Glacial acetic acid[a,c]	Water

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Tetracycline	Water	Water
Ticarcillin	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Ticarcillin-clavulanate	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Tigecycline	Water	Water
Tinidazole	DMSO[a,l]	Water
Tizoxanide	DMSO[a,l]	DMSO[a,l]
Tobramycin	Water	Water
Trimethoprim	0.05 mol/L lactic[a] or hydrochloric[a] acid, 10% of final volume	Water (may need heat)
Trimethoprim (if lactate)	Water	Water
Trospectomycin	Water	Water
Ulifloxacin (prulifloxacin)	DMSO[a]	Water
Vaborbactam	90% DMSO[a]/10% water	Water
Vancomycin	Water	Water
Xeruborbactam	**Water**	**Water**
Zidebactam	Water	Water
Zoliflodacin	DMSO	Water

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; **CAMHB-HSD, cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2-7.4); DTT, DL-dithiothreitol;** DMSO, dimethyl sulfoxide; pH, negative logarithm of hydrogen ion concentration.

Footnotes

a. Consult the safety data sheets before working with any antimicrobial reference standard powder, solvent, or diluent. Some of the compounds (eg, solvents such as DMSO, methanol) are more toxic than others and may necessitate handling in a chemical fume hood.

b. Although these solvents and diluents are recommended, users should always confirm with the manufacturer.

c. For glacial acetic acid, use 1/2 volume of water, then add glacial acetic acid dropwise until dissolved, not to exceed 2.5 µL/mL.

d. Saline – a solution of 0.85% to 0.9% NaCl (w/v).

e. Anhydrous sodium carbonate is used at a weight of exactly 10% of the ceftazidime to be used. The sodium carbonate is dissolved in solution in most of the necessary water. The antimicrobial agent is dissolved in this sodium carbonate solution, and water is added to the desired volume. The solution is to be used as soon as possible, but it can be stored up to six hours at no more than 25°C.

f. For each 1.5 mg of ceftobiprole, add 110 µL of a 10:1 mixture of DMSO and glacial acetic acid. Vortex vigorously for one minute, then intermittently for 15 minutes. Dilute to 1 mL with distilled water.

g. The formulation of colistin reference standard powder used in antimicrobial susceptibility tests is colistin sulfate and not colistin methane sulfonate (sulfomethate).

Table 6A. (Continued)

h. Starting stock solutions of dalbavancin and telavancin should be prepared at concentrations no higher than 1600 µg/mL. Intermediate 100× concentrations should then be diluted in DMSO. Final 1:100 dilutions should then be made directly into CAMHB supplemented with 0.002% (v/v) polysorbate-80, so the final concentration of DMSO in the wells is no greater than 1%. See also Table 8B.

i. Exebacase is an enzyme that requires special handling. Frozen stock solutions should be thawed in a 25°C water bath with gentle mixing every 30 seconds. Thawing should not take more than five minutes. The thawed stock solution and any subsequently prepared dilutions in CAMHB supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (DTT; pH 7.2-7.4) (CAMHB-HSD) should be kept chilled in an ice bucket or refrigerated at 2 to 8°C for no more than one hour while broth microdilution MIC panels are prepared. MIC panels should be frozen within 15 minutes of preparation. Any remaining unused stock solution should be discarded.

j. To prepare one liter of CAMHB-HSD, 250 mL horse serum is added to 750 mL sterile CAMHB. Next, 500 µL CAMHB is removed, and 500 µL DTT is added. CAMHB should be prepared according to manufacturer instructions.

k. The diammonium salt of moxalactam is very stable, but it is almost pure R isomer. Moxalactam for clinical use is a 1:1 mixture of R and S isomers. Therefore, the salt is dissolved in 0.04 mol/L HCl and allowed to react for 1.5 to 2 hours to convert it to equal parts of both isomers.

l. Final concentration of DMSO should not exceed 1%. This may be accomplished as follows: 1) prepare the stock solution at 10 times higher concentration than planned stock solution (ie, prepare at 12 800 µg/mL, rather than 1280 µg/mL); 2) add 1.8 mL sterile water to each agar deep; 3) add 0.2 mL of each antibiotic dilution to each agar deep.

m. Alternatively, nitrofurantoin is dissolved in DMSO.

n. Starting stock solutions of oritavancin should be prepared at concentrations no higher than 1600 µg/mL in 0.002% polysorbate-80 in water. Intermediate 100× oritavancin concentrations should then be prepared in 0.002% polysorbate-80 in water. Final 1:100 dilutions should be made directly into CAMHB supplemented with 0.002% polysorbate-80, so the final concentration of polysorbate-80 in the wells is 0.002%.

o. Must be made fresh on the day of use.

p. Starting stock solutions of tedizolid should be prepared at concentrations no higher than 1600 µg/mL. Intermediate 100× concentrations should be diluted in DMSO. Final 1:100 dilutions should be made directly into CAMHB, so that the final concentration of DMSO in the wells is no greater than 1%. Also see Table 8B.

NOTE: Information in black boldface type is new or modified since the previous edition.

Table 6B. Preparing Stock Solutions for Antimicrobial Agents Provided With Activity Expressed as Units

Antimicrobial Agent	Pure Agent	Calculation for µg/mg	Example
Potassium penicillin G	0.625 µg/unit[1]	Multiply the activity expressed in units/mg by 0.625 µg/unit.	Activity units/mg • 0.625 µg/unit = Activity µg/mg (eg, 1592 units/mg • 0.625 µg/unit = 995 µg/mg)
Sodium penicillin G	0.6 µg/unit[1]	Multiply the activity expressed in units/mg by 0.6 µg/unit.	Activity units/mg • 0.6 µg/unit = Activity µg/mg (eg, 1477 units/mg • 0.6 µg/unit = 886.2 µg/mg)
Polymyxin B	10 000 units/mg = 10 units/µg = 0.1 µg/unit[2]	Multiply the activity expressed in units/mg by 0.1 µg/unit.	Activity units/mg • 0.1 µg/unit = Activity µg/mg (eg, 8120 units/mg • 0.1 µg/unit = 812 µg/mg)
		Divide the activity expressed in units/mg by 10 units/µg.	Activity units/mg / 10 units/µg = Activity µg/mg (eg, 8120 units/mg / 10 units/mg = 812 µg/mg)
Colistin sulfate[a]	30 000 units/mg = 30 units/µg = 0.03333 µg/unit[2]	Multiply the activity expressed in units/mg by 0.03333 µg/unit.	Activity units/mg • 0.03333 µg/unit = Activity µg/mg (eg, 20 277 units/mg • 0.03333 µg/unit = 676 µg/mg)
		Divide the activity expressed in units/mg by 30 units/µg.	Activity units/mg / 30 units/µg = Activity µg/mg (eg, 20 277 units/mg / 30 units/µg = 676 µg/mg)
Streptomycin	785 units/mg[3]	Divide the number of units given for the powder by 785. This gives the percent purity of the powder. Multiply the percent purity by 850, which is the amount in the purest form of streptomycin. This result equals the activity factor in µg/mg.	([Potency units/mg] / [785 units/mg]) • (850 µg/mg) = Potency µg/mg (eg, [751 units/mg / 785 units/mg] • 850 µg/mg = 813 µg/mg) If powder contains 2.8% water: 813 • (1 – 0.028) = potency 813 • 0.972 = 790 µg/mg

Footnote

a. Do not use colistin methanesulfonate for *in vitro* antimicrobial susceptibility tests.

References for Table 6B

1 Geddes AM, Gould IM. Benzylpenicillin (penicillin G). In: Grayson ML, ed. *Kucers' The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic and Antiviral Drugs*. 6th ed. CRC Press, Taylor & Francis Group; 2010:5-58.

2 Polymyxins. In: Kucers A, Crowe SM, Grayson ML, Hoy JF, eds. *The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic and Antiviral Drugs*. 5th ed. Butterworth-Heinemann; 1997:667-675.

3 United States Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Laboratory QA/QC Division. *Bioassay for the detection, identification and quantitation of antimicrobial residues in meat and poultry tissue*. Microbiology Laboratory Guidebook (MLG) 34.03; 2011.

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Table 6C. Preparing Solutions and Media Containing Combinations of Antimicrobial Agents

Antimicrobial Agent	Combination Tested	Preparation	Example
Amikacin-fosfomycin	5:2 ratio (amikacin:fosfomycin)	Prepare 10× starting concentration as 5:2 ratio and dilute as needed. **NOTE:** Media should be supplemented with 25 µg/mL glucose-6-phosphate.	
Amoxicillin-clavulanate	2:1 ratio (amoxicillin:clavulanate)	Prepare 10× starting concentration as 2:1 ratio and dilute as needed.	For a starting concentration of 128/64 in the panel, prepare a 10× stock concentration of 2560 µg/mL for amoxicillin and 1280 µg/mL for clavulanate. Then combine equal amounts of each to the first dilution tube, which will then contain 1280/640 µg/mL of the combination. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Ampicillin-sulbactam	2:1 ratio (ampicillin:sulbactam)	Same as amoxicillin-clavulanate.	
Aztreonam-avibactam	Fixed concentration of avibactam at 4 µg/mL	Prepare 10× starting concentration of aztreonam at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of avibactam 80 µg/mL to each of the diluted tubes.	For a starting concentration of 128/4 in the panel, prepare a 10× stock concentration of aztreonam at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of avibactam at 80 µg/mL. Then add an equal volume of the avibactam 80 µg/mL solution to each diluted tube of aztreonam. For example, 5 mL of 2560 µg/mL aztreonam + 5 mL of 80 µg/mL avibactam = 10 mL of 1280/40 µg/mL aztreonam-avibactam. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Aztreonam-nacubactam	1:1 ratio (aztreonam:nacubactam)	Prepare 10× starting concentration as 1:1 ratio and dilute as needed.	For a starting concentration of 128/128 in the panel, prepare a 20× stock concentration of 2560 µg/mL for aztreonam and 2560 µg/mL for nacubactam. Combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 µg/mL of the combination. Prepare 2-fold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Cefepime-enmetazobactam	Fixed concentration of enmetazobactam at 8 mg/L	Prepare 10× starting concentration of cefepime at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of enmetazobactam 160 µg/mL to each of the diluted tubes.	For a starting concentration of 128/8 in the panel, prepare a 10× stock concentration of cefepime at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of enmetazobactam at 160 µg/mL. Then add an equal volume of the enmetazobactam 160 µg/mL solution to each diluted tube of cefepime. For example, 5 mL of 2560 µg/mL cefepime + 5 mL of 160 µg/mL enmetazobactam = 10 mL of 1280/80 µg/mL cefepime-enmetazobactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.
Cefepime-nacubactam	1:1 ratio (cefepime:nacubactam)	Prepare 10× starting concentration as 1:1 ratio and dilute as needed.	For a starting concentration of 128/128 in the panel, prepare a 20× stock concentration of 2560 µg/mL for cefepime and 2560 µg/mL for nacubactam. Combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 µg/mL of the combination. Prepare 2-fold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.
Cefepime-taniborbactam	Fixed concentration of taniborbactam at 4 µg/mL	Prepare 10x starting concentration of cefepime at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of taniborbactam 80 µg/mL to each of the diluted tubes.	For a starting concentration of 128/4 in the panel, prepare a 10x stock concentration of cefepime at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of taniborbactam at 80 µg/mL. Then add an equal volume of the taniborbactam 80 µg/mL solution to each diluted tube of cefepime. For example, 5 mL of 2560 µg/mL cefepime + 5 mL of 80 µg/mL taniborbactam = 10 mL of 1280/40 µg/mL cefepime-taniborbactam. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Cefepime-tazobactam	Fixed concentration of tazobactam at 8 µg/mL	Prepare 10× starting concentration of cefepime at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of tazobactam 160 µg/mL to each of the diluted tubes.	For a starting concentration of 128/8 in the panel, prepare a 10× stock concentration of cefepime at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of tazobactam at 160 µg/mL. Then add an equal volume of the tazobactam 160 µg/mL solution to each diluted tube of cefepime. For example, 5 mL of 2560 µg/mL cefepime + 5 mL of 160 µg/mL tazobactam = 10 mL of 1280/80 µg/mL cefepime-tazobactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Cefepime-zidebactam	1:1 ratio (cefepime:zidebactam)	Prepare 10× starting concentration as 1:1 ratio and dilute as needed.	For a starting concentration of 128/128 in the panel, prepare a 20× stock concentration of 2560 µg/mL for cefepime and 2560 µg/mL for zidebactam. Then combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 µg/mL of the combination. Prepare 2-fold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.
Ceftaroline-avibactam	Fixed concentration of avibactam at 4 µg/mL	Same as aztreonam-avibactam.	
Ceftazidime-avibactam	Fixed concentration of avibactam at 4 µg/mL	Same as aztreonam-avibactam.	
Ceftibuten-avibactam	**Fixed concentration of avibactam at 4 µg/mL**	**Same as aztreonam-avibactam.**	
Ceftibuten-ledaborbactam	**Fixed concentration of ledaborbactam at 4 µg/mL**	**Same as aztreonam-avibactam.**	
Ceftolozane-tazobactam	Fixed concentration of tazobactam at 4 µg/mL	Same as aztreonam-avibactam.	
Imipenem-relebactam	Fixed concentration of relebactam at 4 µg/mL	Same as aztreonam-avibactam.	
Meropenem-nacubactam	1:1 ratio (meropenem:nacubactam)	Prepare 10× starting concentration as 1:1 ratio and dilute as needed.	For a starting concentration of 128/128 in the panel, prepare a 20× stock concentration of 2560 µg/mL for meropenem and 2560 µg/mL for nacubactam. Combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 µg/mL of the combination. Prepare 2-fold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.
Meropenem-vaborbactam	Fixed concentration of vaborbactam at 8 µg/mL	Prepare 10× starting concentration of meropenem at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of vaborbactam 160 µg/mL to each of the diluted tubes.	For a starting concentration of 64/8 µg/mL in the panel, prepare a 10× stock concentration of meropenem at 1280 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of vaborbactam at 160 µg/mL. Then add an equal volume of the vaborbactam 160 µg/mL solution to each diluted tube of meropenem. For example, 5 mL of 1280 µg/mL meropenem + 5 mL of 160 µg/mL vaborbactam = 10 mL of 640/80 µg/mL meropenem-vaborbactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Meropenem-xeruborbactam	**Fixed concentration of xeruborbactam at 8 µg/mL**	**Prepare 10× starting concentration of meropenem at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of xeruborbactam 160 µg/mL to each of the diluted tubes.**	**For a starting concentration of 64/8 µg/mL in the panel, prepare a 10× stock concentration of meropenem at 1280 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of xeruborbactam at 160 µg/mL. Then add an equal volume of the xeruborbactam 160 µg/mL solution to each diluted tube of meropenem. For example, 5 mL of 1280 µg/mL meropenem + 5 mL of 160 µg/mL xeruborbactam = 10 mL of 640/80 µg/mL meropenem-xeruborbactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.**
Piperacillin-tazobactam	Fixed concentration of tazobactam at 4 µg/mL	Same as aztreonam-avibactam.	
Sulbactam-durlobactam	Fixed concentration of durlobactam at 4 ug/mL	Prepare 10× starting concentration of sulbactam at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of durlobactam 80 µg/mL to each of the diluted tubes.	For a starting concentration of 128/4 in the panel, prepare a 10× stock concentration of sulbactam at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed. Prepare a stock concentration of durlobactam at 80 µg/mL. Then add an equal volume of the durlobactam 80 µg/mL solution to each diluted tube of sulbactam. For example, 5 mL of 2560 µg/mL sulbactam + 5 mL of 80 µg/mL clavulanate = 10 mL of 1280/40 µg/mL sulbactam-durlobactam. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Ticarcillin-clavulanate	Fixed concentration of clavulanate at 2 µg/mL	Prepare 10× starting concentration of ticarcillin at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of clavulanate 40 µg/mL to each of the diluted tubes.	For a starting concentration of 128/2 in the panel, prepare a 10× stock concentration of ticarcillin at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed. Prepare a stock concentration of clavulanate at 40 µg/mL. Then add an equal volume of the clavulanate 40 µg/mL solution to each diluted tube of ticarcillin. For example, 5 mL of 2560 µg/mL ticarcillin + 5 mL of 40 µg/mL clavulanate = 10 mL of 1280/20 µg/mL ticarcillin-clavulanate. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Trimethoprim-sulfamethoxazole	1:19 ratio (trimethoprim:sulfamethoxazole)	Prepare a 10× starting concentration of trimethoprim at 1600 µg/mL (or at 1280 µg/mL that will need dilution to 160 µg/mL). Prepare a 10× starting concentration of sulfamethoxazole at a $\log_2$ multiple of 1520 µg/mL (eg, 1520, 3040, or 6080 µg/mL) depending on the starting concentration needed.	For a starting concentration of 8/152 in the panel, prepare a 10× concentration of trimethoprim at 160 µg/mL. Prepare a 10× starting concentration of sulfamethoxazole at 3040 µg/mL. Add an equal volume of the 160 µg/mL trimethoprim and the 3040 µg/mL sulfamethoxazole to the first dilution tube, and then dilute by serial 2-fold dilutions as usual. For example, 5 mL of 160 µg/mL trimethoprim and 5 mL of 3040 µg/mL sulfamethoxazole = 10 mL of 80/1520 trimethoprim-sulfamethoxazole. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Quinupristin-dalfopristin	Preparation usually not necessary, because drug powder is received as combination.		

NOTE 1: To prepare intermediate dilutions of antimicrobial agents, a convenient formula to use is $C_1 \bullet V_1 = C_2 \bullet V_2$, where C_1 is the concentration of stock solution of the antimicrobial agent (usually 1280 µg/mL or greater); V_1 is the unknown volume that will be needed to make the intermediate concentration; C_2 is the intermediate concentration needed; and V_2 is the volume of the intermediate stock solution needed. For example, to prepare 20 mL of a 40 µg/mL solution from a 1280 µg/mL stock solution:

$$C_1 \bullet V_1 = C_2 \bullet V_2$$

$$1280\ \mu g/mL \bullet V_1 = 40\ \mu g/mL \bullet 20\ mL$$

$$V_1 = \frac{40\ \mu g/mL \bullet 20\ mL}{1280\ \mu g/mL}$$

$$V_1 = 0.625\ mL$$

Therefore, add 0.625 mL of the 1280 µg/mL stock solution to 19.375 mL of diluent (usually water) for a final volume of 20 mL of a 40 µg/mL solution.

NOTE 2: Information in black boldface type is new or modified since the previous edition.

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Table 7. Preparing Dilutions of Antimicrobial Agents to Be Used in Agar Dilution Susceptibility Tests

Antimicrobial Solution										
Step	Concentration, µg/mL	Source	Volume, mL	+	Diluent, mL	=	Intermediate Concentration, µg/mL	=	Final Concentration at 1:10 Dilution in Agar, µg/mL	Log_2
	5120	Stock	-		-		5120		512	9
1	5120	Stock	2		2		2560		256	8
2	5120	Stock	1		3		1280		128	7
3	5120	Stock	1		7		640		64	6
4	640	Step 3	2		2		320		32	5
5	640	Step 3	1		3		160		16	4
6	640	Step 3	1		7		80		8	3
7	80	Step 6	2		2		40		4	2
8	80	Step 6	1		3		20		2	1
9	80	Step 6	1		7		10		1	0
10	10	Step 9	2		2		5		0.5	−1
11	10	Step 9	1		3		2.5		0.25	−2
12	10	Step 9	1		7		1.25		0.125	−3

NOTE: This table is modified from Ericsson HM, Sherris JC. Antibiotic sensitivity testing: report of an international collaborative study. *Acta Pathol Microbiol Scand B Microbiol Immunol*. 1971;217(suppl):1+.

When serial twofold dilution minimal inhibitory concentrations are being prepared and tested, the actual dilution scheme is:

128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0.0039063, 0.0019531 µg/mL, etc.

For convenience only, and not because these are the actual concentrations tested, it was decided to use the following values in these tables:

128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, 0.03, 0.016, 0.008, 0.004, 0.002 µg/mL, etc.

The values that appear in the tables are equivalent to the actual values tested, eg, 0.12 µg/mL = 0.125 µg/mL, 0.016 µg/mL = 0.015625 µg/mL.

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Table 8A. Preparing Dilutions of Antimicrobial Agents to Be Used in Broth Dilution Susceptibility Tests

Antimicrobial Solution								
Step	Concentration,[a] µg/mL	Source	Volume,[a] mL	+	CAMHB[b] Volume,[c] mL	=	Final Concentration, µg/mL	Log_2
1	5120	Stock	1		9		512	9
2	512	Step 1	1		1		256	8
3	512	Step 1	1		3		128	7
4	512	Step 1	1		7		64	6
5	64	Step 4	1		1		32	5
6	64	Step 4	1		3		16	4
7	64	Step 4	1		7		8	3
8	8	Step 7	1		1		4	2
9	8	Step 7	1		3		2	1
10	8	Step 7	1		7		1	0
11	1	Step 10	1		1		0.5	−1
12	1	Step 10	1		3		0.25	−2
13	1	Step 10	1		7		0.125	−3

Abbreviation: CAMHB, cation-adjusted Mueller-Hinton broth.

Footnotes

a. See Table 7 for the dilution scheme when serial twofold dilution minimal inhibitory concentrations are being prepared and tested.

b. Adjustment with cations, if necessary, occurs before this step.

c. The volumes selected can be any multiple of these figures, depending on the number of tests to be performed.

NOTE: This table is modified from Ericsson HM, Sherris JC. Antibiotic sensitivity testing: report of an international collaborative study. *Acta Pathol Microbiol Scand B Microbiol Immunol*. 1971;217(suppl):1:+.

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Table 8B. Preparing Dilutions of Water-Insoluble Antimicrobial Agents to Be Used in Broth Dilution Susceptibility Tests

Antimicrobial Solution										
Step	Concentration, µg/mL	Source	Volume, mL	+	Solvent, mL (eg, DMSO)	=	Intermediate Concentration, µg/mL	=	Final Concentration at 1:100, µg/mL	Log_2
1	1600	Stock					1600		16	4
2	1600	Stock	0.5		0.5		800		8.0	3
3	1600	Stock	0.5		1.5		400		4.0	2
4	1600	Stock	0.5		3.5		200		2.0	1
5	200	Step 4	0.5		0.5		100		1.0	0
6	200	Step 4	0.5		1.5		50		0.5	−1
7	200	Step 4	0.5		3.5		25		0.25	−2
8	25	Step 7	0.5		0.5		12.5		0.125	−3
9	25	Step 7	0.5		1.5		6.25		0.0625	−4
10	25	Step 7	0.5		3.5		3.1		0.03	−5
11	3.1	Step 10	0.5		0.5		1.6		0.016	−6
12	3.1	Step 10	0.5		1.5		0.8		0.008	−7
13	3.1	Step 10	0.5		3.5		0.4		0.004	−8
14	0.4	Step 13	0.5		0.5		0.2		0.002	−9

Abbreviation: DMSO, dimethyl sulfoxide.

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Appendix A. Suggestions for Confirming Antimicrobial Susceptibility Test Results and Organism Identification for Agents Approved by the US Food and Drug Administration for Clinical Use

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
			Action Steps:		
			• Confirm ID and susceptibility.[a] • Report to infection prevention. • Check with public health department to determine appropriate reporting and isolate referral procedures. • Save isolate. **NOTE:** It may be appropriate to notify infection prevention of preliminary findings before confirmation of results.	• Confirm ID and susceptibility if uncommon in the institution.[a] • Check with infection prevention in the facility to determine whether special reporting procedures or additional actions are needed. • Check with public health department to determine appropriate reporting and isolate referral procedures.	• Confirm ID and susceptibility if uncommon in the institution.[a] • Check with infection prevention in the facility to determine whether special reporting procedures or additional action are needed.
Any Enterobacterales	β-lactam combination agents	Ceftazidime-avibactam – R **Imipenem-relebactam – I or R** Meropenem-vaborbactam – I or R		X	
	Cephems	**Cefiderocol – I or R**	**X**		
	Carbapenems	Any carbapenem – I or R[b]		X	
	Aminoglycosides	Amikacin, gentamicin, and tobramycin – R			X
		Plazomicin – R (except *P. mirabilis*)	X		
	Lipopeptides	Colistin/polymyxin B – R[c]	X		

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Escherichia coli, *Klebsiella pneumoniae*, *K. oxytoca*, and *Proteus mirabilis*	Cephems	Cephalosporin III/IV - I/SDD or R			X
Salmonella and *Shigella* spp.[d]	Cephems	Cephalosporin III - I or R		X	
	Macrolides	Azithromycin - R		X	
	Fluoroquinolones	Any fluoroquinolone - I or R		X	
Acinetobacter baumannii complex	**Cephems**	**Cefiderocol - I or R**	**X**		
	Carbapenems	Any carbapenem[c] - I or R			X
	Lipopeptides	Colistin/polymyxin B - R	X		
Pseudomonas aeruginosa	β-lactam combination agents	**Ceftazidime-avibactam - R** Ceftolozane-tazobactam - I or R **Imipenem-relebactam - I or R**			**X**
	Cephems	**Cefiderocol - I or R**	**X**		
	Carbapenems	Any carbapenem[c]- I or R			X
	Aminoglycosides	Amikacin and tobramycin - R			X
	Lipopeptides	Colistin/polymyxin B - R	X		
Stenotrophomonas maltophilia	**Cephems**	**Cefiderocol - NS**	**X**		
	Folate pathway antagonists	Trimethoprim-sulfamethoxazole - I or R			X

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Haemophilus influenzae	Penicillins	Ampicillin – R and ß-lactamase negative		X	
	ß-lactam combination agents	Amoxicillin-clavulanate – R		X	
		Ceftolozane-tazobactam – NS	X		
	Cephems	Cephalosporin III/IV – NS Ceftaroline – NS	X		
	Carbapenems	Any carbapenem – NS	X		
	Fluoroquinolones	Any fluoroquinolone – NS	X		
	Pleuromutilins	Lefamulin – NS	X		
Neisseria gonorrhoeae	Cephems	Cephalosporin III/IV – NS		X	
	Macrolides	Azithromycin – NS			X
	Fluoroquinolones	Ciprofloxacin – I or R			X
Enterococcus spp.	Glycopeptides	Vancomycin – R[c]			X
	Lipoglycopeptides (Vancomycin-susceptible *E. faecalis* only)	Dalbavancin – NS Oritavancin – NS Telavancin – NS	X		
	Lipopeptides	Daptomycin – SDD, I, or R		X	
	Oxazolidinones	Linezolid – R Tedizolid – NS		X	
	Aminoglycosides	Gentamicin high level – R Streptomycin high level – R			X

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Staphylococcus aureus	Penicillinase-stable penicillins	Oxacillin – R			X
	Cephems	Ceftaroline – SDD or R		X	
	Glycopeptides	Vancomycin – I[e]		X	
		Vancomycin – R	X		
	Lipoglycopeptides	Dalbavancin – NS Oritavancin – NS Telavancin – NS	X		
	Lipopeptides	Daptomycin – NS		X	
	Streptogramins	Quinupristin-dalfopristin (MSSA only) – I or R		X	
	Oxazolidinones	Linezolid – R Tedizolid – I or R		X	
	Pleuromutilins	Lefamulin – NS	X		
Staphylococcus spp. other than *S. aureus*	Glycopeptides	Vancomycin – I or R[f]		X	
	Lipopeptides	Daptomycin – NS		X	
	Oxazolidinones	Linezolid – R		X	

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Streptococcus pneumoniae	Penicillins	Amoxicillin or penicillin (nonmeningitis) - R			X
	Cephems	Cephalosporin III/IV (nonmeningitis) - R			X
		Ceftaroline (nonmeningitis) - NS	X		
	Carbapenems	Any carbapenem - I, R, or NS		X	
	Glycopeptides	Vancomycin - NS	X		
	Fluoroquinolones	Any fluoroquinolone - I or R		X	
	Streptogramins	Quinupristin-dalfopristin - I or R		X	
	Ansamycins	Rifampin - I or R		X	
	Oxazolidinones	Linezolid - NS	X		
	Pleuromutilins	Lefamulin - NS	X		
Streptococcus, β-hemolytic group	Penicillins	Ampicillin or penicillin - NS	X		
	Cephems	Cephalosporin III/IV - NS Ceftaroline - NS	X		
	Carbapenems	Any carbapenem - NS	X		
	Glycopeptides	Vancomycin - NS	X		
	Lipoglycopeptides	Dalbavancin - NS Oritavancin - NS Telavancin - NS	X X X		
	Lipopeptides	Daptomycin - NS	X		
	Streptogramins	Quinupristin-dalfopristin (*S. pyogenes* only) - I or R		X	
	Oxazolidinones	Linezolid - NS Tedizolid - NS	X X		

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Streptococcus, viridans group	Carbapenems	Any carbapenem – NS	X		
	Glycopeptides	Vancomycin – NS	X		
	Lipoglycopeptides	Dalbavancin (*S. anginosus* group only) – NS	X		
		Oritavancin – NS	X		
		Telavancin – NS	X		
	Streptogramins	Quinupristin-dalfopristin – I or R	X		
	Oxazolidinones	Linezolid – NS	X		
		Tedizolid – NS	X		
Neisseria meningitidis	Penicillins	Ampicillin or penicillin – I		X	
		Ampicillin or penicillin – R	X		
	Cephems	Cephalosporin III– NS	X		
	Carbapenems	Meropenem – NS	X		
	Macrolides	Azithromycin – NS		X	
	Tetracyclines	Minocycline – NS		X	
	Fluoroquinolones	Any fluoroquinolone – I or R		X	
	Phenicols	Chloramphenicol – I or R		X	
	Ansamycins	Rifampin – I or R		X	
Bacteroides spp. and *Parabacteroides* spp.	**ß-lactam combination agents**	**Imipenem-relebactam – I or R**		**X**	
	Carbapenems	Any carbapenem – I or R		X	
	Nitroimidazoles	Metronidazole – I or R		X	

Abbreviations: I, intermediate; ID, identification; mCIM, modified carbapenem inactivation method; MIC, minimal inhibitory concentration; MSSA, methicillin (oxacillin)-susceptible *Staphylococcus aureus;* NS, nonsusceptible; NWT, non-wild-type; R, resistant; SDD, susceptible-dose dependent.

Appendix A. (Continued)

Footnotes

a. Ensure antimicrobial susceptibility test results and organism identification are accurate and reproducible. Consider the following steps:

   1. Check for transcription errors, contamination, or defective panel, plate, or card.

   2. Check previous reports on the patient to determine if the isolate was encountered and confirmed earlier.

   3. Repeat organism identification and antimicrobial susceptibility tests with initial method to ensure they reproduce. For category I and II, the laboratory may elect to skip step 3 and go to steps 4 and 5. For category III, repeat and/or confirmatory testing may not be needed if resistance is common in the institution.

   4. Confirm organism identification with second method performed in-house or at a referral laboratory.

   5. Confirm antimicrobial susceptibility test results with second method (eg, in-house or referral laboratory). The second method might be a CLSI reference method (eg, broth microdilution, agar dilution, or disk diffusion) or a US Food and Drug Administration–cleared commercial test.

b. Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (eg, MI-Cs in the intermediate or resistant category than those with meropenem or doripenem MICs. MICs for these agents may be elevated due to mechanisms other than carbapenemases among these organisms. A phenotypic test such as mCIM or CarbaNP may be used to identify carbapenemase-producing isolates (see Tables 3A and 3B).

c. Excludes organisms with intrinsic resistance to listed agents as described in Appendix B.

d. When submitting the report to a public health department, include antimicrobial susceptibility test results for *Salmonella* spp. that are intermediate or resistant to third-generation cephalosporins (cephalosporin III) and/or intermediate or resistant to fluoroquinolone or resistant to nalidixic acid.

e. *S. aureus* isolates demonstrating vancomycin MICs 4 µg/mL may represent testing variation and need not be reported or submitted to public health department; *S. aureus* isolates demonstrating MICs > 4 µg/mL should be reported to the local public health department.

f. There are some *Staphylococcus* spp. other than *S. aureus* for which vancomycin MICs may test within the intermediate range (MIC 8–16 µg/mL). In contrast, vancomycin-resistant *Staphylococcus* spp. (MIC ≥ 32 µg/mL) are rare.

NOTE 1: NS: A category used for isolates for which only a susceptible interpretive criterion has been designated because of the absence or rare occurrence of resistant strains. Isolates that have MICs above or zone diameters below the value indicated for the susceptible breakpoint should be reported as nonsusceptible.

NOTE 2: An isolate that is interpreted as nonsusceptible does not necessarily mean that the isolate has a resistance mechanism. It is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution subsequent to the time the susceptible-only breakpoint is set.

NOTE 3: For strains yielding results in the “nonsusceptible” category, organism identification and antimicrobial susceptibility test results should be confirmed (see footnote a).

NOTE 4: Information in black boldface type is new or modified since the previous edition.

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Appendix B. Intrinsic Resistance

Intrinsic resistance is defined as inherent or innate (not acquired) antimicrobial resistance, which is reflected in wild-type antimicrobial patterns of all or almost all representatives of a species. Intrinsic resistance is so common that susceptibility testing is unnecessary. For example, *Citrobacter* spp. are intrinsically resistant to ampicillin.

These tables can be helpful in at least three ways: 1) they provide a way to evaluate the accuracy of testing methods; 2) they aid in the recognition of common phenotypes; and 3) they can assist with verification of cumulative antimicrobial susceptibility test data. In the tables, an "R" occurring with an antimicrobial agent–organism combination means that strains should test resistant. A small percentage (1% to 3%) may appear susceptible due to method variation, mutation, or low levels of resistance expression.

Each laboratory should decide which agents to test and report in consultation with the antimicrobial stewardship team **and other relevant institutional stakeholders.** If tested, the result for an antimicrobial agent–organism combination listed as having intrinsic resistance should be reported as resistant. Consideration may be given to adding comments regarding intrinsic resistance of agents not tested. See Appendix A, footnote a.

Appendix B. (Continued)

B1. Enterobacterales

Organism \ Antimicrobial Agent	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporin II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
Citrobacter freundii	R	R	R		R	R	R						
Citrobacter koseri, *Citrobacter amalonaticus* group[a]	R			R									
Enterobacter cloacae complex[b]	R	R	R		R	R							
Escherichia coli	There is no intrinsic resistance to β-lactams in this organism.												
Escherichia hermannii	R			R									
Hafnia alvei	R	R	R		R	R						R[c]	
Klebsiella (formerly *Enterobacter*) *aerogenes*	R	R	R		R	R							
Klebsiella pneumoniae, *Klebsiella oxytoca*, *Klebsiella variicola*	R			R									
Morganella morganii	R	R			R		R	[d]		R	R	R	
Proteus mirabilis	There is no intrinsic resistance to penicillins and cephalosporins in this organism.							[d]	R	R	R	R	
Proteus penneri	R				R		R	[d]	R	R	R	R	
Proteus vulgaris	R				R		R	[d]	R	R	R	R	
Providencia rettgeri	R	R			R			[d]	R	R	R	R	
Providencia stuartii	R	R			R			[d]	R	R	R	R	[e]
Raoultella spp.[f]	R			R									

Appendix B. (Continued)

B1. Enterobacterales (Continued)

Organism \ Antimicrobial Agent	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporin II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
Salmonella and *Shigella* spp.	There is no intrinsic resistance to β-lactams in these organisms; refer to **WARNING** below for reporting.												
Serratia marcescens	R	R	R		R	R	R				R	R	g
Yersinia enterocolitica	R	R		R	R								

Abbreviations: **MIC, minimal inhibitory concentration;** R, resistant.

WARNING: For *Salmonella* spp. and *Shigella* spp., aminoglycosides, first- and second-generation cephalosporins, and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.

Footnotes

a. *Citrobacter amalonaticus* group includes *C. amalonaticus*, *C. farmeri*, and *C. sedlakii*.

b. *E. cloacae* complex includes *Enterobacter asburiae*, *Enterobacter cloacae*, and *Enterobacter hormaechei*. Other members of the complex include *Enterobacter kobei* and *Enterobacter ludwigii*, for which antimicrobial susceptibility testing data are not available.

c. Colistin and polymyxin B resistance also applies to *Hafnia paralvei*.

d. *Proteus* spp., *Providencia* spp., and *Morganella* spp. may have elevated minimal inhibitory concentrations to imipenem by mechanisms other than by production of carbapenemases. Isolates that test as susceptible should be reported as susceptible.

e. *P. stuartii* should be considered resistant to gentamicin, netilmicin, and tobramycin but not intrinsically resistant to amikacin.

f. *Raoultella* spp. includes *R. ornithinolytica*, *R. terrigena*, and *R. planticola*.

g. *Serratia marcescens* may have elevated MICs to tobramycin. Isolates that test susceptible should be reported as susceptible.

Appendix B. (Continued)

B1. Enterobacterales (Continued)

NOTE 1: Cephalosporins III, cefepime, cefiderocol, aztreonam, ticarcillin-clavulanate, piperacillin-tazobactam, imipenem-relebactam, ceftazidime-avibactam, meropenem-vaborbactam, and the carbapenems are not listed because there is no intrinsic resistance in Enterobacterales.

NOTE 2: Enterobacterales are also intrinsically resistant to clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin), lipoglycopeptides (oritavancin, teicoplanin, telavancin), linezolid, tedizolid, quinupristin-dalfopristin, rifampin, and macrolides (erythromycin, clarithromycin, and azithromycin). However, there are some exceptions with macrolides (eg, *Salmonella* and *Shigella* spp. with azithromycin).

NOTE 3: Information in black boldface type is new or modified since the previous edition.

Appendix B. (Continued)

B2. Non-Enterobacterales

Organism \ Antimicrobial Agent	Ampicillin, Amoxicillin	Piperacillin	Ticarcillin	Ampicillin-sulbactam	Amoxicillin- clavulanate	Piperacillin-tazobactam	Cefotaxime	Ceftriaxone	Ceftazidime	Cefepime	Aztreonam	Imipenem	Meropenem	Ertapenem	Polymyxin B Colistin	Aminoglycosides	Tetracyclines/ Tigecycline	Trimethoprim	Trimethoprim-sulfamethoxazole	Chloramphenicol	Fosfomycin
Acinetobacter baumannii/ Acinetobacter calcoaceticus complex	R				R						R			R				R		R	R
Burkholderia cepacia complex[a]	R	R	R	R	R	a	a	a		a	a	a		R	R	a		a			R
Pseudomonas aeruginosa	R			R	R		R	R						R			R	R	R	R	
Stenotrophomonas maltophilia	R	R	R	R	R	R	R	R			R	R	R	R		R	[b]	R			R

Abbreviation: MIC, minimal inhibitory concentration; R, resistant.

Footnotes

a. *B. cepacia* complex isolates have chromosomal genes that must undergo mutational changes before expressing resistance. It is not known how often these mutations occur during growth. Intrinsic resistance implies the presence of resistance mechanisms in natural or wild-type strains that result in phenotypic resistance for all or nearly all strains. Environmental *B. cepacia* complex strains lacking mutations do not express resistance mechanisms, resulting in low MICs to many antimicrobial agents, whereas clinical strains that express resistance genes, such as those from cystic fibrosis patients, have high MIC values to these same antimicrobial agents. There is insufficient clinical evidence to confirm whether strains that test susceptible *in vitro*, despite the presence of resistance mechanisms, will respond *in vivo*. Therefore, intrinsic resistance to the footnoted antibiotics (listed as resistant in previous editions of M100) cannot be confirmed.

b. *S. maltophilia* is intrinsically resistant to tetracycline but not to doxycycline, minocycline, or tigecycline.

NOTE 1: These nonfermentative gram-negative bacteria are also intrinsically resistant to penicillin (ie, benzylpenicillin), cephalosporins I (cephalothin, cefazolin), cephalosporin II (cefuroxime), cephamycins (cefoxitin, cefotetan), clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin), linezolid, macrolides (erythromycin, azithromycin, clarithromycin), quinupristin-dalfopristin, and rifampin.

NOTE 2: Information in black boldface type is new or modified since the previous edition.

Appendix B. (Continued)

B3. Staphylococci

Organism \ Antimicrobial Agent	Novobiocin	Fosfomycin	Fusidic Acid
S. aureus *S. lugdunensis*	There is no intrinsic resistance in these species.		
S. epidermidis			
S. haemolyticus			
S. saprophyticus	R	R	R
S. capitis		R	
S. cohnii	R		
S. xylosus	R		

Abbreviations: MRS, methicillin (oxacillin)-resistant staphylococci; R, resistant.

NOTE 1: These gram-positive bacteria are also intrinsically resistant to aztreonam, polymyxin B/colistin, and nalidixic acid.

NOTE 2: MRS, as defined by cefoxitin or oxacillin testing, as appropriate to the species, are considered resistant to other β-lactam agents, ie, penicillins, β-lactam combination agents, cephems with the exception of ceftaroline, and carbapenems. This is because most cases of documented MRS infections have responded poorly to β-lactam therapy, or because convincing clinical data that document clinical efficacy for those agents have not been presented.

Appendix B. (Continued)

B4. Enterococcus spp.

Organism \ Antimicrobial Agent	Cephalosporins	Vancomycin	Teicoplanin	Aminoglycosides	Clindamycin	Quinupristin-dalfopristin	Trimethoprim	Trimethoprim-sulfamethoxazole	Fusidic Acid
E. faecalis	R[a]			R[a]	R[a]	R	R	R[a]	R
E. faecium	R[a]			R[a]	R[a]		R	R[a]	R
E. gallinarum/*E. casseliflavus*	R[a]	R		R[a]	R[a]	R	R	R[a]	R

Abbreviation: R, resistant.

a. **Warning:** For *Enterococcus* spp., cephalosporins, aminoglycosides (except for high-level resistance testing), clindamycin, and trimethoprim-sulfamethoxazole may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.

NOTE: These gram-positive bacteria are also intrinsically resistant to aztreonam, polymyxin B/colistin, and nalidixic acid.

Appendix B. (Continued)

B5. Anaerobic Gram-Positive Bacilli

Organism \ Antimicrobial Agent	Vancomycin	Aminoglycosides
Clostridium and *Clostridioides* spp.		R
Clostridium innocuum	R	R

Abbreviation: R, resistant.

B6. Anaerobic Gram-Negative Bacilli

Organism \ Antimicrobial Agent	Aminoglycosides	Penicillin	Ampicillin	Quinolones
Bacteroides spp.	R	R	R	
Fusobacterium canifelinum	R			R

Abbreviation: R, resistant.

Appendix C. QC Strains for Antimicrobial Susceptibility Tests

QC Strain	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
Acinetobacter baumannii NCTC 13304[a,b]	OXA-27 (carbapenemase)	β-lactam combination agents	β-lactam combination agents		
Bacteroides fragilis ATCC®[c] 25285	β-lactamase positive		All anaerobes		
Bacteroides thetaiotaomicron ATCC® 29741	β-lactamase positive		All anaerobes		
Clostridioides (formerly *Clostridium*) *difficile* ATCC® 700057	β-lactamase negative		Gram-positive anaerobes		
Eggerthella lenta (formerly *Eubacterium lentum*) ATCC® 43055			All anaerobes		• Growth on Brucella medium not optimal • No longer required when establishing new QC ranges due to organism variability
Enterococcus faecalis ATCC® 29212			Nonfastidious gram-positive bacteria	• Vancomycin agar • HLAR tests • High-level mupirocin resistance MIC test	• Assess suitability of medium for sulfonamide or trimethoprim MIC and disk diffusion tests.[d] • Assess suitability of cation content in each batch/lot of MHB for daptomycin broth microdilution. Agar dilution has not been validated for daptomycin.
E. faecalis ATCC® 33186					Alternative to *E. faecalis* ATCC® 29212 to assess suitability of MHA for sulfonamide or trimethoprim disk diffusion tests.[d]

Appendix C. (Continued)

QC Strain	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
E. faecalis ATCC® 51299	• *vanB* (vancomycin resistant) • Resistant to high-level aminoglycosides			• Vancomycin agar • HLAR tests	
Escherichia coli ATCC® 25922	β-lactamase negative	• Nonfastidious gram-negative bacteria • *Neisseria meningitidis*	• Nonfastidious gram-negative bacteria • *N. meningitidis*		
E. coli ATCC® 35218[a,b,1]	TEM-1	β-lactam combination agents	β-lactam combination agents		
E. coli NCTC 13353[a,b,2]	• CTX-M-15 (ESBL) • **OXA-1**	β-lactam combination agents	β-lactam combination agents		
E. coli NCTC 13846	MCR-1		Nonfastidious gram-negative bacteria		
E. coli ATCC® BAA-3170™ (formerly *E. coli* AR Bank #0349 *mcr-1*)[3]	MCR-1		Nonfastidious gram-negative bacteria	• Colistin broth disk elution • Colistin agar test	
Haemophilus influenzae ATCC® 10211					Assess each batch/lot of HTM for growth capabilities.
H. influenzae ATCC® 49247	BLNAR	• *H. influenzae* • *Haemophilus parainfluenzae*	• *H. influenzae* • *H. parainfluenzae*		
H. influenzae ATCC® 49766	Ampicillin susceptible	• *H. influenzae* • *H. parainfluenzae*	• *H. influenzae* • *H. parainfluenzae*		More reproducible than *H. influenzae* ATCC® 49247 with selected β-lactam agents
Klebsiella pneumoniae ATCC® 700603[a,b,1,4]	• SHV-18 (ESBL) • OXA-2 • Mutations in OMPK35 and OMPK37	β-lactam combination agents	β-lactam combination agents	ESBL tests	**May demonstrate 2 colony morphologies: 1) opaque and cream colored and 2) translucent. Both colony morphologies can be used.**

Appendix C. (Continued)

QC Strain	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
K. pneumoniae ATCC® BAA-1705™[a,b]	• KPC-2 (carbapenemase) • TEM • SHV	β-lactam combination agents	β-lactam combination agents	Carbapenemase tests	
K. pneumoniae ATCC® BAA-1706™	• Resistant to carbapenems by noncarbapenemase mechanism			Carbapenemase tests	
K. pneumoniae ATCC® BAA-2146™	NDM			Carbapenemase tests	
K. pneumoniae ATCC® BAA-2814™[a,b] (previously B21[KP1074])	• KPC-3 (carbapenemase) • SHV-11 • TEM-1	β-lactam combination agents	β-lactam combination agents		Higher MIC (see Table 5A-2) and better indicator of antimicrobial agent stability than *K. pneumoniae* BAA-1705™
Neisseria gonorrhoeae ATCC® 49226	CMRNG	*N. gonorrhoeae*	*N. gonorrhoeae*		
Pseudomonas aeruginosa ATCC® 27853[e]	Inducible AmpC β-lactamase	Nonfastidious gram-negative bacteria	Nonfastidious gram-negative bacteria		Assess suitability of cation content in each batch/lot of CAMHB.
Staphylococcus aureus ATCC® 25923	• β-lactamase negative • *mecA* negative • *mupA* negative	Nonfastidious gram-positive bacteria		• High-level mupirocin resistance disk diffusion test • ICR disk diffusion test (D-zone test)	Little value in MIC testing due to its extreme susceptibility to most drugs
S. aureus ATCC® 29213	• Weak β-lactamase-producing strain • *mecA* negative • *mupA* negative		Nonfastidious gram-positive bacteria	• Oxacillin salt agar • High-level mupirocin resistance MIC test • ICR MIC test • Penicillin zone-edge test	Assess suitability of cation content in each batch/lot of MHB for daptomycin broth microdilution.

Appendix C. (Continued)

QC Strain	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
S. aureus ATCC® 43300	*mecA* positive	Cefoxitin disk diffusion testing	Cefoxitin MIC testing	Oxacillin salt agar	
S. aureus ATCC® BAA-976™	*msrA*-mediated macrolide-only resistance			ICR MIC test and disk approximation test (D-zone test)	
S. aureus ATCC® BAA-977™	Inducible *ermA*-mediated macrolide resistance			ICR MIC test and disk approximation test (D-zone test)	
S. aureus ATCC® BAA-1708™	*mupA*-mediated high-level mupirocin resistance			High-level mupirocin resistance test	
Streptococcus pneumoniae ATCC® 49619	Penicillin intermediate by altered penicillin-binding protein	• *S. pneumoniae* • *Streptococcus* spp. • *N. meningitidis*	• *S. pneumoniae* • *Streptococcus* spp. • *N. meningitidis*	ICR MIC test	

Abbreviations: ATCC®, American Type Culture Collection; BLNAR, β-lactamase negative, ampicillin-resistant; CAMHB, cation-adjusted Mueller-Hinton broth; CMRNG, chromosomally mediated penicillin-resistant *Neisseria gonorrhoeae*; ESBL, extended-spectrum β-lactamase; HLAR, high-level aminoglycoside resistance; HTM, *Haemophilus* test medium; ICR, inducible clindamycin resistance; MHA, Mueller-Hinton agar; MHB, Mueller-Hinton broth; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; QC, quality control.

Footnotes

a. Careful attention to organism maintenance (eg, minimal subcultures) and storage (eg, −60°C or below) is especially important for these QC strains because spontaneous loss of the plasmid encoding the β-lactamase has been documented. If stored at temperatures above −60°C or if repeatedly subcultured, these strains may lose their resistance characteristics and QC results may be outside the acceptable ranges.

b. To confirm the integrity of the QC strain, test one of the single β-lactam agents highlighted in orange in Tables 4A-2 and 5A-2 by either a disk diffusion or MIC test when the strain is first subcultured from a frozen or lyophilized stock culture. In-range results for the single agent indicate the QC strain is reliable for QC of β-lactam combination agents. It is not necessary to check the QC strain again with a single agent until a new frozen or lyophilized stock culture is put into use.

c. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

d. Disk diffusion and MIC end points should be easy to read as 80% or greater reduction in growth if the medium has acceptable levels of thymidine.

e. May develop resistance to β-lactam antimicrobial agents after repeated subcultures. Minimize this risk by subculturing from a frozen or lyophilized stock culture at least monthly or whenever the strain demonstrates results outside the acceptable range.

Appendix C. (Continued)

NOTE: Routine QC strains listed in Tables 2A through 2J (in "Routine QC Recommendations" boxes at the top of each page) are tested regularly (ie, daily or weekly) to ensure the test system is working and produces results that fall within specified ranges listed in M100. The routine QC strains recommended in this document should be included if a laboratory performs CLSI reference disk diffusion or MIC testing as described herein. For commercial test systems, manufacturer's recommendations should be followed for all QC procedures. Other QC strains are used to assess particular characteristics of a test or test system in select situations or may represent alternative QC strains. For example, *H. influenzae* ATCC® 10211 is more fastidious than *H. influenzae* ATCC® 49247 or *H. influenzae* ATCC® 49766 and is used to ensure HTM can adequately support the growth of patient isolates of *H. influenzae* and *H. parainfluenzae*. QC strains may possess susceptibility or resistance characteristics specific for one or more special tests listed in M02[5] and M07.[6] They can be used to assess a new test, for training new personnel, and for competence assessment, and it is not necessary to include them in routine daily or weekly antimicrobial susceptibility testing QC programs.

References for Appendix C

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2 Woodford N, Ward ME, Kaufmann ME, et al. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum β-lactamases in the UK. *J Antimicrob Chemother*. 2004;54(4):735-743.

3 Centers for Disease Control and Prevention. CDC & FDA Antibiotic Resistance Isolate Bank. Accessed 10 January 2023. https://wwwn.cdc.gov/arisolatebank/

4 Rasheed JK, Anderson GJ, Yigit H, et al. Characterization of the extended-spectrum beta-lactamase reference strain, *Klebsiella pneumoniae* K6 (ATCC® 700603), which produces the novel enzyme SHV-18. *Antimicrob Agents Chemother*. 2000;44(9):2382-2388.

5 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

6 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

NOTE 2: Information in black boldface type is new or modified since the previous edition.

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Appendix D. Anaerobe Cumulative Antibiogram[1]

NOTE: Isolates collected from selected US hospitals from 1 January 2013 to 31 December 2016.[a]

D1. *Bacteroides* spp. and *Parabacteroides* spp.

Anaerobic Organisms	Number of Strains	Ampicillin-sulbactam		Number of Strains	Piperacillin-tazobactam		Number of Strains	Cefoxitin		Number of Strains	Ertapenem		Number of Strains	Imipenem		Number of Strains	Meropenem	
Percent susceptible (%S) and percent resistant (%R)[b]		%S	%R		%S	%R		%S	%R		%S	%R		%S	%R		%S	%R
Breakpoints, µg/mL		≤ 8/4	≥ 32/16		≤ 16/4	≥ 128/4		≤ 16	≥ 64		≤ 4	≥ 16		≤ 4	≥ 16		≤ 4	≥ 16
B. fragilis	129	84	2	1030	96	1	830	100	0	133	82	14	189	97	1	1505	93	5
B. thetaiotaomicron	76	82	5	252	87	0	258	13	54	-	-	-	70	100	0	328	99	0
B. ovatus	30	80	3	206	94	0	177	20	34	19[c]	84[c]	16[c]	49	100	0	236	95	1
B. vulgatus	20[c]	45[c]	15[c]	168	92	0	153	73	14	—	—	—	35	97	0	171	96	4
B. uniformis	19[c]	84[c]	0[c]	78	96	0	72	85	10	—	—	—	19[c]	100[c]	0[c]	93	100	0
Parabacteroides distasonis	27[c]	59[c]	19[c]	92	95	1	82	29	43	—	—	—	26[c]	100[c]	0	119	97	2

Appendix D. (Continued)

D1. *Bacteroides* spp. and *Parabacteroides* spp. (Continued)

Anaerobic Organisms	Number of Strains	Clindamycin		Number of Strains	Moxifloxacin		Number of Strains	Metronidazole	
Percent susceptible (%S) and percent resistant (%R)[b]		%S	%R		%S	%R		%S	%R
Breakpoints, µg/mL		≤ 2	≥ 8		≤ 2	≥ 8		≤ 8	≥ 32
B. fragilis	1013	26	22	256	61	32	1140	100	0
B. thetaiotaomicron	328	28	49	70	54	36	322	100	0
B. ovatus	207	46	51	59	41	25	236	100	0
B. vulgatus	171	53	46	29[c]	31[c]	45[c]	186	100	0
B. uniformis	87	45	48	25[c]	48[c]	40[c]	89	100	0
Parabacteroides distasonis	108	43	44	37	62	35	118	100	0

Footnotes

a. Data were generated from unique isolates from patient specimens submitted to Tufts Medical Center, Boston, Massachusetts; International Health Management Associates, Inc., Schaumburg, Illinois; R.M. Alden Research Laboratory, Culver City, California; Creighton University School of Medicine, Omaha, Nebraska; Mayo Clinic College of Medicine and Science, Rochester, Minnesota; and the Centers for Disease Control and Prevention, Atlanta, Georgia. All testing was performed by the agar dilution method. Information and analysis of previous versions of this table have been published.

b. Intermediate category is not shown but can be derived by subtraction of %S and %R for each antimicrobial agent from %100.

c. Calculated from fewer than the CLSI document M39[1] recommendation of 30 isolates.

Reference for D1

[1] CLSI. *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data*. 5th ed. CLSI guideline M39. Clinical and Laboratory Standards Institute; 2022.

Appendix D. (Continued)

NOTE: Isolates collected from selected US hospitals from 1 January 2013 to 31 December 2016.[a]

D2. Anaerobic Organisms Other Than *Bacteroides* spp. and *Parabacteroides* spp.

Anaerobic Organisms	Number of Strains	Ampicillin-sulbactam		Number of Strains	Piperacillin-tazobactam		Number of Strains	Imipenem		Number of Strains	Meropenem		Number of Strains	Penicillin	
Percent susceptible (%S) and percent resistant (%R)[b]		%S	%R		%S	%R		%S	%R		%S	%R		%S	%R
Breakpoints, µg/mL		≤ 8/4	≥ 32/16		≤ 32/4	≥ 128/4		≤ 4	≥ 16		≤ 4	≥ 16		≤ 0.5	≥ 2
Prevotella spp.	29[c]	97[c]	3[c]	63	100	0	29[c]	100	0	92	98	0	63	100	0
Fusobacterium spp.	20[c]	100[c]	0[c]	55	96	2	75	95	4	20[c]	100[c]	0[c]	–[d]	–[d]	–[d]
Anaerobic gram-positive cocci[e]	–[d]	–[d]	–[d]	1853	99	1	134	99	0	1647	100	0	1647	100	0
Cutibacterium (formerly *Propionibacterium*) *acnes*[f]	–[d]	–[d]	–[d]	18[c]	100[c]	0[c]	17[c]	94[c]	0[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]
Clostridium perfringens	15[c]	100[c]	0	410	100	0	23[c]	100[c]	0[c]	417	100	0	402	90	4
Clostridioides (formerly *Clostridium*) *difficile*[g]	76	99	0	542	93	0	480	69	4	609	99	0	533	6	37
Other *Clostridium* spp.	–[d]	–[d]	–[d]	439	94	1	71	99	0	390	100	0	390	69	13

Appendix D. (Continued)

D2. Anaerobic Organisms Other Than *Bacteroides* spp. and *Parabacteroides* spp. (Continued)

Anaerobic Organisms	Number of Strains	Clindamycin		Number of Strains	Moxifloxacin		Number of Strains	Metronidazole	
Percent susceptible (%S) and percent resistant (%R)[b]		%S	%R		%S	%R		%S	%R
Breakpoints in μg/mL		≤ 2	≥ 8		≤ 2	≥ 8		≤ 8	≥ 32
Prevotella spp.	29[c]	69[c]	28[c]	92	66	25	92	99	0
Fusobacterium spp.	75	77	21	75	68	23	75	95	5
Anaerobic gram-positive cocci[e]	1826	97	3	300	72	21	1692	100	0
C. (formerly *P.*) *acnes*[f]	17[c]	53[c]	35[c]	114	95	4	18[c]	0[c]	100[c]
C. perfringens	425	83	12	23[c]	83[c]	9[c]	425	100	0
Clostridioides (formerly *Clostridium*) *difficile*[g]	1013	32	38	480	74	25	1343	100	0
Other *Clostridium* spp.	461	67	25	71	62	35	461	100	0

Appendix D. (Continued)

Footnotes

a. Data were generated from unique isolates from patient specimens submitted to Tufts Medical Center, Boston, Massachusetts; International Health Management Associates, Inc., Schaumburg, Illinois; R.M. Alden Research Laboratory, Culver City, California; Creighton University School of Medicine, Omaha, Nebraska; Mayo Clinic College of Medicine and Science, Rochester, Minnesota; and the Centers for Disease Control and Prevention, Atlanta, Georgia. All testing was performed by the agar dilution method. Information and analysis of previous versions of this table have been published.

b. Intermediate category is not shown but can be derived by subtraction of %S and %R for each antimicrobial agent from %100.

c. Calculated from fewer than the CLSI document M39[1] recommendation of 30 isolates.

d. A dash (–) indicates that data were not available.

e. Anaerobic gram-positive cocci include *Peptococcus, Peptostreptococcus, Finegoldia, Peptoniphilus,* and *Anaerococcus* species.

f. 80 isolates of *Cutibacterium* (formerly *Propionibacterium*) *acnes* from two of the sites generated MIC values for rifampin ≤ 0.03 µg/mL using the agar dilution method. There are no interpretive breakpoints for this organism/antimicrobial agent combination.

g. *Clostridioides* (formerly *Clostridium*) *difficile* isolates are from an intestinal source; these results do not imply efficacy for intraluminal infections. Vancomycin minimal inhibitory concentrations for isolates were < 4 µg/mL.

Reference for D2

[1] CLSI. *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data*. 5th ed. CLSI guideline M39. Clinical and Laboratory Standards Institute; 2022.

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Appendix E. Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints

The evolving science of pharmacokinetics-pharmacodynamics has become increasingly important in recent years in determining minimal inhibitory concentration (MIC) breakpoints. Recently approved susceptible or susceptible-dose dependent (SDD) breakpoints for a number of agents have been based on a specific dosage regimen(s); these dosage regimens are listed in the table below. Proper application of the breakpoints necessitates drug exposure at the site of infection that corresponds to or exceeds the expected systemic drug exposure at the dose listed in adult patients with normal renal function. This information should be shared with pharmacists, infectious diseases staff, and others making dosing recommendations for the institution.

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2A. Enterobacterales				
Amikacin	**≤ 4 µg/mL**	**15 mg/kg administered every 24 h**	**N/A**	
Ampicillin (used to predict results for amoxicillin)	≤ 8 µg/mL	Ampicillin: 2 g parenterally administered every 4–6 h or Amoxicillin: 1-2 g parenterally administered every 6 h	N/A	
Ampicillin (used to predict results for amoxicillin; salmonellosis, shigellosis, and uncomplicated UTIs due to *E. coli* and *P. mirabilis*.	≤ 8 µg/mL	Ampicillin: 500 mg orally administered every 6 h or Amoxicillin: 250 mg orally administered every 8 h or 500 mg every 12 h	N/A	
Amoxicillin-clavulanate	≤ 8/4 µg/mL	1.2 g administered parenterally every 6 h 875/125 mg orally administered every 12 h or 500/125 mg every 8 h (only for uncomplicated UTIs or when completing therapy for systemic infection)	N/A	
Ampicillin-sulbactam	≤ 8/4 µg/mL	3 g parenterally administered every 6 h	N/A	
Azithromycin (*Salmonella enterica* ser. Typhi and *Shigella* spp.)	≤ 16 µg/mL	500 mg administered daily	N/A	
Aztreonam	≤ 4 µg/mL	1 g administered every 8 h	N/A	
Cefazolin (*E. coli*, *K. pneumoniae*, and *P. mirabilis* for infections other than uncomplicated UTIs only)	≤ 2 µg/mL	2 g administered every 8 h	N/A	
Cefazolin (*E. coli*, *K. pneumoniae*, and *P. mirabilis* for uncomplicated UTIs only)	≤ 16 µg/mL	1 g administered every 12 h	N/A	
Ceftaroline	≤ 0.5 µg/mL	600 mg administered every 12 h	N/A	

Appendix E. (Continued)

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2A. Enterobacterales (Continued)				
Cefepime	≤ 2 µg/mL	1 g administered every 12 h	4 µg/mL	1 g administered every 8 h or 2 g administered every 12 h
			8 µg/mL or zone diameter: 19–24 mm	2 g administered every 8 h (Because it is not possible to correlate specific zone diameters with specific MICs, an isolate with a zone diameter in the SDD range should be treated as if it might be an MIC of 8 µg/mL.)
Cefiderocol	≤ 4 µg/mL	2 g every 8 h administered over 3 h	N/A	
Cefotaxime	≤ 1 µg/mL	1 g administered every 8 h	N/A	
Ceftriaxone	≤ 1 µg/mL	1 g administered every 24 h	N/A	
Cefoxitin	≤ 8 µg/mL	8 g per day (eg, 2 g administered every 6 h)	N/A	
Cefuroxime	≤ 8 µg/mL	1.5 g administered every 8 h	N/A	
Ceftazidime	≤ 4 µg/mL	1 g administered every 8 h	N/A	
Ceftazidime-avibactam	≤ 8/4 µg/mL	2.5 g (2 g ceftazidime + 0.5 g avibactam) every 8 h administered over 2 h	N/A	
Ceftizoxime	≤ 1 µg/mL	1 g administered every 12 h	N/A	
Ceftolozane-tazobactam	≤ 2/4 µg/mL	3 g administered every 8 h (pneumonia) 1.5 g administered every 8 h (other indications)	N/A	
Ciprofloxacin	≤ 0.25 µg/mL	400 mg IV or 500 mg orally administered every 12 h	N/A	
Colistin or polymyxin B	≤ 2 µg/mL[a]	See International Consensus Guidelines[1] for dosage recommendations.	N/A	
Doripenem	≤ 1 µg/mL	500 mg administered every 8 h	N/A	
Ertapenem	≤ 0.5 µg/mL	1 g administered every 24 h	N/A	
Gentamicin	**≤ 2 µg/mL**	**7 mg/kg administered every 24 h**	**N/A**	
Imipenem	≤ 1 µg/mL	500 mg administered every 6 h or 1 g every 8 h	N/A	
Imipenem-relebactam (excluding family Morganellaceae)	≤ 1/4 µg/mL	1.25 g administered every 6 h	N/A	

Appendix E. (Continued)

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2A. Enterobacterales (Continued)				
Levofloxacin	≤0.5 µg/mL	750 mg administered every 24 h	N/A	
Meropenem	≤1 µg/mL	1 g administered every 8 h	N/A	
Meropenem-vaborbactam	≤4/8 µg/mL	4 g (2 g meropenem + 2 g vaborbactam) every 8 h administered over 3 h	N/A	
Piperacillin-tazobactam	≤8/4 µg/mL	3.375-4.5 g administered every 6 h as a 30-minute infusion	16/4 µg/mL	4.5 g administered every 6 h as a 3-h infusion or 4.5 g administered every 8 h as a 4-h infusion
Plazomicin (excluding family Morganellaceae)	**≤ 2 µg/mL**	**15 mg/kg administered every 24 h over 30 minutes**	**N/A**	
Tobramycin	**≤ 2 µg/mL**	**7 mg/kg administered every 24 h**	**N/A**	
Table 2B-1. *Pseudomonas aeruginosa*				
Amikacin	**≤ 16 µg/mL**	**15 mg/kg administered every 24 h**	**N/A**	
Aztreonam	≤8 µg/mL	1 g administered every 6 h or 2 g every 8 h	N/A	
Cefepime	≤8 µg/mL	1 g administered every 8 h or 2 g every 12 h	N/A	
Cefiderocol	≤4 µg/mL	2 g every 8 h administered over 3 h	N/A	
Ceftazidime	≤8 µg/mL	1 g administered every 6 h or 2 g every 8 h	N/A	
Ceftazidime-avibactam	≤8/4 µg/mL	2.5 g (2 g ceftazidime + 0.5 g avibactam) administered every 8 h over 2 h	N/A	
Ceftolozane-tazobactam	≥4/4	3 g administered every 8 h (pneumonia) 1.5 g administered every 8 h (other indications)	N/A	
Ciprofloxacin	≤0.5 µg/mL	400 mg IV administered every 8h	N/A	
Colistin or polymyxin B	≤2 µg/mL[a]	See International Consensus Guidelines[1] for dosage recommendations	N/A	
Doripenem	≤2 µg/mL	500 mg administered every 8 h	N/A	
Imipenem	≤2 µg/mL	1 g administered every 8 h or 500 mg every 6 h	N/A	
Imipenem-relebactam	≤2/4 µg/mL	1.25 g administered every 6 h	N/A	
Levofloxacin	≤1 µg/mL	750 mg administered every 24 h	N/A	
Meropenem	≤2 µg/mL	1 g administered every 8 h	N/A	
Piperacillin	≤16 µg/mL	**4** g administered every 6 h **over 30 minutes or over 3 h**	N/A	
Piperacillin-tazobactam	≤16/4 µg/mL	**4.5** g administered every 6 h **over 30 minutes or over 3 h**	N/A	
Ticarcillin-clavulanate	≤16/2 µg/mL	3 g administered every 6 h	N/A	
Tobramycin	**≤ 1 µg/mL**	**7 mg/kg administered every 24 h**	**N/A**	
Table 2B-2. *Acinetobacter* spp.				
Cefiderocol	≤4 µg/mL	2 g every 8 h administered over 3 h (for *A. baumannii* complex only)	N/A	
Colistin or polymyxin B	≤2 µg/mL[a]	See International Consensus Guidelines[1] for dosage recommendations	N/A	
Doripenem	≤2 µg/mL	500 mg administered every 8 h	N/A	
Imipenem	≤2 µg/mL	500 mg administered every 6 h	N/A	
Meropenem	≤2 µg/mL	1 g administered every 8 h or 500 mg every 6 h	N/A	

Appendix E. (Continued)

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2B-4. *Stenotrophomonas maltophilia*				
Cefiderocol	≤ 1 µg/mL	2 g every 8 h administered over 3 h	N/A	
Table 2C. *Staphylococcus* spp.				
Ceftaroline (*S. aureus* only)	≤ 1 µg/mL	600 mg administered every 12 h	2-4 µg/mL	600 mg every 8 h administered over 2 h
Dalbavancin (*S. aureus* only)	≤ 0.25 µg/mL	1500 mg (single dose) or 1000 mg (two doses) IV administered over 30 minutes followed one week later by 500 mg IV administered over 30 minutes	N/A	
Lefamulin (*S. aureus* only)	≤ 0.25 µg/mL	150 mg IV or 600 mg orally administered every 12 h	N/A	
Oritavancin (*S. aureus* only)	≤ 0.12 µg/mL	1200 mg IV administered once	N/A	
Tedizolid (*S. aureus* only)	≤ 0.5 µg/mL	200 mg administered every 24 h	N/A	
Telavancin (*S. aureus* only)	≤ 0.12 µg/mL	10 mg/kg administered every 24 h	N/A	
Table 2D. *Enterococcus* spp.				
Ampicillin (used to predict results for amoxicillin)	≤ 8 µg/mL	Ampicillin: 2 g parenterally administered every 4 -6 h or Amoxicillin: 1-2 g parenterally administered every 6 h	N/A	
Ampicillin (used to predict results for amoxicillin)	≤ 8 µg/mL	Ampicillin: 500 mg orally administered every 6 h or Amoxicillin: 250 mg orally administered every 8 h or 500 mg every 12 h	N/A	
Dalbavancin (vancomycin-susceptible *E. faecalis* only)	≤ 0.25 µg/mL	1500 mg (single dose) or 1000 mg (two doses) IV administered over 30 minutes followed one week later by 500 mg IV administered over 30 minutes	N/A	
Daptomycin (*E. faecium* only)	N/A	N/A	≤ 4 µg/mL	8-12 mg/kg administered every 24 h
Daptomycin (*Enterococcus* spp. other than *E. faecium*)	≤ 2 µg/mL	6 mg/kg administered every 24 h	N/A	
Oritavancin	≤ 0.12 µg/mL	1200 mg IV administered once	N/A	
Tedizolid	≤ 0.5 µg/mL	200 mg administered every 24 h	N/A	
Telavancin	≤ 0.25 µg/mL	10 mg/kg administered every 24 h	N/A	

Appendix E. (Continued)

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae*				
Amoxicillin-clavulanate	≤ 2/1	875/125 mg orally administered every 12 h or 500/125 mg every 8 h		
Ampicillin	≤ 1 µg/mL	2 g IV administered every 4 h (meningitis)	N/A	
Ampicillin-sulbactam	≤ 2/1 µg/mL	3 g IV administered every 6 h	N/A	
Ceftaroline (*H. influenzae* only)	≤ 0.5 µg/mL	600 mg administered every 12 h	N/A	
Ceftolozane-tazobactam (*H. influenzae* only)	≤ 0.5/4 µg/mL	3 g IV administered every 8 h	N/A	
Lefamulin (*H. influenzae* only)	≤ 2 µg/mL	150 mg IV or 600 mg orally administered every 12 h	N/A	
Table 2F. *Neisseria gonorrhoeae*				
Azithromycin	≤ 1 µg/mL	1 g single dose	N/A	
Table 2G. *Streptococcus pneumoniae*				
Amoxicillin (nonmeningitis)	≤ 2 µg/mL	500 mg administered orally every 8 h or 875 mg administered orally every 12 h	N/A	
Amoxicillin-clavulanate (nonmeningitis)	≤ 2/1 µg/mL	500 mg administered orally every 8 h or 875 mg administered orally every 12 h (based on amoxicillin dosage)	N/A	
Ceftaroline (nonmeningitis)	≤ 0.5 µg/mL	600 mg administered every 12 h	N/A	
Lefamulin	≤ 0.25 µg/mL	150 mg IV or 600 mg orally administered every 12 h	N/A	
Penicillin (nonmeningitis)	≤ 2 µg/mL	2 million units administered every 4 h (12 million units per day)	N/A	
Penicillin parenteral (meningitis)	≤ 0.06 µg/mL	3 million units administered every 4 h	N/A	

Appendix E. (Continued)

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2H-1. *Streptococcus* spp. ß-Hemolytic Group				
Ceftaroline	≤ 0.5 µg/mL	600 mg administered every 12 h	N/A	
Dalbavancin (*S. pyogenes*, *S. agalactiae*, and *S. dysgalactiae* only)	≤ 0.25 µg/mL	1500 mg (single dose) or 1000 mg (two doses) IV administered over 30 minutes followed one week later by 500 mg IV administered over 30 minutes	N/A	
Oritavancin	≤ 0.25 µg/mL	1200 mg IV administered once	N/A	
Tedizolid (*S. pyogenes* and *S. agalactiae* only)	≤ 0.5 µg/mL	200 mg administered every 24 h	N/A	
Telavancin	≤ 0.12 µg/mL	10 mg/kg administered every 24 h	N/A	
Table 2H-2. *Streptococcus* spp. Viridans Group				
Dalbavancin (*S. anginosus* group only)	≤ 0.25 µg/mL	1500 mg (single dose) or 1000 mg (two doses) IV administered over 30 minutes followed one week later by 500 mg IV administered over 30 minutes	N/A	
Oritavancin	≤ 0.25 µg/mL	1200 mg IV administered once	N/A	
Tedizolid (*S. anginosus* group only)	≤ 0.25 µg/mL	200 mg administered every 24 h	N/A	
Telavancin	≤ 0.06 µg/mL	10 mg/kg administered every 24 h	N/A	
Table 2I. *Neisseria meningitidis*				
Ampicillin	≤ 0.12 µg/mL	2 g administered every 4 h	N/A	
Table 2J. Anaerobes				
Imipenem-relebactam	≤ 4/4 µg/mL	1.25 g administered every 6 h	N/A	

Abbreviations: IV, intravenous; MIC, minimal inhibitory concentration; N/A, not applicable; SDD, susceptible-dose dependent; UTI, urinary tract infection.

Footnote

a. MIC ≤ 2 µg/mL for colistin and polymyxin B corresponds to intermediate category.

NOTE: Information in black boldface type is new or modified since the previous edition.

Reference for Appendix E

[1] Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy*. 2019;39(1):10-39.

Appendix F. Susceptible-Dose Dependent Interpretive Category

Abbreviations for Appendix F

AST	antimicrobial susceptibility testing
FDA	US Food and Drug Administration
MIC	minimal inhibitory concentration
QC	quality control
SDD	susceptible-dose dependent

Susceptible-dose dependent (SDD) is recommended instead of "intermediate" for several drug and organism combinations for which there are multiple approved or routinely used dosing options:

- Enterobacterales: cefepime, piperacillin, and piperacillin-tazobactam
- *Staphylococcus aureus*: ceftaroline
- *Enterococcus faecium*: daptomycin

SDD highlights the option of using higher doses or alternative dosing regimens by which to achieve a higher dose exposure for the treatment of infections caused by isolates when the minimal inhibitory concentration (MIC) or the zone diameter is in the SDD range.

What does SDD mean?

SDD is a category defined by a breakpoint that implies that susceptibility of an isolate depends on the dosing regimen that is used in the patient. To achieve levels that are likely to be clinically effective against isolates for which the susceptibility testing results (either MICs or zone diameters) are in the SDD category, it is necessary to use a dosing regimen (ie, higher doses, more frequent doses, or both) that results in higher drug exposure than that achieved with the dose that was used to establish the susceptible breakpoint. Consideration should be given to the maximum, literature-supported dosage regimens, because higher exposure gives the highest probability of adequate coverage of an SDD isolate. Appendix E lists the doses used when establishing SDD categories. The drug label should be consulted for recommended doses and adjustment for organ function.

NOTE: The concept of SDD has been included within the intermediate category definition for antimicrobial agents. However, this is often overlooked or not understood by clinicians and microbiologists when an intermediate result is reported. The SDD category may be assigned when doses well above those used to calculate the susceptible breakpoint are supported by the literature, widely used clinically, and/or approved and for which sufficient data to justify the designation exist and have been reviewed. When the intermediate category is used, its definition remains unchanged.

Appendix F. (Continued)

Why is SDD being used now?

- There is a growing need to refine susceptibility reporting to maximize clinicians' use of available drugs.
- Intermediate too often means "resistant" to clinicians because they do not appreciate the full definition of "intermediate."
- SDD is more specific and conveys what we know—a higher dose can be considered for isolates with MICs (or zones of inhibition) that fall in this interpretive category.
- SDD is already well established for use in antifungal susceptibility testing.
- Antibiotic stewardship programs, which emphasize dosage regimen and duration of therapy options, are increasing awareness of appropriate use of antibiotics. Personnel from these programs should be able to describe the significance to clinicians of an SDD result.

How should this change be implemented?

- Meet with the appropriate practitioners at your institution (eg, members of the antimicrobial stewardship team **and other relevant institutional stakeholders**) to explain SDD and determine a plan for implementation, if appropriate.
- Talk to the manufacturer of your antimicrobial susceptibility testing (AST) device to determine how to implement reporting SDD on your device.
  - **NOTE:** Because the US Food and Drug Administration (FDA) does not yet recognize the SDD interpretive category and commercial manufacturers must use FDA breakpoints, the manufacturer cannot adopt the CLSI SDD breakpoints. However, for most systems, you can manually change the breakpoints and implement, following a verification study.
- Work with your laboratory information system staff to report "SDD" or dose ("D") when MICs or zone diameters are in the SDD range. Some laboratory information systems may handle only a single character and use of "D" for "dose" may be appropriate. Ideally, this could be translated to SDD on the final patient report. Regardless of approach, make certain that SDD will be transmitted to the hospital information system and appropriately displayed on reports viewed by clinicians.
- Distribute user-specific educational materials to laboratory staff and clinicians receiving AST results from your laboratory. Examples of these materials can be found on the CLSI Subcommittee on Antimicrobial Susceptibility Testing webpage at www.clsi.org.

Appendix F. (Continued)

Additional Questions and Answers:

1. Q: Does CLSI recommend a comment to be reported with the new SDD breakpoints?

   A: If a laboratory chooses to report a comment explaining the SDD range, CLSI recommends the following: "The interpretive criterion for susceptible is based on a dosage regimen of [dose] (refer to Appendix E). The interpretive criterion for SDD is based on dosage regimens that result in higher antimicrobial exposure, either higher doses or more frequent doses, or both."

2. Q: Will all intermediate ranges become SDD?

   A: No, the SDD category will be implemented for drug and organism combinations only when there is sufficient evidence to suggest alternative approved dosage regimens may be appropriate for organisms that have MICs or zone diameters between the susceptible and resistant categories.

3. Q: Will SDD be applied to other antimicrobial agents?

   A: CLSI will examine the SDD category possibility for additional drug and organism combinations for which multiple dosing options exist and have been well studied.

4. Q: How do we perform a verification study before implementing the new breakpoints on our AST device?

   A: Guidelines for performance of such a verification study are available (see CLSI document M52[1]).[2]

5. Q: Does SDD apply to all patients and specimen types (eg, pediatric, geriatric, immunosuppressed)?

   A: Yes, in terms of laboratory reporting. Clinicians must decide how to use an SDD result for a specific patient while considering all other clinical and physiological parameters for that patient.

6. Q: Is any special QC needed once the SDD breakpoints are implemented?

   A: No, currently recommended routine QC is sufficient.

7. Q: Will it be necessary to report SDD on proficiency testing survey samples?

   A: Sponsors of proficiency testing surveys are aware of the difficulties encountered by laboratories in implementing newer CLSI breakpoints. It is highly unlikely that there will be a mandate to report SDD in the near future, but it would be best to check with your proficiency testing survey provider.

Appendix F. (Continued)

8. Q: If we can implement the revised breakpoints but cannot facilitate reporting of SDD, can we report “intermediate” instead of SDD?

   A: A decision related to this question should be made following consultation **with the antimicrobial stewardship team and other relevant institutional stakeholders.**

9. Q: If we can implement the revised breakpoints but cannot facilitate reporting of SDD, can we report an MIC or zone diameter without an interpretation?

   A: A zone diameter should never be reported without an interpretation because there is a high risk of misinterpretation of this value, which poses patient safety issues. There is a lesser danger of reporting an MIC without an interpretation, but this should not be done without an accompanying qualifying comment. See answer to question 8, above.

10. Q: What does the dosing information that is given with breakpoints mean?

    A: The evolving science of pharmacokinetics-pharmacodynamics has become increasingly important in recent years in determining MIC breakpoints. Recently approved susceptible or SDD breakpoints for a number of agents have been based on a specific dosage regimen(s); these dosage regimens are listed in Appendix E. Proper application of the breakpoints necessitates drug exposure at the site of infection that corresponds to or exceeds the expected systemic drug exposure, at the dose listed, in adult patients with normal renal function. This information should be shared with pharmacists, infectious diseases staff, and others making dosing recommendations for the institution.

NOTE: Information in black boldface type is new or modified since the previous edition.

References for Appendix F

[1] CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.

[2] Patel J, Sharp S, Novak-Weekley S. Verification of antimicrobial susceptibility testing methods: a practical approach. *Clin Microbiol Newslett*. 2013;35(13):103-109.

Appendix G. Epidemiological Cutoff Values

Abbreviations for Appendix G

ECV	epidemiological cutoff value
MIC	minimal inhibitory concentration
NWT	non-wild-type
WT	wild-type

G1 CLSI Epidemiological Cutoff Value Additions/Revisions Since 2015

Antimicrobial Agent	Date of Addition/Revision (M100 edition)	Comment
Anaerobes		
Vancomycin	January 2015 (M100-S25)	For use with *Cutibacterium* (formerly *Propionibacterium*) *acnes*.

G2 Defining Epidemiological Cutoff Values

G2.1 Definitions

epidemiological cutoff value (ECV) – the minimal inhibitory concentration (MIC) or zone diameter value that separates microbial populations into those with and without phenotypically detectable resistance (non-wild-type [NWT] or wild-type [WT], respectively). The ECV defines the highest MIC or smallest zone diameter for the WT population of isolates.

EXAMPLE:

Interpretive Category	ECVs	
	MIC, µg/mL	Zone Diameter, mm
Wild-type	≤ 4	≥ 20
Non-wild-type	≥ 8	≤ 19

- **wild-type (WT)** – an interpretive category defined by an ECV that describes the microbial population with no phenotypically detectable mechanisms of resistance or reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.

- **non-wild-type (NWT)** – an interpretive category defined by an ECV that describes the microbial population with phenotypically detectable mechanisms of resistance and reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.

Appendix G. (Continued)

G2.2 Epidemiological Cutoff Values vs Clinical Breakpoints

ECVs are based on *in vitro* data only, using MIC or zone diameter distributions. ECVs are not clinical breakpoints, and the clinical relevance of ECVs for a particular patient has not yet been identified or approved by CLSI or any regulatory agency. By contrast, clinical breakpoints are established using MIC distributions, pharmacokinetic/pharmacodynamic data, and clinical outcome data, when available (as described in CLSI document M23[1]).

"Caution": Zone diameter (disk diffusion) and MIC values for which ECVs are defined are not to be interpreted or reported as susceptible, intermediate, or resistant but rather as WT or NWT. The ECVs should not be used as clinical breakpoints.

G2.3 Establishing Epidemiological Cutoff Values

ECVs are determined by collecting and merging MIC distribution data obtained by testing microbes from a variety of sources and then applying statistical techniques for estimating the MIC at the upper end of the WT distribution. Subsequently, corresponding zone diameter data from disk diffusion testing are examined and a disk diffusion ECV is determined, when appropriate. To ensure reliability, ECVs are estimated while accounting for both biological (strain-to-strain) variation and MIC/disk assay variation within and between laboratories. They are based on the assumption that the WT distribution of a particular antimicrobial agent–organism combination does not vary geographically or over time.

Several conditions must be fulfilled to generate reliable ECVs. The most important are:

- An ECV can be determined only within a single species for a single agent because of the genetic diversity between species within a genus.
- All MIC values included in the dataset must have been determined using a standard reference method (eg, the CLSI MIC broth dilution method as described in M07,[2] which is also the method outlined in an international reference standard[3]). Similarly, the standard reference disk diffusion method as described in M02[4] must be used when zone diameter ECVs are defined.
- Data must be sourced from at least three separate laboratories and at least 100 unique isolates must be included in the merged dataset.

Appendix G. (Continued)

- MIC values contributed from an individual laboratory dataset should be "on scale" (ie, the MIC is not below the lowest or above the highest concentration tested), whenever possible. This is particularly important for MICs of the presumptive WT strains. Before merging data from individual laboratories, the MIC distribution from each laboratory must be inspected, and if the lowest concentration tested is also the mode, the data must be excluded.
  - Once acceptable data are merged, there are several methods that can be used to estimate the ECV.
    - Visual inspection is the simplest method and is generally acceptable for MIC distributions when there is clear separation of WT and NWT strains. When there is obvious overlap between WT and NWT strains, visual inspection is too subjective to set a reliable ECV.
    - Statistical methods are preferred because they remove potential observer bias from the estimation. The two most widely referenced statistical methods are those described by Turnidge et al.[5] and by Kronvall.[6]
  - Establishment of ECVs from MIC distributions may be supplemented with molecular tests for known resistance genes. The detection of a resistance gene per se in strains with MICs at or below the ECV does not necessarily contradict the choice of ECV, unless it can be accompanied by evidence that the gene is being expressed. In such cases, the ECV may need to be reassessed.

G2.4 Epidemiological Cutoff Value Use by the Medical Microbiology Laboratory

The need for testing and interpreting drug and organism combinations with an ECV but no clinical breakpoint must be discussed with appropriate clinical specialists (eg, antibiotic stewardship, infectious diseases, and pharmacy). While ECVs do not predict clinical outcome, laboratories may consider noting WT or NWT MIC (or zone diameter) interpretations on laboratory reports. Many physicians may choose not to consider using antimicrobial agents with an NWT interpretation, if other therapeutic options are available. However, it is critical that laboratories refrain from reporting report WT as susceptible, or NWT as resistant, as there are insufficient clinical data to support this practice. ECVs may be used to signal the emergence of resistance, although this application for ECVs is best suited to public health laboratories and surveillance studies.

Appendix G. (Continued)

References for G2

1 CLSI. *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*. 5th ed. CLSI guideline M23.Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

3 ISO. *Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases*. ISO 20776-1. International Organization for Standardization; 2019.

4 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

5 Turnidge J, Kahlmeter G, Kronvall G. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. *Clin Microbiol Infect*. 2006;12(5):418-425.

6 Kronvall G. Normalized resistance interpretation as a tool for establishing epidemiological MIC susceptibility breakpoints. *J Clin Microbiol*. 2010;48(12):4445-4452.

G3 Epidemiological Cutoff Value Tables

"Caution": Zone diameter (disk diffusion) and MIC values for which ECVs are defined are not to be interpreted or reported as susceptible, intermediate, or resistant but rather as WT or NWT. The ECVs should not be used as clinical breakpoints.

ECVs listed in Table G1 are applicable only to the species indicated. Currently, there are insufficient data to support their use with other species.

Table G1. ECVs for Specific Anaerobic Species

Antimicrobial Agent	MIC ECV, µg/mL		Comment
	WT	NWT	
Vancomycin	≤ 2	≥ 4	For use with *Cutibacterium* (formerly *Propionibacterium*) *acnes*[1-4] and *Clostridioides* (formerly *Clostridium*) *difficile*.[5-7]

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type.

Appendix G. (Continued)

References for Table G1

1 Citron DM, Kwok YY, Appleman MD. In vitro activity of oritavancin (LY333328), vancomycin, clindamycin, and metronidazole against *Clostridium perfringens, Propionibacterium acnes,* and anaerobic gram-positive cocci. *Anaerobe*. 2005;11(1-2):93-95.

2 Goldstein EJ, Citron DM, Merriam CV, Warren YA, Tyrrell KL, Fernandez HT. *In vitro* activities of the new semisynthetic glycopeptide telavancin (TD-6424), vancomycin, daptomycin, linezolid, and four comparator agents against anaerobic gram-positive species and *Corynebacterium* spp. *Antimicrob Agents Chemother*. 2004;48(6):2149-2152.

3 Oprica C, Nord CE; ESCMID Study Group on Antimicrobial Resistance in Anaerobic Bacteria. European surveillance study on the antibiotic susceptibility of *Propionibacterium acnes*. *Clin Microbiol Infect*. 2005;11(3):204-213.

4 Tyrrell KL, Citron DM, Warren YA, Fernandez HT, Merriam CV, Goldstein EJ. In vitro activities of daptomycin, vancomycin, and penicillin against *Clostridium difficile, C. perfringens, Finegoldia magna,* and *Propionibacterium acnes*. *Antimicrob Agents Chemother*. 2006;50(8):2728-2731.

5 Snydman DR, McDermott LA, Jacobus NV, et al. U.S.-based National Sentinel Surveillance Study for the epidemiology of *Clostridium difficile*-associated diarrheal isolates and their susceptibility to fidaxomicin. *Antimicrob Agents Chemother*. 2015;59(10):6437-6443.

6 Goldstein EJ, Citron DM, Tyrrell KL, Merriam CV. Comparative in vitro activities of SMT19969, a new antimicrobial agent, against *Clostridium difficile* and 350 gram-positive and gram-negative aerobic and anaerobic intestinal flora isolates. *Antimicrob Agents Chemother*. 2013;57(10):4872-4876.

7 Goldstein EJ, Babakhani F, Citron DM. Antimicrobial activities of fidaxomicin. *Clin Infect Dis*. 2012;55(suppl 2):S143-S148.

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Appendix H. Using Molecular Assays for Resistance Detection

Abbreviations for Appendix H

AST	antimicrobial susceptibility testing
ESBL	extended-spectrum ß-lactamase
MIC	minimal inhibitory concentration
MRSA	methicillin (oxacillin)-resistant *Staphylococcus aureus*
N/A	not applicable
PBP2a	penicillin-binding protein 2a
VRE	vancomycin-resistant enterococci

Antimicrobial resistance and susceptibility are complex, and current *in vitro* methods have been developed to predict a microorganism's response to antibacterial therapy *in vivo*. Standardized phenotypic methods have evolved over many decades, but faster and potentially more reliable nucleic acid– and protein-based methods have been recently developed to detect antimicrobial resistance. The current challenge for medical laboratories is to integrate molecular assays for antimicrobial resistance determinants with conventional antimicrobial susceptibility testing (AST) procedures, sometimes despite an incomplete understanding of test limitations.

The tables in this appendix provide a practical approach for testing and reporting results among medical laboratories that routinely use molecular techniques (with or without a phenotypic test) for detecting antimicrobial resistance. Antimicrobial resistance is genetically complex and based on available data. Molecular methods are often used as a screening tool (eg, methicillin [oxacillin]-resistant *Staphylococcus aureus* [MRSA] from nasal swabs) or as a rapid adjunct to traditional phenotypic methods (eg, KPC from instrument-flagged blood culture bottles). Interpretation necessitates critical thinking and an understanding of the dynamics between detecting "resistance" determinants and testing phenotypic "susceptibility." Detecting a resistance marker does not necessarily predict therapeutic failure of antimicrobial agents. The gene may be nonfunctional or expressed at clinically insignificant levels. Conversely, the absence of the genetic marker does not necessarily indicate susceptibility, because technical issues may interfere with detection (eg, inhibition of amplification, emergence of genetic variants). In some cases, a molecular approach may be superior to traditional phenotypic methods, such as in the case of low *in vitro* expression, heteroresistance, or poor growth masking higher minimal inhibitory concentrations (MICs). Overall, laboratorians should attempt to apply a consistent approach to molecular-based methods and aim to resolve discordant results with repeat or supplementary testing, by referral to a reference laboratory or by reporting both results in accordance with institutional policies.

As understanding of the molecular mechanisms of antimicrobial resistance continues to develop, more sophisticated approaches to molecular detection of antimicrobial resistance in the medical microbiology laboratory will undoubtedly emerge. The following tables will be updated as needed to ensure the provision of relevant guidance as methods evolve.

Appendix H. (Continued)

Table H1. Strategies for Reporting Methicillin (Oxacillin) Results When Using Molecular and Phenotypic AST Methods for *S. aureus*

Indication	Targets	Methods	Specimen Types	Results		Suggestions for Resolution	Consider reporting as[a]:	Comments[b]
				Genotype or Predicted Phenotype	Observed Colony Phenotype (if tested)			
Detecting methicillin (oxacillin) resistance in *S. aureus*	PBP2a	Latex agglutination, immuno-chromatography	Colony	PBP2a positive	Cefoxitin R	N/A	Methicillin (oxacillin) R	1
				PBP2a negative	Cefoxitin S	N/A	Methicillin (oxacillin) S	1
				PBP2a positive	Cefoxitin S	Confirm isolate identification, repeat latex agglutination and AST, and consider *mecA* colony NAAT, if available.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	1-2
				PBP2a negative	Cefoxitin R	Confirm isolate identification, repeat latex agglutination and AST, and consider *mecA* colony NAAT, if available.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	1
	mecA	NAAT, microarray hybridization, ISH	Colony, blood culture broth, surveillance specimen	*mecA* detected	Cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] R) and consider reporting molecular result per institutional protocol.	3-6
				mecA not detected	Cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] S) and consider reporting molecular result per institutional protocol.	3-6
				mecA detected	Cefoxitin S	Confirm isolate identification, repeat AST, and repeat or perform *mecA* colony NAAT, if available. If mixed specimen, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	2-5, 8-9
				mecA not detected	Cefoxitin R	Confirm isolate identification, repeat AST, and repeat or perform *mecA* colony NAAT, if available. If mixed specimen, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	3, 7

Appendix H. (Continued)

Table H1. (Continued)

Indication	Targets	Methods	Specimen Types	Results		Suggestions for Resolution	Consider reporting as[a]:	Comments[b]
				Genotype or Predicted Phenotype	Observed Colony Phenotype (if tested)			
Detecting methicillin (oxacillin) resistance in *S. aureus* (Continued)	SCC*mec*-*orfX* functional regions only	NAAT	Blood culture broth, surveillance specimen	SCC*mec* detected	Cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] R) and consider reporting molecular result per institutional protocol.	3-6
				SCC*mec* not detected	Cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] S) and consider reporting molecular result per institutional protocol.	3-6
				SCC*mec* detected	Cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	2, 10
				SCC*mec* not detected	Cefoxitin R	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	7, 11

Appendix H. (Continued)

Table H1. (Continued)

Indication	Targets	Methods	Specimen Types	Results: Genotype or Predicted Phenotype	Results: Observed Colony Phenotype (if tested)	Suggestions for Resolution	Consider reporting as[a]:	Comments[b]
Detection of methicillin (oxacillin) resistance in *S. aureus* (Continued)	SCC*mec*-*orfX* junctional regions and *mecA* and/or other targets	NAAT	Blood culture broth, surveillance specimen	SCC*mec* AND *mecA* or other target detected	Cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] R) and consider reporting molecular result per institutional protocol.	3-6
				SCC*mec* AND *mecA* or other target not detected	Cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] S) and consider reporting molecular result per institutional protocol.	3-6
				SCC*mec* AND *mecA* or other target detected	Cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	2
				SCC*mec* AND *mecA* or other target not detected	Cefoxitin R	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	3, 11

Abbreviations: AST, antimicrobial susceptibility testing; ISH, *in situ* hybridization; MSSA, methicillin (oxacillin)-susceptible *Staphylococcus aureus*; MRSA, methicillin (oxacillin)-resistant *S. aureus*; N/A, not applicable; NAAT, nucleic acid amplification test; PBP2a, penicillin-binding protein 2a; PCR, polymerase chain reaction; R, resistant; S, susceptible.

Appendix H. (Continued)

Table H1. (Continued)

Comments

(1) False-positive and false-negative PBP2a latex bead agglutination results have been observed.[1]

(2) Rare *mecA*-positive *S. aureus* isolates will test susceptible to cefoxitin.[2,3]

(3) *mecC* or *mecA* variant gene-mediated methicillin (oxacillin) resistance may not be detected by *mecA* PCR.[4,5]

(4) The simultaneous presence of *mecA*-positive *Staphylococcus* spp. (other than *S. aureus*) and MSSA may result in false-positive MRSA molecular results.[6,7]

(5) Strains harboring unstable SCC*mec* insertions may lose *mecA* during culture.[8]

(6) Compared with culture, the sensitivity of molecular methods may be higher, while the specificity may be lower.

(7) Occasional false-negative *mecA* results have been reported for direct blood culture molecular assays.[9]

(8) For ISH assays with a cefoxitin induction step, false-positive *mecA* results should be rare.[10]

(9) In polymicrobial cultures, the presence of *mecA* cannot be attributed to a specific isolate.

(10) Strains harboring an SCC*mec* remnant lacking the *mecA* gene (*mecA* dropout) or mutant *mecA* allele may test positive in assays that target only SCC*mec-orfX* junctional regions. Laboratories using molecular tests that detect only SCC*mec-orfX* junctional region targets may consider adding a disclaimer to the report stating the proportion of false-positive results related to *mecA* dropouts observed in isolates from the patient population served.[11]

(11) Multiple SCC*mec* types exist; depending on the design of the assay, some SCC*mec* variants may not be detected.[12]

Footnotes

a. Isolates that test as methicillin resistant are also oxacillin resistant, and the term “methicillin R” is synonymous with “oxacillin R.”

b. In addition to the specific possibilities listed in the comments, genotype and/or phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes or mutations, and/or wild-type reversions of resistance targets.

Appendix H. (Continued)

Table H1. (Continued)

References for Table H1

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[3] Swenson JM, Tenover FC; Cefoxitin Disk Study Group. Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. *J Clin Microbiol*. 2005;43(8):3818-3823.

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[7] Shore AC, Rossney AS, O'Connell B, et al. Detection of staphylococcal cassette chromosome *mec*-associated DNA segments in multiresistant methicillin-susceptible *Staphylococcus aureus* (MSSA) and identification of *Staphylococcus epidermidis* ccrAB4 in both methicillin-resistant *S. aureus* and MSSA. *Antimicrob Agents Chemother*. 2008;52(12):4407-4419.

[8] Wong H, Louie L, Lo RY, Simor AE. Characterization of *Staphylococcus aureus* isolates with a partial or complete absence of staphylococcal cassette chromosome elements. *J Clin Microbiol*. 2010;48(10):3525-3531.

[9] Beal SG, Ciurca J, Smith G, et al. Evaluation of the nanosphere verigene gram-positive blood culture assay with the VersaTREK blood culture system and assessment of possible impact on selected patients. *J Clin Microbiol*. 2013;51(12):3988-3992.

[10] Salimnia H, Fairfax MR, Lephart P, et al. An international, prospective, multicenter evaluation of the combination of AdvanDx *Staphylococcus* QuickFISH BC with mecA XpressFISH for detection of methicillin-resistant *Staphylococcus aureus* isolates from positive blood cultures. *J Clin Microbiol*. 2014;52(11):3928-3932.

[11] Stamper PD, Louie L, Wong H, Simor AE, Farley JE, Carrol KC. Genotypic and phenotypic characterization of methicillin-susceptible *Staphylococcus aureus* isolates misidentified as methicillin-resistant *Staphylococcus aureus* by the BD GeneOhm MRSA assay. *J Clin Microbiol*. 2011(4):1240-1244.

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Appendix H. (Continued)

Table H2. Strategies for Reporting Vancomycin Results When Using Molecular and Phenotypic Antimicrobial Susceptibility Testing Methods for *Enterococcus* spp.

Indication	Targets	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Genotype or Predicted Phenotype	Observed Phenotype (if tested)			
Detection of vancomycin-resistant enterococci	*vanA* *vanB*	NAAT or array hybridization technology	Blood culture broth or surveillance cultures	*vanA* and/or *vanB* detected	Vancomycin R	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	1-3
				vanA and/or *vanB* not detected	Vancomycin S	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	
				vanA and/or *vanB* detected	Vancomycin S	Confirm isolate identification to species level (eg, *Enterococcus faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R.	1-3
				vanA and/or *vanB* not detected	Vancomycin R	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R.	4

Appendix H. (Continued)

Table H2. (Continued)

Indication	Targets	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Genotype or Predicted Phenotype	Observed Phenotype (if tested)			
Detection of vancomycin-resistant enterococci (Continued)	*vanA*	NAAT	Surveillance cultures	*vanA* detected	Vancomycin R	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	1-2
				vanA not detected	Vancomycin S	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	5
				vanA detected	Vancomycin S	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If the discrepancy is not resolved by suggested testing, report as vancomycin R.	1-2
				vanA not detected	Vancomycin R	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If the discrepancy is not resolved by suggested testing, report as vancomycin R.	4-5

Abbreviations: AST, antimicrobial susceptibility testing; N/A, not applicable; NAAT, nucleic acid amplification test; R, resistance; S, susceptible; VRE, vancomycin-resistant enterococci.

Comments

(1) *vanA* may be present in nonenterococcal species.[1]

(2) Vancomycin-variable *Enterococcus faecium* isolates were recently revealed in Canada. They carry wild-type *vanA* but initially test as vancomycin susceptible with a culture-based method. They can convert to a resistant phenotype during vancomycin treatment.[2,3]

(3) The *vanB* gene has been found in several commensal nonenterococcal bacteria, which may lead to misclassification of vancomycin-susceptible enterococci as resistant in surveillance cultures containing mixed bacterial species.[4]

Appendix H. (Continued)

Table H2. (Continued)

(4) Constitutive low-level vancomycin resistance can be detected phenotypically (2–32 µg/mL) from the presence of *vanC*, an intrinsic resistance characteristic of *Enterococcus gallinarum (vanC1*) and *Enterococcus casseliflavus* (*vanC2–C4*).[5]

(5) Targeting *vanA* only may miss regional *vanB*-carrying VRE.[6]

Footnote

a. In addition to the specific possibilities referenced in the comments, genotype and/or phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

References for Table H2

1 Patel R. Enterococcal-type glycopeptide resistance genes in non-enterococcal organisms. *FEMS Microbiol Lett*. 2000;185(1):1-7.

2 Gagnon S, Lévesque S, Lefebvre B, Bourgault AM, Labbé AC, Roger M. vanA-containing *Enterococcus faecium* susceptible to vancomycin and teicoplanin because of major nucleotide deletions in Tn1546. *J Antimicrob Chemother*. 2011;66(12):2758–2762.

3 Thaker MN, Kalan L, Waglechner N, et al. Vancomycin-variable enterococci can give rise to constitutive resistance during antibiotic therapy. *Antimicrob Agents Chemother*. 2015;59(3):1405-1410.

4 Ballard SA, Grabsch EA, Johnson PD, Grayson ML. Comparison of three PCR primer sets for identification of *vanB* gene carriage in feces and correlation with carriage of vancomycin-resistant enterococci: interference by *vanB*-containing anaerobic bacilli. *Antimicrob Agents Chemother*. 2005;49(1):77-81.

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Appendix H. (Continued)

Table H3. Reporting Results From Extended-Spectrum ß-Lactamase Resistance and Carbapenemase Molecular Tests for Enterobacterales

Indication	Targets	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Molecular Target Results	Observed Phenotype (if tested)			
Detection of ESBL resistance in Enterobacterales (in an isolate susceptible to all carbapenems)	ESBL type CTX-M, SHV, TEM	NAAT, microarray	Colony, blood culture	Detection of any ESBL target	R to all 3rd- and 4th-generation cephalosporins tested (eg, ceftriaxone R, cefotaxime R, ceftazidime R, cefepime R)	N/A	Report phenotypic results as found (if available); consider reporting presence of molecular target per institutional protocol.	1-12
				Detection of any ESBL target	S to all 3rd- and 4th-generation cephalosporins tested (eg, ceftriaxone S, cefotaxime S, ceftazidime S, cefepime S)	Repeat molecular and phenotypic tests. If blood culture, check for mixed culture. If mixed, test isolates individually and report phenotypic results as found.	If the discrepancy is not resolved, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported.	1-12
				Detection of *CTX-M* ESBL target	Variable resistance to 3rd- and 4th-generation cephalosporins (eg, ceftriaxone R, cefotaxime R, ceftazidime R or S, cefepime R or S)	Expected phenotype for some *CTX-M* strains. Check cefepime using a reference method if S.	Report phenotypic results as found, including reference cefepime result; consider reporting presence of molecular target per institutional protocol.	1-12
				Detection of *TEM* or *SHV* ESBL target	Variable resistance to 3rd- and 4th-generation cephalosporins (eg, ceftriaxone R or S, cefotaxime R or S, ceftazidime R or S, cefepime R or S).	Expected phenotype for some *TEM*/*SHV* strains. Check cefepime using a reference method if S.	Report phenotypic results as found, including reference cefepime result; consider reporting presence of molecular target per institutional protocol.	1-12

Appendix H. (Continued)

Table H3. (Continued)

Indication	Targets	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Molecular Target Results	Observed Phenotype (if tested)			
Detection of ESBL resistance in Enterobacterales (in an isolate susceptible to all carbapenems) (Continued)				No detection of ESBL targets	Resistance to 3rd-generation cephalosporins and variable resistance to 4th-generation cephalosporins (eg, ceftriaxone R, cefotaxime R, ceftazidime R, cefepime R or S)	Likely non-tested broad spectrum ß-lactamase (eg, AmpC, carbapenemase, or other ESBL); consider repeating molecular tests and checking cefepime using reference method if S.	Report phenotypic results as found, including reference cefepime result if tested.	1-12
Detection of carbapenem resistance in Enterobacterales	KPC, OXA-48-like, VIM, NDM, or IMP Or Phenotypic evidence of a carbapenemase (eg, mCIM or CarbaNP positive)	NAAT, microarray	Colony, blood culture	Detection of any tested carbapenemase target	Resistance to all carbapenems (eg, meropenem R, imipenem R, doripenem R, ertapenem R)	N/A	Report phenotypic results as found (if available); consider reporting presence of molecular target per institutional protocol.	1-4, 12-14
				Detection of any tested carbapenemase target	Susceptible to all carbapenems except ertapenem (variable) (eg, meropenem S, imipenem S, doripenem S, ertapenem R or S)	Repeat molecular and phenotypic tests. If blood culture, check for mixed culture. If mixed, test isolates individually and report phenotypic results as found; consider a phenotypic test for carbapenemase activity (such as CarbaNP or mCIM).	If the discrepancy is not resolved, repeat AST should be performed using a reference method and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the S range will be effective, or whether the molecular assays are completely accurate.	1-4, 12-15

Appendix H. (Continued)

Table H3. (Continued)

Indication	Targets	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Molecular Target Results	Observed Phenotype (if tested)			
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP Or Phenotypic evidence of a carbapenemase (eg, mCIM or CarbaNP positive)	NAAT, microarray	Colony, blood culture	Detection of any tested carbapenemase target or phenotypic detection of carbapenemase production	Susceptibility (S or SDD) to 3rd- and/or 4th-generation cephalosporins but intermediate or resistant to at least one carbapenem tested	Repeat molecular and phenotypic tests.	If the discrepancy is not resolved, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution: "Current clinical and laboratory evidence is insufficient to conclude whether cephalosporin therapy of carbapenemase-carrying strains with an MIC in the S/SDD range will be effective."	1-4, 12-14

Appendix H. (Continued)

Table H3. (Continued)

Indication	Targets	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Molecular Target Results	Observed Phenotype (if tested)			
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP Or Phenotypic evidence of a carbapenemase (eg, mCIM or CarbaNP positive)	NAAT, microarray	Colony, blood culture	No detection of tested carbapenemase targets	Susceptible to all carbapenems except ertapenem (eg, meropenem S, imipenem S, doripenem S, ertapenem R)	Likely ESBL/AmpC and porin alteration, especially for *Enterobacter* spp.; consider a phenotypic test for carbapenemase activity (eg, CarbaNP or mCIM); carbapenemase unlikely if negative, although rare carbapenemases (eg, GES-types, are still possible).	If carbapenemase activity is detected, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the susceptible range will be effective or whether the molecular assays are completely accurate. Otherwise report phenotypic results as found.	1-4, 12-15

Appendix H. (Continued)

Table H3. (Continued)

Indication	Targets	Methosd	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Molecular Target Results	Observed Phenotype (if tested)			
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP Or Phenotypic evidence of a carbapenemase (eg, mCIM or CarbaNP positive)	NAAT, microarray	Colony, blood culture	No detection of tested carbapenemase targets	Resistance to any carbapenems except ertapenem (eg, meropenem R, imipenem R, doripenem R, ertapenem R or S)	Possible other carbapenemase. If blood culture, check for mixed culture. If mixed, test isolates individually and report as found; consider repeating molecular and AST and performing a phenotypic test for carbapenemase activity (eg, CarbaNP or mCIM).	If carbapenemase activity is detected, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the S range will be effective or whether the molecular assays are completely accurate. Otherwise report phenotypic results as found.	1-4, 12-16

Abbreviations: AST, antimicrobial susceptibility testing; ESBL, extended-spectrum β-lactamase; mCIM, modified carbapenem inactivation method; MIC, minimal inhibitory concentration; N/A, not applicable; NAAT, nucleic acid amplification test; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

Comments

(1) Multiple β-lactamases may be carried by individual bacterial isolates. Most carbapenemase-producing bacteria are resistant to 3rd- and 4th-generation cephalosporins, although bacteria producing some carbapenemase enzymes (eg, OXA-48 and SME) may not test resistant unless they co-produce an ESBL or AmpC β-lactamase.

(2) Molecular assays can detect the presence of specific β-lactamase genes but cannot exclude the presence of other β-lactamase genes or resistance mechanisms, or novel variants with changes in primer or probe annealing sites. Therefore, phenotypic resistance should always be reported.

Appendix H. (Continued)

Table H3. (Continued)

(3) Isolates with phenotypic susceptibility despite the presence of a resistance determinant may indicate the potential for resistance to emerge during therapy.

(4) These are provisional guidelines based on general principles; however, the performance characteristics of many individual research use–only assays are presently unknown.

(5) Susceptibility of TEM/SHV-carrying strains to ß-lactam combinations is variable.

(6) Susceptibility of ESBL-carrying strains to cefepime is variable.

(7) Susceptibility of ESBL-carrying strains to ß-lactam combination agents is variable.

(8) Some strains carrying CTX-M ESBLs remain susceptible to ceftazidime.

(9) Some strains carrying TEM/SHV-derived ESBLs remain susceptible to cefotaxime and ceftriaxone.

(10) Some molecular assays for AmpC may not reliably distinguish between chromosomal and plasmid-encoded genes in some bacterial species.

(11) Most strains with derepressed AmpC expression remain susceptible to cefepime.

(12) These recommendations are based on cephalosporin and carbapenem breakpoints in M100.

(13) The susceptibility to other carbapenems of ertapenem-resistant strains with ESBL or AmpC enzymes and reduced porin expression that do not contain carbapenemase genes or express carbapenemase activity may be reported as measured in phenotypic susceptibility assays.

(14) Rapid tests for carbapenemase activity (eg, CarbaNP) may not detect OXA-48-like and some other carbapenemases.

(15) Caution is advised. Current clinical evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the susceptible range will be effective.

(16) Some isolates of Enterobacterales, in particular but not exclusively *Morganella* spp., *Proteus* spp., and *Providencia* spp., may exhibit intrinsic low-level resistance to imipenem on a non–carbapenemase-mediated basis.

Footnote

a. In addition to the specific possibilities listed in the comments, genotype and/or phenotype discrepancies could arise as a consequence of mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

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Appendix I. Cefiderocol Broth Preparation and Reading Broth Microdilution Minimal Inhibitory Concentration End Points

Abbreviations for Appendix I

CAMHB cation-adjusted Mueller-Hinton broth
ID-CAMHB iron-depleted cation-adjusted Mueller-Hinton broth
pH negative logarithm of hydrogen ion concentration

I1 Supplements

I1.1 Calcium and Magnesium Stock Solutions

Refer to M07[1] for cation stock solution preparation.

I1.2 Zinc Stock Solution

The steps for preparing zinc stock solution are listed below.

Step	Action	Comments
1	Dissolve 0.29 g $ZnSO_4 \cdot 7H_2O$ in 100 mL deionized water.	This solution contains 0.65 mg Zn^{++}/mL (10 mmol Zn^{++}/mL). Verify that the deionized water has an iron content of ≤ 0.03 mg/L.
2	Sterilize the solution by membrane filtration.	
3	Store the solution at 15 to 25°C in a sterile single-use plastic container.	Previously used glass containers should be avoided to prevent inadvertent iron contamination.

Appendix I. (Continued)

I2 Iron-depleted Cation-adjusted Mueller-Hinton Broth[a]

The steps for preparing iron-depleted cation-adjusted Mueller-Hinton broth (ID-CAMHB) are listed below.[2]

Step	Action	Comments
1	Prepare the CAMHB.	Follow manufacturer's instructions.
2	Autoclave the media and let cool to room temperature.	
3	Add 100 g chelating resin to 1 L autoclaved CAMHB.[2]	Removes polyvalent metal cations in the medium- to low-level concentrations (range, 0–0.18 mg/L).[2]
4	Stir the solution at room temperature for approximately **6** hours using a magnetic stir bar.	
5	Filter the solution using a 0.2-µm filter.	Removes the resin. It is recommended that testing for residual iron levels of the filtrate should be conducted at this step to confirm that the iron content does not exceed 0.03 mg/L. Residual iron content can be measured with a commercial iron detection kit capable of detecting low levels of iron (0.02 mg/L). If iron levels exceed 0.03 mg/L, restart the procedure at the chelation step 3 above.
6	Check the pH to determine whether it is 7.3 ± 0.1.	If the pH is above 7.4, adjust it using 1 or 6 N HCl (use of 6 N HCl will minimize the volume required to adjust the pH). If the pH is below 7.2, use 2.5 N NaOH.
7	Add the cation to achieve final concentrations in the following ranges: • Ca^{++} 20–25 mg/L • Mg^{++} 10–12.5 mg/L • Zn^{++} 0.5–1.0 mg/L	The final concentration of iron in ID-CAMHB prepared using this method should be ≤ 0.03 mg/L. Refer to M07[1] for calculating the amount of Ca^{++}, Mg^{++}, and the table below for calculating the amount of Zn^{++} needed.

Appendix I. (Continued)

I2 ID-CAMBH (Continued)

Step	Action	Comment
8	Check the pH to determine whether it is 7.3 ± 0.1.	If the pH exceeds 7.4, adjust it using 1 or 6 N HCl (use of 6 N HCl will minimize the volume required to adjust the pH). If the pH is below 7.2, use 2.5 N NaOH.
9	Filter the final product using a 0.2-µm filter.	
10	Store the media at 4 to 8°C for up to 2 months.	

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; ID-CAMHB, iron-depleted cation-adjusted Mueller-Hinton broth.

Example for adding Zn^{++} back to CAMHB that contains below-detectable concentrations (< 0.0001 mg/L) of Zn^{++} after chelation in step 3[2]:

Step	Action	Comments
1	Calculate the amount of Zn^{++} needed using this formula: Final amount needed − amount in medium = amount to be added	For Zn^{++}, the final amount needed is 0.5–1 mg/L. 1 mg/L − 0 mg/L = 1 mg/L
2	Add 1.54 mL Zn^{++} stock per L (1.54 mL for each 1 mg/L).	C = concentration, V = volume $C_1 \bullet V_1$ = desired $C_2 \bullet$ final V_2 0.65 mg/mL $Zn^{++} \bullet$ V1 = 1 mg Zn^{++} /1000 mL • 1000 mL V_1 = 1 mg ÷ 0.65 mg/mL V_1= 1.54 mL of Zn^{++} stock
3	Proceed with steps 8 and 9 above.	

Appendix I. (Continued)

I3 Determining Broth Microdilution End Points

The steps for reading and interpreting broth microdilution end points for cefiderocol when tested with ID-CAMHB are listed below.

Step	Action	Comments
1	Read the MIC as the lowest concentration of antimicrobial agent that completely inhibits organism growth in the tubes or microdilution wells as detected by the unaided eye.	See step 2 for exceptions. Viewing devices intended to facilitate reading microdilution tests and recording results may be used as long as there is no compromise in the ability to discern growth in the wells.
2	Compare the amount of growth in the wells containing the cefiderocol with the amount of growth in the growth-control well containing ID-CAMHB (no antimicrobial agent).	For a test to be considered valid, acceptable growth (definite turbidity or button) must occur in the growth-control well (see Figure I1). Trailing may occur in some organisms (eg, *Acinetobacter* spp.) and should be ignored when a tiny button or light or faint turbidity relative to the growth control may be observed. Read the MIC as the first well in which growth is significantly reduced (see Figure I2).
3	Interpret the results.	Refer to the appropriate portion of Tables 2 for breakpoints.

Abbreviations: ID-CAMHB, iron-depleted cation-adjusted Mueller-Hinton broth; MIC, minimal inhibitory concentration.

NOTE: Information in black boldface type is new or modified since the previous edition.

Footnote

a. Ensure all reagents (eg, deionized water to prepare acid and base and cation solutions) have been verified as having an iron content of ≤ 0.03 mg/L.

Appendix I. (Continued)

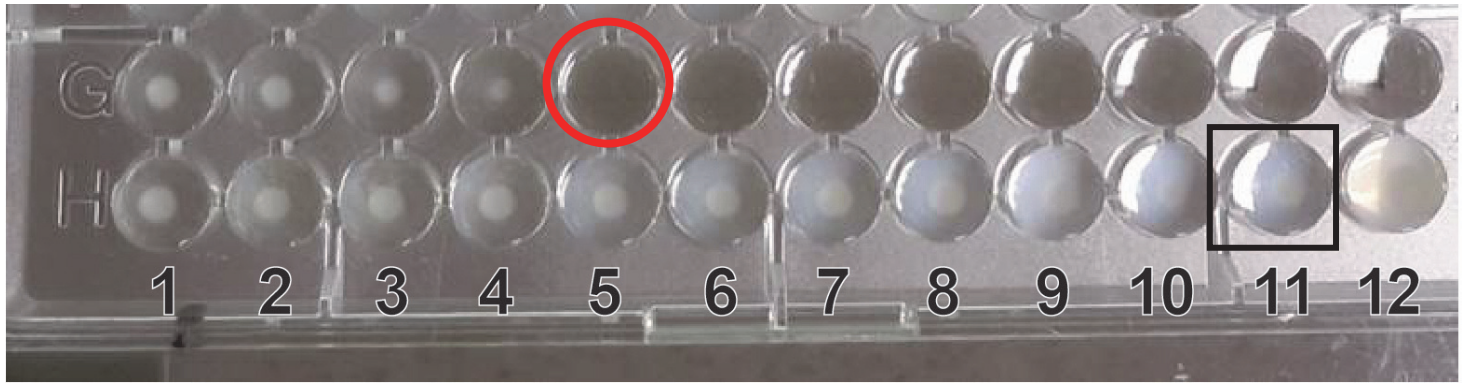

Figure I1. Cefiderocol Test With a Clear End Point. The cefiderocol concentrations in wells G1 to G12 are 0.03 to 64 µg/mL. Row G shows the cefiderocol MIC at 0.5 µg/mL in well G5 (red circle). The growth-control well is H11 (black box).

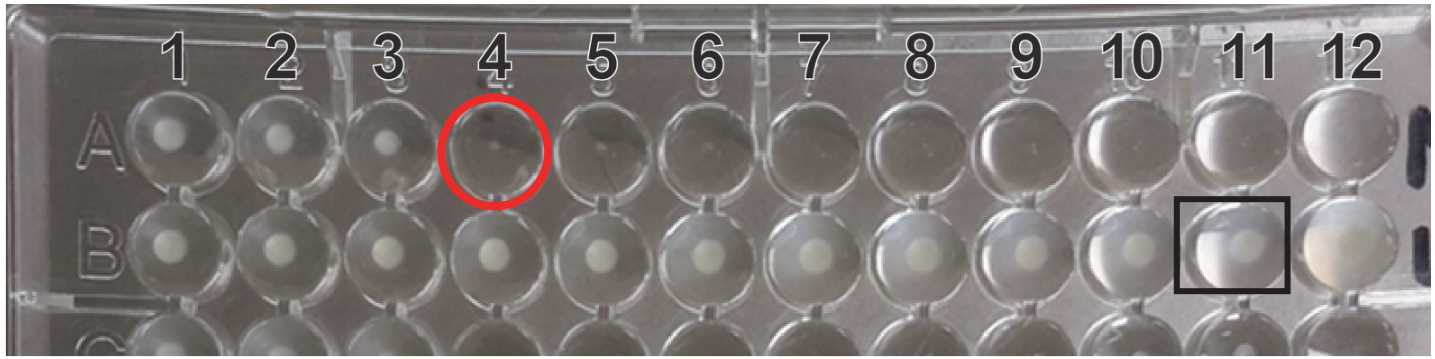

Figure I2. Cefiderocol Test With a Trailing End Point. The cefiderocol concentrations in wells A1 to A12 are 0.03 to 64 µg/mL. Row A shows the cefiderocol MIC at 0.25 µg/mL in well A4 (red circle). The growth control well is B11 (black box).

References for Appendix I

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

2 Hackel, MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis*. 2019;94(4):321-325.

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Glossary I (Part 1). ß-Lactams: Class and Subclass Designations and Generic Names

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and some agents are no longer available for human use.

Antimicrobial Class	Antimicrobial Subclasses		Agents Included; Generic Names
Penicillins	Penicillinase-labile penicillins[a]	Penicillin	Penicillin
		Aminopenicillins	Amoxicillin Ampicillin
		Carboxypenicillins	Carbenicillin Ticarcillin
		Ureidopenicillins	Azlocillin Piperacillin
	Penicillinase-stable penicillins[b]		Cloxacillin Dicloxacillin Nafcillin Oxacillin
	Amdinocillin		Mecillinam
ß-lactam combination agents			Amoxicillin-clavulanate Ampicillin-sulbactam Aztreonam-avibactam Aztreonam-nacubactam (1:1) Cefepime-enmetazobactam (4:1) Cefepime-nacubactam (1:1) Cefepime-taniborbactam Cefepime-tazobactam (1:1) Cefepime-zidebactam Ceftaroline-avibactam Ceftazidime-avibactam **Ceftibuten-avibactam** **Ceftibuten-ledaborbactam** Ceftolozane-tazobactam Imipenem-relebactam Meropenem-nacubactam (1:1) Meropenem-vaborbactam **Meropenem-xeruborbactam** Piperacillin-tazobactam Sulbactam-durlobactam Ticarcillin-clavulanate

Glossary I (Part 1). (Continued)

Antimicrobial Class	Antimicrobial Subclasses	Agents Included; Generic Names
Cephems (parenteral)	Cephalosporins I[c]	Cefazolin Cephalothin Cephapirin Cephradine
	Cephalosporins II[c]	Cefamandole Cefonicid Cefuroxime (parenteral)
	Cephalosporins III[c]	Cefoperazone Cefotaxime Ceftazidime Ceftizoxime Cetriaxone
	Cephalosporins IV[c]	Cefepime Cefpirome
	Cephalosporins with anti-MRSA activity	Ceftaroline Ceftobiprole
	Cephamycins	Cefmetazole Cefotetan Cefoxitin
	Oxacephem	Moxalactam
	Siderophore cephalosporin	Cefiderocol
Cephems (oral)	Cephalosporins	Cefaclor Cefadroxil Cefdinir Cefditoren Cefetamet Cefixime Cefpodoxime Cefprozil Ceftibuten Cefuroxime (oral) Cephalexin Cephradine
	Carbacephem	Loracarbef
Monobactams		Aztreonam
Penems	Carbapenems	Biapenem Doripenem Ertapenem Imipenem Meropenem Razupenem Tebipenem
	Penems	Faropenem Sulopenem

Abbreviations: MRSA, methicillin (oxacillin)-resistant *Staphylococcus aureus;* FDA, US Food and Drug Administration.

Glossary I (Part 1). (Continued)

Footnotes

a. Hydrolyzed by staphylococcal penicillinase.

b. Not hydrolyzed by staphylococcal penicillinase.

c. Cephalosporins I, II, III, and IV are sometimes referred to as first-, second-, third-, and fourth-generation cephalosporins, respectively. Cephalosporins III and IV are also referred to as “extended-spectrum cephalosporins.” This does not imply activity against extended-spectrum ß-lactamase–producing gram-negative bacteria.

NOTE: Information in black boldface type is new or modified since the previous edition.

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Glossary I (Part 2). Non-β-Lactams: Class and Subclass Designations and Generic Names

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and some agents are no longer available for human use.

Antimicrobial Class	Antimicrobial Subclasses	Agents Included; Generic Names
Aminocyclitols		Spectinomycin
Aminoglycosides		Amikacin Gentamicin Kanamycin Netilmicin Plazomicin Streptomycin Tobramycin
Aminoglycoside-fosfomycin		Amikacin-fosfomycin
Ansamycins	Rifamycins	Rifabutin Rifapentine Rifampin Rifaximin
Lysins	Lysin with antistaphylococcal activity	Exebacase
Folate pathway antagonists	Dihydrofolate reductase inhibitors	Iclaprim Sulfonamides Trimethoprim Trimethoprim-sulfamethoxazole
	Sulfonamides	Sulfamethoxazole Sulfisoxazole
	Combination	Trimethoprim-sulfamethoxazole
Fosfomycins		Fosfomycin
Glycopeptides	Glycopeptide	Vancomycin
	Lipoglycopeptides	Dalbavancin Oritavancin Teicoplanin Telavancin
	Lipoglycodepsipeptide	Ramoplanin
Lincosamides		Clindamycin Lincomycin
Lipopeptides		Daptomycin Surotomycin
	Polymyxins	Colistin Polymyxin B
Macrocyclic lactone		Fidaxomicin

Glossary I (Part 2). (Continued)

Antimicrobial Class	Antimicrobial Subclasses	Agents Included; Generic Names
Macrolides		Azithromycin Clarithromycin Dirithromycin Erythromycin
	Fluoroketolide	Solithromycin
	Ketolides	Nafithromycin Telithromycin
Nitroheterocyclics	Nitrofuran	Nitrofurantoin
	Nitroimidazoles	Metronidazole Secnidazole Tinidazole
	Thiazolides	Nitazoxanide Tizoxanide
Oxazolidinones		Linezolid Tedizolid
Peptide	Magainin	Pexiganan
Phenicols		Chloramphenicol Thiamphenicol
Pleuromutilins		Lefamulin Retapamulin
Pseudomonic acid		Mupirocin
Quinolones		Cinoxacin Garenoxacin Nalidixic acid
	Benzoquinolizine	Levonadifloxacin
	Fluoroquinolones	Besifloxacin Ciprofloxacin Clinafloxacin Delafloxacin Enoxacin Finafloxacin Fleroxacin Gatifloxacin Gemifloxacin Grepafloxacin Levofloxacin Lomefloxacin Moxifloxacin Norfloxacin Ofloxacin Ozenoxacin Pefloxacin Sparfloxacin Trovafloxacin Ulifloxacin (prulifloxacin)

Glossary I (Part 2). (Continued)

Antimicrobial Class	Antimicrobial Subclasses	Agents Included; Generic Names
Quinolonyl oxazolidinone		Cadazolid
Spiropyrimidinetrione		Zoliflodacin
Steroid	Fusidane	Fusidic acid
Streptogramins		Quinupristin-dalfopristin
Tetracyclines		Doxycycline Minocycline Tetracycline
	Fluorocycline	Eravacycline
	Glycylcycline	Tigecycline
	Aminomethylcycline	Omadacycline
Triazaacenaphthylene		Gepotidacin

Abbreviation: FDA, US Food and Drug Administration.

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Glossary II. Antimicrobial Agent Abbreviations, Routes of Administration, and Drug Class

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and some agents are no longer available for human use.

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Amikacin	AN	AN, AK, Ak, AMI, AMK, AKN		X	X		Aminoglycoside
Amikacin-fosfomycin	AKF	AKF	X[d]				Aminoglycoside-fosfomycin
Amoxicillin	AMX	AMX, Amx, AMOX, AC, AML, A	X				Penicillin
Amoxicillin-clavulanate	AMC	AMC, Amc, A/C, AUG, Aug, XL, AML	X				ß-lactam combination agent
Ampicillin	AM	AM, Am, AMP, AP	X	X	X		Penicillin
Ampicillin-sulbactam	SAM	SAM, A/S, AMS, AB			X		ß-lactam combination agent
Azithromycin	AZM	AZM, Azi, AZI, AZ, ATH	X		X		Macrolide
Azlocillin	AZL	AZ, Az, AZL		X	X		Penicillin
Aztreonam	ATM	ATM, AZT, Azt, AT, AZM			X		Monobactam
Aztreonam-avibactam	AZA	AZA			X		ß-lactam combination agent
Aztreonam-nacubactam	ANC	ANC			X		ß-lactam combination agent
Besifloxacin	BES	BES				X	Fluoroquinolone
Biapenem	BPM	BPM			X		Carbapenem
Cadazolid	CDZ	CDZ	X				Quinolonyl oxazolidinone
Carbenicillin (indanyl salt) Carbenicillin	CB	CB, Cb, BAR, CAR, CRB, PY	X	X	X		Penicillin
Cefaclor	CEC	CEC, CCL, Cfr, FAC, CF, CFC	X				Cephem
Cefadroxil	CFR	CFR, FAD, CDX	X				Cephem
Cefamandole	MA	MA, CM, Cfm, FAM, CMD		X	X		Cephem

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Cefazolin	CZ	CZ, CFZ, Cfz, FAZ, KZ, CZN		X	X		Cephem
Cefdinir	CDR	CDR, Cdn, DIN, CD, CFD	X				Cephem
Cefditoren	CDN	CDN, DIT, FD	X				Cephem
Cefepime	FEP	FEP, Cpe, PM, CPM		X	X		Cephem
Cefepime-enmetazobactam	FPE	FPE			X		ß-lactam combination agent
Cefepime-nacubactam	FNC	FNC			X		ß-lactam combination agent
Cefepime-taniborbactam	FTB	FTB			X		ß-lactam combination agent
Cefepime-tazobactam	FPT	FPT			X		ß-lactam combination agent
Cefepime-zidebactam	FPZ	FPZ			X		ß-lactam combination agent
Cefetamet	CAT	CAT, FET	X				Cephem
Cefiderocol	FDC	FDC			X		Siderophore ß-lactam
Cefixime	CFM	CFM, FIX, Cfe, IX	X				Cephem
Cefmetazole	CMZ	CMZ, CMZS, CMT, Cmz		X	X		Cephem
Cefonicid	CID	CID, Cfc, FON, CPO		X	X		Cephem
Cefoperazone	CFP	CFP, Cfp, CPZ, PER, FOP, CP		X	X		Cephem
Cefotaxime	CTX	CTX, TAX, Cft, FOT, CT		X	X		Cephem
Cefotetan	CTT	CTT, CTN, Ctn, CTE, TANS, CN		X	X		Cephem
Cefoxitin	FOX	FOX, CX, Cfx, FX		X	X		Cephem
Cefpirome	CPO	CPO, CPR, CR		X	X		Cephem
Cefpodoxime	CPD	CPD, Cpd, POD, PX	X				Cephem
Cefprozil	CPR	CPR, CPZ, FP	X				Cephem
Ceftaroline	CPT	CPT, Cpt, CTR			X		Cephem
Ceftaroline-avibactam	CPA	CPA			X		ß-lactam combination agent
Ceftazidime	CAZ	CAZ, Caz, TAZ, TZ		X	X		Cephem
Ceftazidime-avibactam	CZA	CZA			X		ß-lactam combination agent

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Ceftibuten	CTB	CTB, TIB, CB, CFB, CFT	X				Cephem
Ceftibuten-avibactam	**CBA**	**CBA**	**X**				**β-lactam combination agent**
Ceftibuten-ledaborbactam	**CLB**	**CLB**	**X**				**β-lactam combination agent**
Ceftizoxime	ZOX	ZOX, CZX, CZ, Cz, CTZ, TIZ		X	X		Cephem
Ceftobiprole	BPR	BPR			X		Cephem
Ceftolozane-tazobactam	CT	CT, C/T, CXT, CLT			X		β-lactam combination agent
Ceftriaxone	CRO	CRO, CTR, FRX, Cax, AXO, TX		X	X		Cephem
Cefuroxime (oral) Cefuroxime (parenteral)	CXM	CXM, CFX, ROX, Crm, FUR, XM	X 	 X	 X		Cephem
Cephalexin	CN	CN, LEX, CFL, CL, CFX	X				Cephem
Cephalothin	CF	CF, Cf, CR, CL, CEP, CE, KF, CEF			X		Cephem
Cephapirin	CP	CP, HAP		X	X		Cephem
Cephradine	RAD	RAD, CH, CED, CE	X				Cephem
Chloramphenicol	C	C, CHL, CL	X		X		Phenicol
Cinoxacin	CIN	CIN, Cn	X				Quinolone
Ciprofloxacin	CIP	CIP, Cp, CI	X		X		Fluoroquinolone
Clarithromycin	CLR	CLR, CLM, CLA, Cla, CH	X				Macrolide
Clinafloxacin	CLX	CFN, CLX, LF, CFL	X		X		Fluoroquinolone
Clindamycin	CM	CC, CM, CD, Cd, CLI, DA	X	X	X		Lincosamide
Cloxacillin	CLO	CX, Clx, CLO, OB, OX	X	X	X		Penicillin
Colistin	CL	CL, CS, CT, CI, CO, COL			X		Lipopeptide
Dalbavancin	DAL	DAL			X		Lipoglycopeptide
Daptomycin	DAP	DAP, Dap, DPC			X		Lipopeptide
Delafloxacin	DLX	DLX, DFX	X		X		Fluoroquinolone
Dicloxacillin	DX	DX, DIC	X				Penicillin
Dirithromycin	DTM	DTM, DT, DIR	X				Macrolide
Doripenem	DOR	DOR, Dor			X		Carbapenem

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Doxycycline	DO	DO, DOX, DC, DOXY, D, DX, Dox, DXT	X		X		Tetracycline
Enoxacin	ENX	ENX, Enx, ENO, ENOX, ENO(F)	X				Fluoroquinolone
Ertapenem	ETP	ETP, Etp		X	X		Carbapenem
Eravacycline	ERV	ERV	X		X		Fluorocycline
Erythromycin	E	E, ERY, EM	X		X		Macrolide
Exebacase	EXE	EXE			X		Antistaphylococcal lysin
Faropenem	FPM	FAR, FARO, FPM, Faro	X				Penem
Fidaxomicin	FDX	FDX	X				Macrocyclic
Finafloxacin	FIN	FIN	X		X	X	Fluoroquinolone
Fleroxacin	FLE	FLE, Fle	X		X		Fluoroquinolone
Fosfomycin	FOS	FOS, FF, FO, FM, Fos	X				Fosfomycin
Fusidic acid	FA	FA, FC, FUS, FD, FU, FAD	X		X	X	Steroidal
Garenoxacin	GRN	GRN, Grn	X		X		Quinolone
Gatifloxacin	GAT	GAT, Gat, GA, GFLX	X		X		Fluoroquinolone
Gemifloxacin	GEM	GEM, Gem	X				Fluoroquinolone
Gentamicin Gentamicin synergy	GM	GM, Gm, CN, GEN GM500, HLG, Gms, GHLR, GMS		X	X		Aminoglycoside
Gepotidacin	GEP	GEP	X		X		Triazaacenaphthylene
Grepafloxacin	GRX	GRX, Grx, GRE, GP	X				Fluoroquinolone
Iclaprim	ICL	ICL, IP			X		Folate pathway antagonist
Imipenem	IPM	IPM, IMI, Imp, IP			X		Carbapenem
Imipenem-relebactam	IMR	IMR, IPR, I/R			X		ß-lactam combination agents
Kanamycin	K	K, KAN, HLK, KM		X	X		Aminoglycoside
Lefamulin	LMU	LMU	X		X		Pleuromutilin
Levofloxacin	LVX	LVX, Lvx, LEV, LEVO, LE	X		X		Fluoroquinolone
Levonadifloxacin	LND	LND			X		Benzoquinolizine
Lincomycin	LIN	L, Lin, LIN, MY		X	X		Lincosamide
Linezolid	LZD	LNZ, LZ, LZD, Lzd	X		X		Oxazolidinone
Lomefloxacin	LOM	LOM, Lmf, LFLX, LOMX	X				Fluoroquinolone

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Loracarbef	LOR	LOR, Lor	X				Cephem
Mecillinam	MEC	MEC, Mec, MM, MEL	X				Penicillin
Meropenem	MEM	MEM, Mer, MERO, MRP, MP			X		Carbapenem
Meropenem-nacubactam	MNC	MNC			X		ß-lactam combination agent
Meropenem-vaborbactam	MEV	MEV			X		ß-lactam combination agent
Meropenem-xeruborbactam	**XEM**	**XEM**			**X**		**ß-lactam combination agent**
Methicillin	ME	ME, MET, DP		X	X		Penicillin
Metronidazole	MET	MET, MTZ, MZ, MRD, MTR	X		X		Nitroimidazole
Minocycline	MI	MI, MIN, Min, MN, MNO, MC, MH	X		X		Tetracycline
Moxalactam	MOX	MOX, Mox		X	X		Cephem
Moxifloxacin	MXF	MXF, Mxf, MX	X		X		Fluoroquinolone
Mupirocin	MUP	MUP, MOP, MU, Mup, PUM				X	Pseudomonic acid
Nafcillin	NF	NF, NAF, Naf		X	X		Penicillin
Nafithromycin	ZMK	ZMK, ZWK	X				Ketolide
Nalidixic acid	NA	NA, NAL	X				Quinolone
Netilmicin	NET	NET, Nt, NC		X	X		Aminoglycoside
Nitazoxanide	NIT	NIT	X				Thiazolide
Nitrofurantoin	FM	FM, F/M, FD, Fd, FT, NIT, NI, F	X				Nitrofuran
Norfloxacin	NX	NX, NOV, NV, NO	X				Fluoroquinolone
Novobiocin	NB	NB				X	Aminocoumarin
Ofloxacin	OFL	OFL, OFX, Ofl, OF	X	X	X		Fluoroquinolone
Omadacycline	OMC	OMC	X		X		Tetracycline
*Oritavancin	ORI	ORI			X		Lipoglycopeptide
Oxacillin	OX	OX, Ox, OXS, OXA	X	X	X		Penicillin
Ozenoxacin	OZN	OZN				X	Fluoroquinolone
Pefloxacin	PEF	PEF, PF, Pef, PE					Fluoroquinolone
Penicillin	P	P, PEN, PV, PG	X	X	X		Penicillin
Pexiganan	PEX	PEX, P/N				X	Peptide

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Piperacillin	PIP	PIP, PI, PP, Pi, PRL		X	X		Penicillin
Piperacillin-tazobactam	TZP	TZP, PTZ, P/T, PTc			X		ß-lactam combination agent
Plazomicin	PLZ	PLZ			X		Aminoglycoside
Polymyxin B	PB	PB, POL, PO			X		Lipopeptide
Quinupristin-dalfopristin	SYN	SYN, Syn, QDA, RP, QDF			X		Streptogramin
Ramoplanin	RAM	RAM	X				Lipoglycodepsipeptide
Razupenem	RZM	RZ, RZM			X		Carbapenem
Rifampin	RA	RA, RIF, Rif, RI, RD, RP, RFP	X		X		Ansamycin
Rifamycin	RIF	RF, RIF	X		X		Ansamycin
Rifapentine	RPT	RPT				X	Pleuromutilin
Rifaximin	RFX	RFX	X				Ansamycin
Secnidazole	SEC	SEC	X				Nitroimidazole
Solithromycin	SOL	SOL	X		X	X	Fluoroketolide
Sparfloxacin	SPX	SPX, Sfx, SPX, SO, SPFX	X				Fluoroquinolone
Spectinomycin	SPT	SPT, SPE, SC, SP, SH, SPC		X	X		Aminocyclitol
Streptomycin Streptomycin synergy	STS	STS, S, STR, StS, SM, ST2000, HLS, SHLR		X	X		Aminoglycoside
Sulbactam-durlobactam	SUD	SUD, SUL			X		ß-lactam combination agent
Sulfonamides	SSS	G, SSS, S3	X		X		Folate pathway antagonist (some PO only)
Sulopenem	SLP	SLP, SPM	X		X		Penem
Surotomycin	SUR	SUR	X				Lipopeptide
Tebipenem	TBP	TBP	X				Carbapenem
Tedizolid	TZD	TZD	X		X		Oxazolidinone
Teicoplanin	TEC	TEC, TPN, Tei, TEI, TP, TPL		X	X		Lipoglycopeptide
Telavancin	TLV	TLV, TLA			X		Lipoglycopeptide
Telithromycin	TEL	TEL	X				Ketolide
Tetracycline	TE	TE, Te, TET, TC	X		X		Tetracycline

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Thiamphenicol	TP	TP	X	X	X		Phenicol
Ticarcillin	TIC	TIC, TC, TI, Ti		X	X		Penicillin
Ticarcillin-clavulanate	TIM	TIM, Tim, T/C, TCC, TLc, TTC			X		ß-lactam combination agent
Tigecycline	TGC	TGC, Tgc			X		Glycylcycline
Tinidazole	TNZ	TNZ	X				Nitroimidazoles
Tinoxanide	TIN	TIN	X				Thiazolide
Tobramycin	TM	TM, NN, TO, To, TOB, TN		X	X		Aminoglycoside
Trimethoprim	TMP	TMP, T, TR, W,TM	X				Folate pathway antagonist
Trimethoprim-sulfamethoxazole	SXT	SXT, SxT, T/S, TS, COT	X		X		Folate pathway antagonist
Trospectomycin	TBR	TBR		X	X		Aminocyclitol
Trovafloxacin	TRO	TVA, Tva, TRV, TV, TRO	X		X		Fluoroquinolone
Ulifloxacin (prulifloxacin)	PRU	PRU, ULI	X				Fluoroquinolone
Vancomycin	VA	VA, Va, VAN, VCM	X		X		Glycopeptide
Zoliflodacin	ZFD	ZFD	X				Spiropyriminetrione

Abbreviations: FDA, US Food and Drug Administration; IM, intramuscular; IV, intravenous; PO, oral.

Footnotes

a. Abbreviations assigned to one or more diagnostic products in the United States. If no diagnostic product is available, abbreviation is that of the manufacturer.

b. Abbreviations used by antimicrobial susceptibility testing device manufacturers may differ from those recommended by CLSI.

c. As available in the United States.

d. Amikacin-fosfomycin is aerosolized and inhaled.

NOTE: Information in black boldface type is new or modified since the previous edition.

M100-Ed33

For Use With M02 and M07

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Glossary III. List of Identical Abbreviations Used for More Than One Antimicrobial Agent in US Diagnostic Products

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and some agents are no longer available for human use.

Abbreviations	Antimicrobial Agents for Which Respective Abbreviations Are Used
AZ	Azithromycin, azlocillin
AZM	Azithromycin, aztreonam
CB, Cb	Ceftibuten, carbenicillin
CD, Cd	Clindamycin, cefdinir
CDN, Cdn	Cefdinir, cefditoren
CF, Cf	Cefaclor, cephalothin
CFM, Cfm	Cefixime, cefamandole
CFR, Cfr	Cefaclor, cefadroxil
CFX, Cfx	Cefoxitin, cefuroxime
CH	Clarithromycin, cephradine
CL	Cephalothin, chloramphenicol
CLX, Clx	Clinafloxacin, cloxacillin
CM	Clindamycin, cefamandole
CN, Cn	Cephalexin, cefotetan, cinoxacin, gentamicin
CP, Cp	Cephapirin, cefoperazone, ciprofloxacin
CPR	Cefpirome, cefprozil
CPZ	Cefprozil, cefoperazone
CT	Ceftolozane-tazobactam, colistin
CZ, Cz	Ceftizoxime, cefazolin
DX	Doxycycline, dicloxacillin
FO	Fleroxacin, fosfomycin
NIT	Nitazoxanide, nitrofurantoin
TC	Tetracycline, ticarcillin
TM	Tobramycin, trimethoprim

Abbreviation: FDA, US Food and Drug Administration.

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