Quality Control of Anti Microbial Susceptibility Test

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Difference between Microbiology and other sections

Reproducibility

The reproducibility or precision of a microbiological test is reduced by two things:

1. *Lack of homogeneity. A* single sample from a patient may contain more than one organism. Repeat culturing may therefore isolate different organisms.

2. *Lack of stability.* As time passes, the microorganisms in a specimen multiply or die at different rates. Repeat culturing may therefore isolate different organisms. To improve precision, therefore, specimens should be tested as soon as possible after collection.

Very Major Error/ Discrepancy

- Reference test result **resistant, method under comparison result sensitive.**
- The patient might be inappropriately treated and die.

Major Error/ Discrepancy

- Reference test result **sensitive, method under comparison result resistant.**
- The patient will not be inappropriately treated but the category result is wrong.

Minor Error/ Discrepancy

- Reference test result **intermediate, method under comparison result sensitive or resistant.**
- Or vice versa.

Internal quality control

An internal quality control programme should be:

- **practical**
- **realistic**
- **economical**

An internal quality control programme should not attempt to evaluate every procedure, reagent, and culture medium on every working day.

It should evaluate each procedure, reagent, and culture medium according to a practical schedule, based on the importance of each item to the quality of the test as a whole.

Internal Quality Control

Control Strains and maintenance

Performance limits for Control Strains

Routine Testing for Control Strains

Frequency of Testing

Control of Media

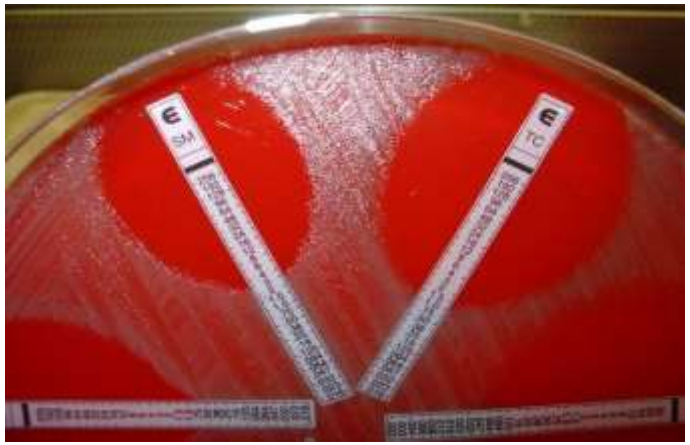
Control of Antimicrobial Disks

Control of Inoculum

Recognition of Atypical Results

AST Methods

- **agar disk diffusion method** provides qualitative interpretive category results of susceptible, intermediate, and resistant
- **microdilution and agar gradient diffusion methods** provide a quantitative result, a minimum inhibitory concentration



Where errors can occur in susceptibility testing

- media
- antimicrobials
- inoculum
- incubation
- equipment
- interpretation

Performance Standards for Antimicrobial Disk Susceptibility Testing

Disk Diffusion
Antimicrobial Susceptibility Test

Muller Hinton Agar
Bacterial Strain
Antimicrobial Disk
Inoculum Preparation
Performing the Disk Diffusion Test

Muller Hinton Agar

pH

Moisture

Effects of Thymidine and Thymine

Effects of variation in divalent cations

pH

The pH of each batch of Müeller-Hinton agar should be checked when the medium is prepared. The exact method used will depend largely on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (e.g., aminoglycosides, quinolones, and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected. The pH can be checked by one of the following means:

* **Macerate a sufficient amount of agar to submerge the tip of a pH electrode.**
* **Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup.**
* **Use a properly calibrated surface electrode.**

Moisture

If, just before use, excess surface moisture is present, the plates should be placed in an incubator (35°C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is lost by evaporation (usually 10 to 30 minutes). The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the petri dish covers when the plates are inoculated.

Effects of Thymidine and Thymine

Media containing excessive amounts of thymidine or thymine can reverse the **inhibitory effect of sulfonamides and trimethoprim**, thus yielding **smaller and less distinct zones**, or even no zone at all, which may result in false-resistance reports. Müeller-Hinton agar that is as low in thymidine content as possible should be used. To evaluate a new lot of Müeller-Hinton agar, ***Enterococcus faecalis* ATCC 29212**, or alternatively, *E. faecalis* ATCC 33186, should be tested with **trimethoprim/sulfamethoxazole disks**. Satisfactory media will provide essentially clear, distinct **zones of inhibition 20 mm or greater in diameter**. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

Table 4: Interpretation of the zones of inhibition obtained from Antimicrobial discs needed for identifying any irregularities in critical indicator "content of thymine and / or thymidine."

Indicator: content of thymine and / or thymidine		
Antimicrobial disc	**Zone greater than 20 mm**	**Zone less than 20 mm**
Sulfamethoxazole / trimethoprim 23.75 / 1.25µg	The medium is with corresponding content of thymine and / or thymidine	The medium hastoo high content of thymidine

Effects of variation in divalent cations

Variation in divalent cations, principally magnesium and calcium, will affect results of **aminoglycoside and tetracycline tests with *P. aeruginosa*** strains. **Excessive cation** content will **reduce zone sizes**, whereas low cation content may result in unacceptably large zones of inhibition. Excess zinc ions may reduce zone sizes of carbapenems. Performance tests with each lot of Müeller-Hinton agar must conform to the control limits.

Table 2: Interpretation of the zones of inhibition obtained from Antimicrobial discs required for identifying any irregularities in critical indicator "content of divalent cations"

Indicator: content of Ca^{2+} and Mg^{2+}		
Antimicrobial Disc	**Zone greater than the upper limit**	**Zone less than the lower limit**
gentamicin 10µg, tobramycin10µg tetracycline 30µg, doxycycline30µg,	the content of Ca^{2+} and Mg^{2+} is too low	the content of Ca^{2+} and Mg^{2+} is too high

Quality control of Mueller-Hinton agar

- Test each new batch of MH agar to ensure that all zones are within ranges.

- Particular problems:

– High or low concentrations of divalent cations (Ca^{2+}, Mg^{2+}) may be indicated by inhibition zones for aminoglycosides with *P. aeruginosa* ATCC 27853 below/above quality control limits, respectively.

– Excess thymine and thymidine may be indicated by inhibition zones for trimethoprim-sulfamethoxazole and *E. faecalis* ATCC 29212 below quality control limits.

MUELLER-HINTON Agar

Media proposed for testing the sensitivity of clinically important pathogens towards antibiotics or sulfonamides.

Quality control

	Inhibition zone diameter in mm acc. to WHO (revised) TEST STRAINS			
Test discs	**Esch. coli ATCC 25922**	**Staph. aureus ATCC 25923**	**Pseud. aeruginosa ATCC 27853**	**Enteroc. faecalis ATCC 33186**
Ampicillin 10 µg	16-22	27-35	-	-
Tetracyclin 30 µg	18-25	19-28	-	-
Gentamicin 10 µg	19-26	19-27	16-21	-
Polymyxin B 300 IU	12-17	7-13	-	-
Sulfamethoxazole 1.25 µg +Trimethoprim 23.75 µg	24-32	24-32	-	> 20

Preparation and storage of media

The medium should have a level depth of 4.0 ± 0.5 mm (approximately 25 mL in a 90 mm circular plate, 31 mL in a 100 mm circular plate, 71 mL in a 150 mm circular plate, 40 mL in a 100 mm square plate).

For agar plates (commercially or in-house prepared) stored in plastic bags or sealed containers, it may be necessary to dry the plates prior to use . This is to avoid excess moisture, which may result in problems with fuzzy zone edges and/or haze within zones.

Media for non-fastidious organisms

Organisms	**Medium**
Enterobacterales *Pseudomonas* spp. *Stenotrophomonas maltophilia* *Acinetobacter* spp. *Staphylococcus* spp. *Enterococcus* spp. *Aeromonas* spp.	Mueller-Hinton agar

Media for fastidious organisms

Organisms	**Medium**
Streptococcus pneumoniae Streptococcus groups A, B, C and G Viridans group streptococci *Haemophilus influenzae* *Moraxella catarrhalis* *Listeria monocytogenes* *Pasteurella multocida* *Campylobacter jejuni* and *coli* *Corynebacterium* spp. *Aerococcus sanguinicola* and *urinae* *Kingella kingae*	Mueller-Hinton agar + 5% mechanically defibrinated horse blood + 20 mg/L β-NAD (MH-F)
Other fastidious organisms	Pending

Reference Strains

Quality Control Strain	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Screening Tests	Other
Enterococcus faecalis ATCC® 51299	•Resistant to vancomycin (*VanB*) and high-level aminoglycosides			•Vancomycin agar High-level aminoglycoside resistance	
Escherichia coli ATCC® 25922	•β-lactamase negative	•Nonfastidious gram-negatives •*Neisseria meningitidis*	•Nonfastidious gram-negatives •*Neisseria meningitidis* •Potential agents of bioterrorism		
Escherichia coli ATCC® 35218	•Contains plasmid-encoded TEM-1 β-lactamase (non-ESBL) [a,b,e,f]	•β-lactam/β-lactamase inhibitor combinations	•β-lactam/β-lactamase inhibitor combinations		
Haemophilus influenzae ATCC® 49247	•BLNAR	•*Haemophilus* spp.	•*Haemophilus* spp.		
Haemophilus influenzae ATCC® 49766	•Ampicillin susceptible	•*Haemophilus* spp. (more reproducible with selected β-lactams)	•*Haemophilus* spp. (more reproducible with selected β-lactams)		
Helicobacter pylori ATCC® 43504			•*Helicobacter pylori*		
Klebsiella pneumoniae ATCC® 700603	•Contains SHV-18 ESBL [b,e,f]	•ESBL screen and confirmatory tests	•ESBL screen and confirmatory tests		
Neisseria gonorrhoeae ATCC® 49226	• CMRNG, chromosomally mediated penicillin resistant	•*Neisseria gonorrhoeae*	•*Neisseria gonorrhoeae*		
Pseudomonas aeruginosa ATCC® 27853[c]	• Contains inducible AmpC β-lactamase	•Nonfastidious gram-negatives	•Nonfastidious gram-negatives •Potential agents of bioterrorism		•Assess suitability of cation content in each batch/lot of Mueller-Hinton for gentamicin MIC and disk diffusion.
Staphylococcus aureus ATCC® 25923	•β-lactamase negative •*mecA* negative •Little value in MIC testing due to extreme susceptibility to most drugs	•Nonfastidious gram-positives			

Antimicrobial Disks

Source of Disks

COA

Lot number

QC recommendation

Storage

***Refrigerate the containers at 8°C or below, or freeze at -14°C or below, in a nonfrost-free freezer until needed. Sealed packages of disks that contain drugs from the ß-lactam class should be stored frozen, except for a small working supply, which may be refrigerated for at most one week. Some labile agents (e.g., imipenem, cefaclor, and clavulanic acid combinations) may retain greater stability if stored frozen until the day of use.**

***The unopened disc containers should be removed from the refrigerator or freezer one to two hours before use, so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts cold disks.**

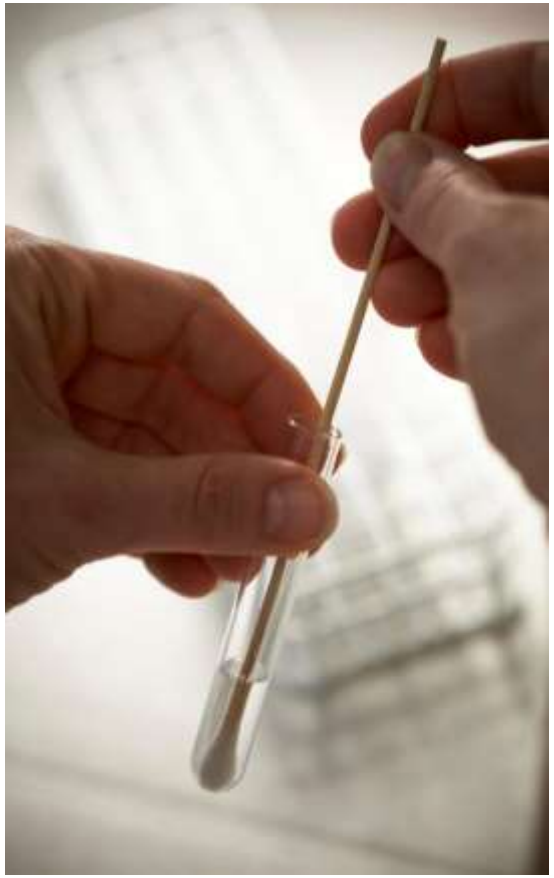
Loss of potency of antimicrobial agents in disks results in reduced inhibition zone diameters and is a common source of error. The following are essential:

Store disks, including those in dispensers, in sealed containers with a moisture-indicating desiccant and protected from light (some agents, including metronidazole, chloramphenicol and the fluoroquinolones, are inactivated by prolonged exposure to light).

Inoculum Preparation

Turbidity Standard

Inoculum



Turbidity Standard

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), should be used. A $BaSO_4$ 0.5 McFarland standard may be prepared as follows:

1. A 0.5-ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2 . 2H_2O$) is added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.
2. The correct density of the turbidity standard should be verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.08 to 0.13 for the 0.5 McFarland standard.
3. The Barium Sulfate suspension should be transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculum.

1. These tubes should be tightly sealed and stored in the dark at room temperature.
2. The barium sulfate turbidity standard should be vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance. If large particles appear, the standard should be replaced. Latex particle suspensions should be mixed by inverting gently, not on a vortex mixer
3. The barium sulfate standards should be replaced or their densities verified monthly.

It is recommended that a photometric device is used to adjust the density of the suspension. The photometric device must be calibrated against a 0.5 McFarland standard according to the manufacturer's instruction.

Alternatively, the density of the suspension can be compared visually to a 0.5 McFarland turbidity standard. To aid comparison, compare the test and standard against a white background with black lines.

Streptococcus pneumoniae is, preferably, suspended from a blood agar plate to the density of a 0.5 McFarland standard. When *Streptococcus pneumoniae* is suspended from a chocolate agar plate, the inoculum must be equivalent to a 1.0 McFarland standard

Inoculum

Direct Colony Suspension Method

Can be used with all most organisms

Recommended method for testing the fastidious organisms:

Hemophillus spp

N.Gonorrhoeae

N.Meningitidis

Streptococci

Staphylococci for potential Meticilin or Oxacilin resistance

Growth Method

Alternative method

Sometime preferable when **colony growth is difficult to suspend directly**

Used for non fastidious organisms (except Staphylococci)

When **fresh (24 hr) colony are not available**

Inoculum

Direct Colony Suspension Method

1. As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from a **18- to 24-hour agar plate** (a **nonselective medium, such as blood agar**, should be used). The suspension is adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer.
2. This approach is the recommended method for testing the **fastidious organisms**, *Haemophilus* spp , *N. gonorrhoeae*, and streptococci, and for testing staphylococci for potential **methicillin or oxacillin resistance**.

Inoculum

Growth Method

The growth method is performed as follows

1. At least **three to five well-isolated colonies** of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing **4 to 5 ml** of a suitable broth medium, such as tryptic soy broth.
2. The broth culture is incubated at **35°C** until it achieves or exceeds the turbidity of the 0.5 McFarland standard (**usually 2 to 6 hours**)
3. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/ml for *E.coli* ATCC 25922. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

Performing the Disk Diffusion Test

Inoculation of Test Plates

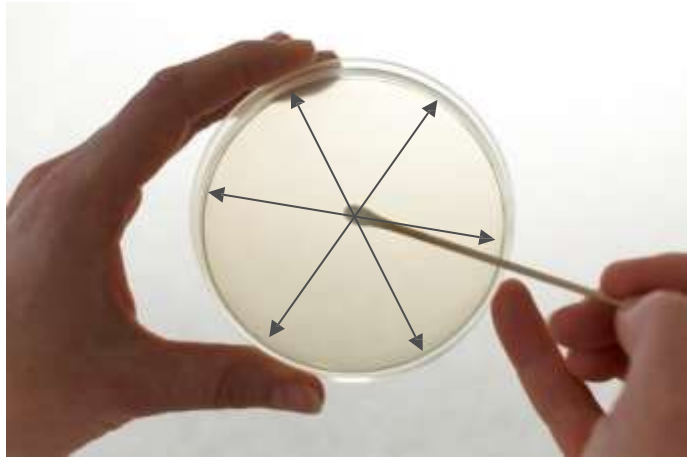
1.Optimally, within **15 minutes after adjusting the turbidity** of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be **rotated several times and pressed firmly on the inside wall of the tube** above the fluid level. This will remove excess inoculum from the swab.

•For Gram-negative bacteria, remove excess fluid by pressing and turning the swab against the inside of the tube to avoid over-inoculation.

•For Gram-positive bacteria, do not press or turn the swab against the inside of the tube.

2. The **dried surface** of a Müeller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is **repeated by streaking two more times**, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.
3. The lid may be left ajar for 3 to 5 minutes, but **no more than 15 minutes**, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided . Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.



Performing the Disk Diffusion Test

Application of Discs to Inoculated Agar Plates

1. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be **pressed down** to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are **no closer than 24 mm from center to center.** Ordinarily, no more than **12 discs should be placed on one 150 mm plate** or more than **5 discs on a 100 mm plate**. Because some of the drug diffuses almost instantaneously, a **disc should not be relocated** once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.
2. The plates are inverted and placed in an incubator set to **35°C within 15 minutes after the discs are applied**. With the exception of *Haemophilus* spp., streptococci and *N. gonorrhoeae*, the plates should not be incubated in an increased CO_2 atmosphere, because the interpretive standards were developed by using ambient air incubation, and CO_2 will significantly alter the size of the inhibitory zones of some agents.

To avoid overlapping zones dispensed predictably small zones (Gm, V) next to those that give larger zones (Cephalosporines)

To be able to detect inducible clindamycin resistance in staphylococci and streptococci, the erythromycin and clindamycin disks must be placed at a distance of **12-20 mm** from edge to edge for **staphylococci** and **12-16 mm** from edge to edge for **streptococci**.

The 15-15-15 minute rule

Follow these instructions for disk diffusion:

- Use the inoculum suspension optimally within **15 minutes** of preparation, and always within 60 minutes.
- Apply disks within **15 minutes** of inoculation.
- Incubate plates within **15 minutes** of disk application.

Table 3	**Incubation conditions for antimicrobial susceptibility test plates**
Organism	**Incubation conditions**
Enterobacterales	35 ± 1°C in air for 18 ± 2 h
Pseudomonas spp.	35 ± 1°C in air for 18 ± 2 h
Stenotrophomonas maltophilia	35 ± 1°C in air for 18 ± 2 h
Acinetobacter spp.	35 ± 1°C in air for 18 ± 2 h
Staphylococcus spp.	35 ± 1°C in air for 18 ± 2 h
Enterococcus spp.	35 ± 1°C in air for 18 ± 2 h (24 h for glycopeptides)
Aeromonas spp.	35 ± 1°C in air for 18 ± 2 h
Streptococcus groups A, B, C and G	35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h
Streptococcus pneumoniae	35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h
Viridans group streptococci	35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h
Haemophilus influenzae	35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h
Moraxella catarrhalis	35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h
Listeria monocytogenes	35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h
Pasteurella multocida	35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h
Campylobacter jejuni and *coli*	See **Appendix A**
Corynebacterium spp.	35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h. Isolates with insufficient growth after 16-20 h are re-incubated immediately and inhibition zones read after a total of 40-44 h incubation.
Aerococcus sanguinicola and *urinae*	35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h. Isolates with insufficient growth after 16-20 h are re-incubated immediately and inhibition zones read after a total of 40-44 h incubation.
Kingella kingae	35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h. Isolates with insufficient growth after 16-20 h are re-incubated immediately and inhibition zones read after a total of 40-44 h incubation.
Other fastidious organisms	Pending

32nd Edition

M100

Performance Standards for Antimicrobial Susceptibility Testing

Test/Report Groups

group A are considered appropriate for inclusion in a routine, primary testing panel, as well as for routine reporting of results for the specific organism groups.

Group B includes antimicrobial agents that may warrant primary testing, but they may be reported only selectively, such as when the organism is **resistant to agents** of the same antimicrobial class, as in group A. Other indications for reporting the result might include a selected **specimen source** (eg, a third-generation cephalosporin for enteric bacilli from CSF or trimethoprim-sulfamethoxazole for urinary tract isolates); a **polymicrobial infection**; infections involving multiple sites; cases of patient **allergy**, intolerance, or failure to respond to an antimicrobial agent in group A; or for infection control purposes or for infection **prevention** .

Group C includes alternative or supplemental antimicrobial agents that may necessitate testing in those institutions that harbor endemic or epidemic strains resistant to several of the primary drugs (especially in the same class, eg, β-lactams); for **treatment of patients allergic** to primary drugs; for **treatment of unusual organisms** (eg, chloramphenicol for extraintestinal isolates of *Salmonella spp.); or for reporting to* infection control as an epidemiological aid.

Group U ("urine") includes certain antimicrobial agents (eg, nitrofurantoin and certain quinolones) that are used only or primarily for treating UTIs. These agents should not be routinely reported against pathogens recovered from other infection sites. An exception to this rule is for *Enterobacteriaceae in Table 1A, in which cefazolin is listed as a surrogate agent for oral cephalosporins. Other antimicrobial* agents with broader indications may be included in group U for specific urinary pathogens (eg, *Enterococcus and ciprofloxacin).*

Group O ("other") includes antimicrobial agents that have a clinical indication for the organism group but are generally not candidates for routine testing and reporting in the United States.

Group Inv. ("investigational") includes antimicrobial agents that are investigational for the organism group and have not yet been approved by the FDA for use in the United States.

- **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) 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:** The intermediate category implies clinical efficacy in anatomical sites where the drugs are physiologically concentrated. **An I with a "^" in Tables 2 indicates agents that have the potential to concentrate at an anatomical site. 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."

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**

Example of Breakpoints and Interpretive Categories as Used in Table 2

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

* Or SDD, if appropriate.

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Redefining susceptibility testing categories **S**, **I** and **R**.

2019

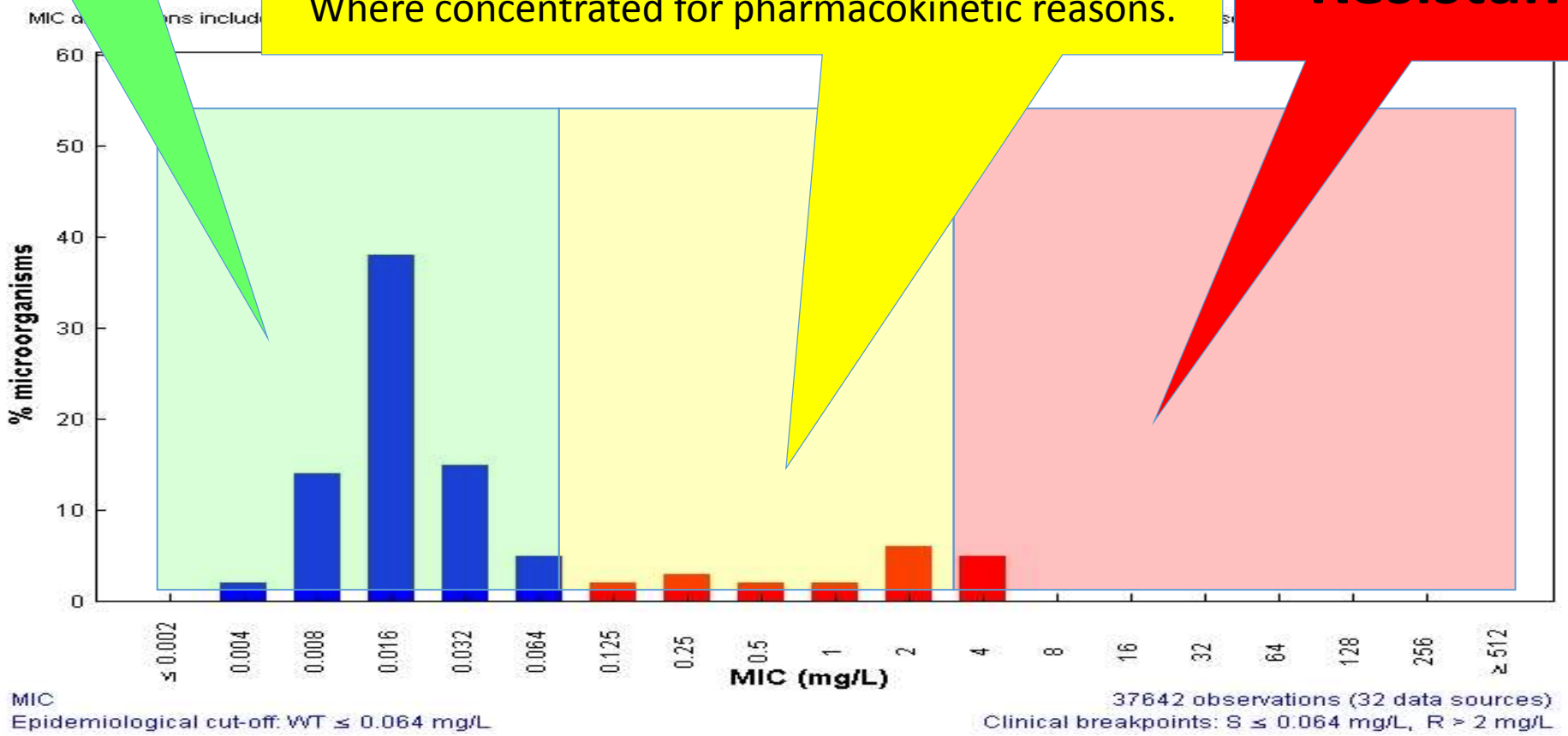
SIR – the old definitions

Susceptible

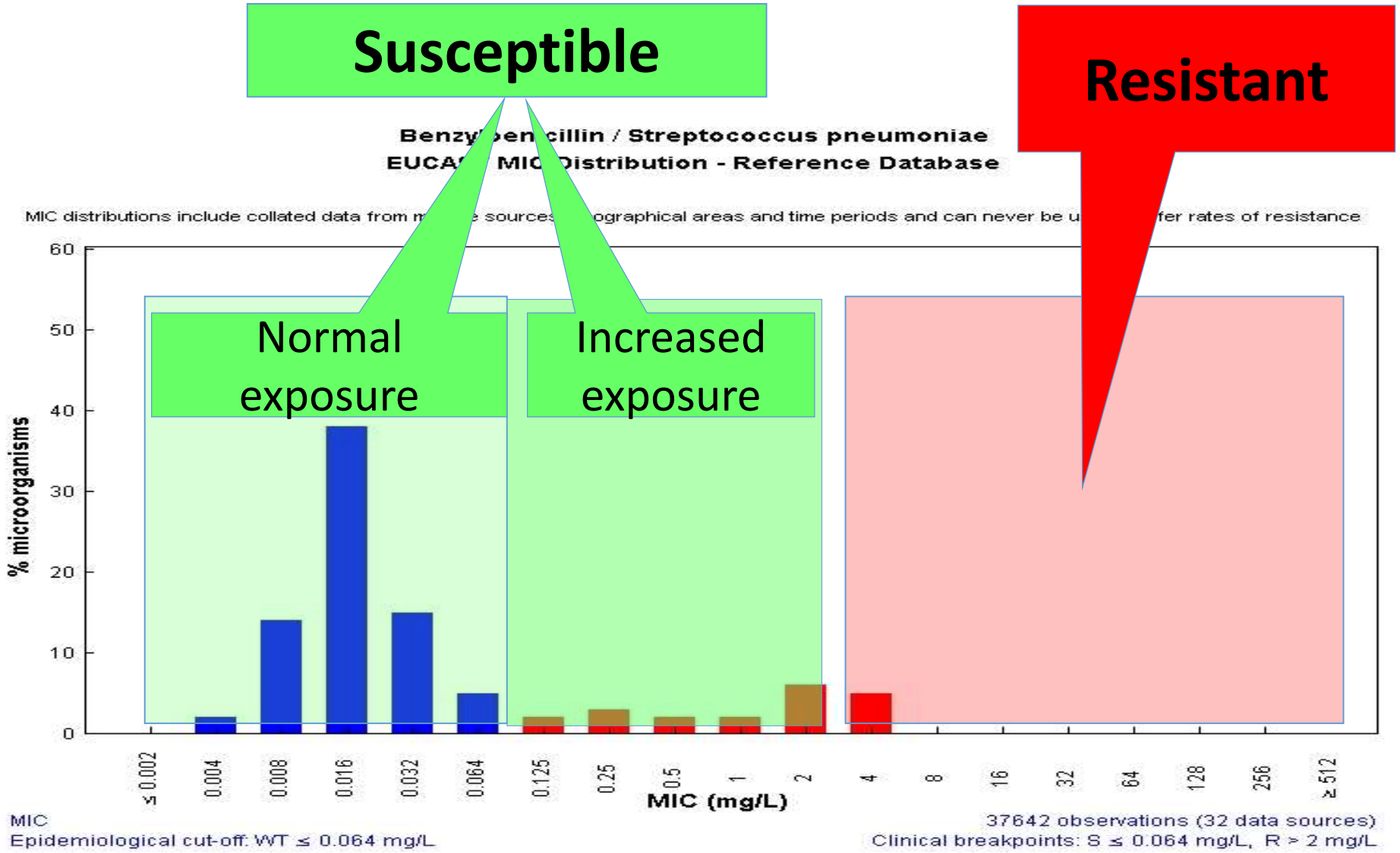
Intermediate

Uncertain effect.
Buffer zone for technical variation.
For a high dose.
Where concentrated for pharmacokinetic reasons.

Resistant



SIR - new definitions 2019



With the modified definition of the ”I-category”….

….the only difference between ”S” and ”I” is the amount of drug at the site of the infection necessary to achieve an adequate clinical response.

The term ”intermediate” is replaced by the term ”Susceptible, increased exposure” but the abreviation in reports is still ”I”.

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, infectious diseases staff, pathology group, pharmacy) 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.

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.

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."**

Location	Organism	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.	1st- and 2nd-generation cephalosporins, cephamycins, and aminoglycosides
Table 2D	*Enterococcus* spp.	Aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole
"Warning": The following antimicrobial agents that are included in this document should not be routinely reported for bacteria isolated from CSF. These antimicrobial agents are not the drugs of choice and may not be effective for treating CSF infections caused by these organisms (ie, the bacteria included in Tables 2A through 2J):		
Tables 2A through 2J	**Bacteria isolated from CSF**	**Agents administered by oral route only, 1st- and 2nd-generation cephalosporins and cephamycins, clindamycin, macrolides, tetracyclines, and fluoroquinolones**

Abbreviation: CSF, cerebrospinal fluid.

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 availability 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” . “Or” indicates cross-susceptibility and cross-resistance is nearly complete (very major error)

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

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	**3H**
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	**3E**

Supplemental Tests (Optional)

Supplemental Test	Organisms	Test Description	Optional for:	Table Location
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, 3B-1
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	**3F**

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

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	**3G**
HLAR by disk diffusion	• *Enterococcus* spp.	Disk diffusion with gentamicin and streptomycin	If screen inconclusive	Broth microdilution, agar dilution MIC	**3J**

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

Surrogate Agent Tests

Surrogate Agent	Organisms	Test Description	Results	Table Location
Cefazolin	• *E. coli* • *Klebsiella 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 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. (excluding *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.	1A, 2C, **3F**
Oxacillin	• *S. pneumoniae*	Disk diffusion	Predicts penicillin susceptibility if oxacillin zone is ≥20 mm. If oxacillin zone is ≤19 mm, penicillin MIC must be done.	1B, 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.

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)

Examples of Equivalent Agent Tests

Agents	Organisms	Identified by	Table Location
Cefotaxime or ceftriaxone	**Enterobacterales**	“Or”	1A and 2A
Colistin or polymyxin B	**Enterobacterales, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* complex**	**“Or”**	**2A, 2B-1, and 2B-2**
Azithromycin or clarithromycin or erythromycin	*Staphylococcus* spp.	“Or”	1A 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	1A and 2C
The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin.	*Haemophilus* spp.	Note listed	1B and 2E
The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin.	Anaerobes	Note listed	2J

Table 1A. Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Nonfastidious Organisms by Microbiology Laboratories in the United States

Group A: Includes antimicrobial agents considered appropriate for inclusion in a routine, primary testing panel, as well as for routine reporting of results for the specific organism group.			
Enterobacterales	*Pseudomonas aeruginosa*	*Staphylococcus* spp.	*Enterococcus* spp.[a]
Ampicillin[b]	Ceftazidime	Azithromycin[c] or clarithromycin[c] or erythromycin[c]	Ampicillin[d] Penicillin[e]
Cefazolin[f]	Gentamicin Tobramycin		
Gentamicin[b] Tobramycin[b]	Piperacillin-tazobactam	Clindamycin[c]	
		Oxacillin[g,h,i,j,k] Cefoxitin[g,h,j] (surrogate test for oxacillin)	
		Penicillin[g]	
		Trimethoprim-sulfamethoxazole	
Group B: Includes antimicrobial agents that may warrant primary testing but may be reported only selectively, such as when the organism is resistant to agents of the same antimicrobial class in Group A.[l]			
Amikacin[b]	Amikacin	Ceftaroline[m]	Daptomycin[l,n]
Amoxicillin-clavulanate Ampicillin-sulbactam	Aztreonam	Daptomycin[l,n]	Linezolid Tedizolid[o]
Azithromycin[p]			
Ceftazidime-avibactam	Cefepime	Linezolid Tedizolid[m]	Vancomycin
Ceftolozane-tazobactam	Ceftazidime-avibactam		
Imipenem-relebactam	**Imipenem-relebactam**		
Meropenem-vaborbactam	Ceftolozane-tazobactam		
Piperacillin-tazobactam			
Cefuroxime	Ciprofloxacin Levofloxacin	Doxycycline Minocycline[c] Tetracycline[q]	
Cefepime	Doripenem Imipenem Meropenem	**Lefamulin[m]**	
Cefotetan Cefoxitin		Vancomycin[l]	
Cefotaxime[b,f] or Ceftriaxone[b,f]			
Ciprofloxacin[b] Levofloxacin[b]		Rifampin[r]	
Doripenem Ertapenem Imipenem Meropenem			
Trimethoprim-sulfamethoxazole[b]			

Table 1A. (Continued)

Group C: Includes alternative or supplemental antimicrobial agents that may require testing in institutions that harbor endemic or epidemic strains resistant to several of the primary drugs, for treatment of patients allergic to primary drugs, for treatment of unusual organisms, or for reporting to infection prevention as an epidemiological aid.			
Enterobacterales	*Pseudomonas aeruginosa*	*Staphylococcus* spp.	*Enterococcus* spp.[a]
Aztreonam Ceftazidime		Chloramphenicol[c]	Gentamicin (high-level resistance testing only)
Ceftaroline		Ciprofloxacin or levofloxacin Moxifloxacin	Streptomycin (high-level resistance testing only)
Chloramphenicol[b,c]			
Tetracycline[q]			Dalbavancin[i,s]
		Gentamicin[t]	Oritavancin[i,s]
		Dalbavancin[i,m]	Telavancin[i,s]
		Oritavancin[i,m]	
		Telavancin[i,m]	
Group U: Includes antimicrobial agents that are used only or primarily for treating UTIs.			
Cefazolin (surrogate test for uncomplicated UTI)[u]		Nitrofurantoin	Ciprofloxacin Levofloxacin
Fosfomycin[v]		Sulfisoxazole	
Nitrofurantoin		Trimethoprim	Fosfomycin[v]
Sulfisoxazole			Nitrofurantoin
Trimethoprim			Tetracycline[q]

Table 1A. (Continued)

Group A: Includes antimicrobial agents considered appropriate for inclusion in a routine, primary testing panel, as well as for routine reporting of results for the specific organism group.			
Acinetobacter spp.	*Burkholderia cepacia* complex	*Stenotrophomonas maltophilia*	Other Non-Enterobacterales[l,w]
Ampicillin-sulbactam	Levofloxacin[l]	Levofloxacin Minocycline Trimethoprim-sulfamethoxazole	Ceftazidime
Ceftazidime	Meropenem		Gentamicin Tobramycin
Ciprofloxacin Levofloxacin	Trimethoprim-sulfamethoxazole		
Doripenem Imipenem Meropenem			
Gentamicin Tobramycin			
Group B: Includes antimicrobial agents that may warrant primary testing but may be reported only selectively, such as when the organism is resistant to agents of the same antimicrobial class in Group A.[l]			
Amikacin	Ceftazidime	Ceftazidime[l]	Amikacin
Piperacillin-tazobactam	Minocycline		Aztreonam
Cefepime			Cefepime
Cefotaxime Ceftriaxone			Ciprofloxacin Levofloxacin
Doxycycline			Imipenem Meropenem
Minocycline			Piperacillin-tazobactam
Trimethoprim-sulfamethoxazole			Trimethoprim-sulfamethoxazole
Group C: Includes alternative or supplemental antimicrobial agents that may require testing in institutions that harbor endemic or epidemic strains resistant to several of the primary drugs, for treatment of patients allergic to primary drugs, for treatment of unusual organisms, or for reporting to infection prevention as an epidemiological aid.			
	Chloramphenicol[c,l]	Chloramphenicol[c,l]	Cefotaxime Ceftriaxone
			Chloramphenicol[c]
Group U: Includes antimicrobial agents that are used only or primarily for treating UTIs.			
Tetracycline[q]			Sulfisoxazole
			Tetracycline[q]

Abbreviations: CSF, cerebrospinal fluid; MIC, minimal inhibitory concentration; UTI, urinary tract infection.

Table 1A. (Continued)

"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.

Table 1B. Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Fastidious Organisms by Microbiology Laboratories in the United States

Haemophilus influenzae*[e] and *Haemophilus parainfluenzae	***Neisseria gonorrhoeae*[l]**	***Streptococcus pneumoniae*[k]**	***Streptococcus* spp. β-Hemolytic Group[q]**	***Streptococcus* spp. Viridans Group[q]**
Group A: Includes antimicrobial agents considered appropriate for inclusion in a routine, primary testing panel, as well as for routine reporting of results for the specific organism group.				
Ampicillin[e,g]	Azithromycin[*,†]	Erythromycin[a,c]	Clindamycin[c,p]	Ampicillin[n,*] Penicillin[n,*]
	Ceftriaxone[†] Cefixime[†]			
	Ciprofloxacin[†]	Penicillin[l] (oxacillin disk)	Erythromycin[a,c,p]	
	Tetracycline[b,†]		Penicillin[o,†] or ampicillin[o,†]	
		Trimethoprim-sulfamethoxazole		
Group B: Includes antimicrobial agents that may warrant primary testing but may be reported only selectively, such as when the organism is resistant to agents of the same antimicrobial class, as in Group A.[d]				
Ampicillin-sulbactam		Cefepime[*] Cefotaxime[l,*] Ceftriaxone[l,*]	Cefepime or cefotaxime or ceftriaxone	Cefepime Cefotaxime Ceftriaxone
Cefotaxime[e] or ceftazidime[e] or ceftriaxone[e]		Clindamycin[c]	Vancomycin	Vancomycin
Ciprofloxacin or levofloxacin or moxifloxacin		Doxycycline		
		Levofloxacin[k] Moxifloxacin[k]		
Meropenem[e]		Meropenem[l,*]		
		Tetracycline[b]		
		Vancomycin[l]		

p. ***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 group B *Streptococcus* is isolated from a pregnant woman with severe penicillin allergy (high risk for anaphylaxis), erythromycin and clindamycin (including inducible clindamycin resistance **[ICR]**) should be tested, and only clindamycin should be reported. **Erythromycin, even when tested for determination of ICR, should not be reported.** See Table 3**H**.

Table 1B. (Continued)

Group C: Includes alternative or supplemental antimicrobial agents that may require testing in institutions that harbor endemic or epidemic strains resistant to several of the primary drugs, for treatment of patients allergic to primary drugs, for treatment of unusual organisms, or for reporting to infection prevention as an epidemiological aid.				
Haemophilus influenzae*[e] and *Haemophilus parainfluenzae	***Neisseria gonorrhoeae*[j]**	***Streptococcus pneumoniae*[k]**	***Streptococcus* spp. β-Hemolytic Group[q]**	***Streptococcus* spp. Viridans Group[q]**
Azithromycin[f] Clarithromycin[f]		Amoxicillin[*] Amoxicillin-clavulanate[*]	Ceftaroline	Ceftolozane-tazobactam
Aztreonam		Cefuroxime[*]	Chloramphenicol[c]	Chloramphenicol[c]
Amoxicillin-clavulanate[f]		Ceftaroline	Daptomycin[r,*]	Clindamycin[c]
Cefaclor[f] Cefprozil[f]		Chloramphenicol[c]	Levofloxacin	Erythromycin[a,c]
Cefdinir[f] or cefixime[f] or cefpodoxime[f]		Ertapenem[*] Imipenem[*]	Linezolid Tedizolid[s]	Linezolid Tedizolid[t]
		Linezolid	Dalbavancin[u,*] Oritavancin[*] Telavancin[*]	Dalbavancin[u,*] Oritavancin[*] Telavancin[*]
Ceftaroline[h]		Rifampin[m]		
Cefuroxime[f]				
Chloramphenicol[c]				
Ertapenem or imipenem				
Rifampin[i]				
Tetracycline[b]				
Trimethoprim-sulfamethoxazole				

Abbreviations: CSF, cerebrospinal fluid; MIC, minimal inhibitory concentration.

[*] MIC testing only; disk diffusion test is unreliable.

[†] Routine testing is not necessary (see footnotes j and o).

Table 1B. (Continued)

"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.

Table 2A. Zone Diameter and MIC Breakpoints for Enterobacterales

M100-2021

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 [5]).**

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.

(5) Positive blood culture broth can be used as the inoculum for direct disk diffusion testing of select antimicrobial agents (see below) against Enterobacterales as described in Table 3E with a standard incubation of 16 to 18 hours, using current disk diffusion breakpoints in Table 2A. For antimicrobial agents not listed below for Enterobacterales, for other genera, and for shorter direct incubation times, eg, 8 to 10 hours, CLSI has not yet evaluated this direct disk diffusion method.

Antimicrobial Agents
Ampicillin
Aztreonam
Ceftazidime
Ceftriaxone
Tobramycin
Trimethoprim-sulfamethoxazole

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

M100-2022

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 [5]).

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® 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) 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.

(2) 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 not be comparable to ampicillin, with poorer efficacy.** In addition, for extraintestinal isolates of *Salmonella* spp., a 3rd-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.

M100-2022

Table 2A. Enterobacterales (Continued)

(3) 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 infectious diseases practitioners, pharmacists, pharmacy and therapeutics committees, infection prevention committees, and the antimicrobial stewardship team.

(4) **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.**

(5) 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.

Table 2A. Enterobacterales (Continued)

Test/Report Group	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											
A	Ampicillin	10 µg	≥17	-	14-16^	≤13	≤8	-	16^	≥32	**(6)** Results of ampicillin testing can be used to predict results for amoxicillin. See general comment (2).
O	Piperacillin	100 µg	≥21	-	18-20^	≤17	≤16	-	32-64^	≥128	
O	Mecillinam	10 µg	≥15	-	12-14^	≤11	≤8	-	16^	≥32	**(7)** For testing and reporting of *E. coli* urinary tract isolates only.
β-LACTAM COMBINATION AGENTS											
B	Amoxicillin-clavulanate	20/10 µg	≥18	-	14-17^	≤13	≤8/4	-	16/8^	≥32/16	
B	Ampicillin-sulbactam	10/10 µg	≥15	-	12-14^	≤11	≤8/4	-	16/8^	≥32/16	
B	Ceftolozane-tazobactam	30/10 µg	≥21	-	18-20^	≤17	≤2/4	-	4/4^	≥8/4	**(8)** Breakpoints are based on a dosage regimen of 1.5 g administered every 8 h.
B	Ceftazidime-avibactam	30/20 µg	≥21	-	-	≤20	≤8/4	-	-	≥16/4	**(9)** Breakpoints are based on a dosage regimen of 2.5 g every 8 h administered over 2 h. **(10)** Confirmatory MIC testing is indicated for isolates with zones of 20-22 mm to avoid reporting false-susceptible or false-resistant results.
B	Imipenem-relebactam	10/25 µg	≥25	-	21-24^	≤20	≤1/4	-	2/4^	≥4/4	**(11) Breakpoints are based on a dosage regimen of 1.25 g administered every 6 h.** **(12) Breakpoints do not apply to the family *Morganellaceae*, which includes but is not limited to the genera *Morganella*, *Proteus*, and *Providencia*.** **(13) 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.**

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Table 2A. Enterobacterales (Continued)

Test/Report Group	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											
A	Ampicillin	10 µg	≥17	-	14-16^	≤13	≤8	-	16^	≥32	**(6)** Results of ampicillin testing can be used to predict results for amoxicillin. **(7) 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.** **(8) Breakpoints when oral ampicillin is used for therapy of uncomplicated UTIs due only to *E. coli*, *P. mirabilis*, *Shigella*, and *Salmonella* 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 (2).
O	Piperacillin		-	-	-	-	**≤8**	**16**	-	**≥32**	**(9) 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.**
O	Mecillinam	10 µg	≥15	-	12-14^	≤11	≤8	-	16^	≥32	**(10)** For testing and reporting of *E. coli* urinary tract isolates only.

Table 2A. Enterobacterales (Continued)

Test/Report Group	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											
(11) 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.											
B	Amoxicillin-clavulanate	20/10 µg	≥18	-	14-17^	≤13	≤8/4	-	16/8^	≥32/16	**(12) Breakpoints are based on a dosage regimen of 1.2 g IV administered every 6 h.** **(13) 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.**
B	Ampicillin-sulbactam	10/10 µg	≥15	-	12-14^	≤11	≤8/4	-	16/8^	≥32/16	**(14) Breakpoints are based on a dosage regimen of 3 g administered parenterally every 6 h.**
B	Ceftolozane-tazobactam	30/10 µg	**≥22**	-	**19-21^**	**≤18**	≤2/4	-	4/4^	≥8/4	(15) 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.**
B	Ceftazidime-avibactam	30/20 µg	≥21	-	-	≤20	≤8/4	-	-	≥16/4	**(16)** Breakpoints are based on a dosage regimen of 2.5 g every 8 h administered over 2 h. **(17)** Confirmatory MIC testing is indicated for isolates with zones of 20-22 mm to avoid reporting false-susceptible or false-resistant results.
B	Imipenem-relebactam	10/25 µg	≥25	-	21-24^	≤20	≤1/4	-	2/4^	≥4/4	**(18)** Breakpoints are based on a dosage regimen of 1.25 g administered every 6 h. **(19)** 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)

Test/Report Group	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 (Continued)											
B	Meropenem-vaborbactam	20/10 µg	≥18	-	15-17^	≤14	≤4/8	-	8/8^	≥16/8	**(14)** Breakpoints are based on a dosage regimen of 4 g every 8 h administered over 3 h.
B	Piperacillin-tazobactam	100/10 µg	≥21	-	18-20^	≤17	≤16/4	-	32/4-64/4^	≥128/4	
O	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.)											

(15) WARNING: For *Salmonella* spp. and *Shigella* spp., 1st- and 2nd-generation cephalosporins and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.

(16) 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 the current breakpoints, routine ESBL testing is no longer necessary before reporting results (ie, it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins from susceptible to resistant). However, ESBL testing may still be useful for epidemiological or infection prevention purposes. For laboratories that have not implemented the current breakpoints, ESBL testing should be performed as described in Table 3A.

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* spp., 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.

(17) *Enterobacter, Klebsiella* (formerly *Enterobacter*) *aerogenes, Citrobacter,* and *Serratia* may develop resistance during prolonged therapy with 3rd-generation cephalosporins as a result of derepression of AmpC β-lactamase. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing repeat isolates may be warranted.

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Table 2A. Enterobacterales (Continued)

Test/Report Group	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 (Continued)											
B	Meropenem-vaborbactam	20/10 µg	≥ 18	-	15-17^	≤ 14	≤ 4/8	-	8/8^	≥ 16/8	(20) Breakpoints are based on a dosage regimen of 4 g every 8 h administered over 3 h.
B	Piperacillin-tazobactam	100/10 µg	**≥ 25**	**21-24**		**≤ 20**	**≤ 8/4**	**16/4**		**≥ 32/4**	**(21) 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.**
O	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.)											

(22) WARNING: For *Salmonella* spp. and *Shigella* spp., 1st- and 2nd-generation cephalosporins and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.

(23) 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 the current breakpoints, routine ESBL testing is no longer necessary before reporting results (ie, it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins from susceptible to resistant). However, ESBL testing may still be useful for epidemiological or infection prevention purposes. For laboratories that have not implemented the current breakpoints, ESBL testing should be performed as described in Table 3A.

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* spp., 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.

(24) *Enterobacter*, *Klebsiella* (formerly *Enterobacter*) *aerogenes*, *Citrobacter*, and *Serratia* may develop resistance during prolonged therapy with 3rd-generation cephalosporins as a result of derepression of AmpC β-lactamase. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing repeat isolates may be warranted.

Table 2A. Enterobacterales (Continued)

Test/Report Group	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)											
A	Cefazolin	30 µg	≥23	-	20-22	≤19	≤2	-	4	≥8	**(18)** 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 (**16**).
U	Cefazolin	30 µg	≥15	-	-	≤14	≤16	-	-	≥32	**(19)** 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).
C	Ceftaroline	30 µg	≥23	-	20-22^	≤19	≤0.5	-	1^	≥2	**(20)** Breakpoints are based on a dosage regimen of 600 mg administered every 12 h.
B	Cefepime	30 µg	≥25	19-24	-	≤18	≤2	4-8	-	≥16	**(21)** 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.

Table 2A. Enterobacterales (Continued)

Test/Report Group	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)											
B B	Cefotaxime or ceftriaxone	30 µg 30 µg	≥ 26 ≥ 23	-	23-25^ 20-22^	≤ 22 ≤ 19	≤ 1 ≤ 1	-	2^ 2^	≥ 4 ≥ 4	**(22)** 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 (16).
B	Cefotetan	30 µg	≥ 16	-	13-15^	≤ 12	≤ 16	-	32^	≥ 64	
B	Cefoxitin	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	**(23)** Breakpoints are based on a dosage regimen of at least 8 g per day (eg, 2 g administered every 6 h).
B	Cefuroxime (parenteral)	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	**(24)** Breakpoints are based on a dosage regimen of 1.5 g administered every 8 h. See comment (16).
C	Ceftazidime	30 µg	≥ 21	-	18-20^	≤ 17	≤ 4	-	8^	≥ 16	**(25)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h. See comment (16).
O	Cefamandole	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	See comment (16).
O	Cefmetazole	30 µg	≥ 16	-	13-15^	≤ 12	≤ 16	-	32^	≥ 64	**(26)** Insufficient new data exist to reevaluate breakpoints listed here.
O	Cefonicid	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	See comment (16).
O	Cefoperazone	75 µg	≥ 21	-	16-20	≤ 15	≤ 16	-	32	≥ 64	See comment (16).
O	Ceftizoxime	30 µg	≥ 25	-	22-24^	≤ 21	≤ 1	-	2^	≥ 4	**(27)** Breakpoints are based on a dosage regimen of 1 g administered every 12 h. See comment (16).
O	Moxalactam	30 µg	≥ 23	-	15-22^	≤ 14	≤ 8	-	16-32^	≥ 64	See comment (16).
Inv.	Cefiderocol	30 µg	≥ 16	-	12-15^	≤ 11	≤ 4	-	8^	≥ 16	**(28)** Breakpoints are based on a dosage regimen of 2 g every 8 h administered over 3 h.

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Table 2A. Enterobacterales (Continued)

Test/Report Group	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)											
B B	Cefotaxime or ceftriaxone	30 µg 30 µg	≥ 26 ≥ 23	-	23-25^ 20-22^	≤ 22 ≤ 19	≤ 1 ≤ 1	-	2^ 2^	≥ 4 ≥ 4	**(29)** 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 (23).
B	Cefotetan	30 µg	≥ 16	-	13-15^	≤ 12	≤ 16	-	32^	≥ 64	
B	Cefoxitin	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	**(30)** Breakpoints are based on a dosage regimen of at least 8 g per day (eg, 2 g administered every 6 h).
B	Cefuroxime (parenteral)	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	**(31)** Breakpoints are based on a dosage regimen of 1.5 g administered every 8 h. See comment (23).
C	Ceftazidime	30 µg	≥ 21	-	18-20^	≤ 17	≤ 4	-	8^	≥ 16	**(32)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h. See comment (23).
O	Cefamandole	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	See comment (23).
O	Cefmetazole	30 µg	≥ 16	-	13-15^	≤ 12	≤ 16	-	32^	≥ 64	**(33)** Insufficient new data exist to reevaluate breakpoints listed here.
O	Cefonicid	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	See comment (23).
O	Cefoperazone	75 µg	≥ 21	-	16-20	≤ 15	≤ 16	-	32	≥ 64	See comment (23).
O	Ceftizoxime	30 µg	≥ 25	-	22-24^	≤ 21	≤ 1	-	2^	≥ 4	**(34)** Breakpoints are based on a dosage regimen of 1 g administered every 12 h. See comment (23).
O	Moxalactam	30 µg	≥ 23	-	15-22^	≤ 14	≤ 8	-	16-32^	≥ 64	See comment (23).
B	Cefiderocol	30 µg	≥ 16	-	**9**-15^	**≤ 8**	≤ 4	-	8^	≥ 16	**(35)** Breakpoints are based on a dosage regimen of 2 g every 8 h administered over 3 h.

Table 2A. Enterobacterales (Continued)

Test/Report Group	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)											
B	Cefuroxime	30 µg	≥23	-	15-22^	≤14	≤4		8-16^	≥32	See comment (29).
U	Cefazolin (surrogate test for oral cephalosporins and uncomplicated UTIs)	30 µg	≥15	-	-	≤14	≤16	-	-	≥32	**(29)** 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.
O	Loracarbef	30 µg	≥18	-	15-17^	≤14	≤8	-	16^	≥32	**(30)** Do not test *Citrobacter*, *Providencia*, or *Enterobacter* spp. with cefdinir or loracarbef by disk diffusion because false-susceptible results have been reported. See comment (29).
O	Cefaclor	30 µg	≥18	-	15-17^	≤14	≤8	-	16^	≥32	See comment (29).
O	Cefdinir	5 µg	≥20	-	17-19^	≤16	≤1	-	2^	≥4	See comments (29) and (30).
O	Cefixime	5 µg	≥19	-	16-18^	≤15	≤1	-	2^	≥4	**(31)** Do not test *Morganella* spp. with cefixime, cefpodoxime, or cefetamet by disk diffusion.
O	Cefpodoxime	10 µg	≥21	-	18-20^	≤17	≤2	-	4^	≥8	See comments (29) and (31).
O	Cefprozil	30 µg	≥18	-	15-17^	≤14	≤8	-	16^	≥32	**(32)** Do not test *Providencia* spp. with cefprozil by disk diffusion because false-susceptible results have been reported. See comment (29).
Inv.	Cefetamet	10 µg	≥18	-	15-17^	≤14	≤4	-	8^	≥16	See comment (31).
Inv.	Ceftibuten	30 µg	≥21	-	18-20^	≤17	≤8	-	16^	≥32	**(33)** For testing and reporting of urinary tract isolates only.

Table 2A. Enterobacterales (Continued)

Test/Report Group	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											
C	Aztreonam	30 µg	≥21	-	18-20^	≤17	≤4	-	8^	≥16	**(34)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h. See comment (16).
CARBAPENEMS											
(35) 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.[4-7] Consultation with an infectious diseases practitioner is recommended for isolates for which the carbapenem MICs or zone diameter results from disk diffusion testing are in the intermediate or resistant ranges. Laboratories using Enterobacterales MIC breakpoints for carbapenems described in M100-S20 (January 2010) should perform the CarbaNP test, mCIM, eCIM, and/or a molecular assay (refer to Tables 3B and 3C for methods) when isolates of Enterobacterales are suspicious for carbapenemase production based on imipenem or meropenem MICs 2-4 µg/mL or ertapenem MIC 2 µg/mL (refer to Tables 3B-1 and 3C-1 for guidance on reporting). After implementing the current breakpoints, these additional tests may not need to be performed other than for epidemiological or infection prevention purposes (ie, it is no longer necessary to edit results for the carbapenems to resistant if a carbapenemase producer is detected). See Appendix H, Table H3 regarding suggestions for reporting when molecular and phenotypic methods are discordant. 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.										
B	Doripenem	10 µg	≥23	-	20-22^	≤19	≤1	-	2^	≥4	**(36)** Breakpoints are based on a dosage regimen of 500 mg administered every 8 h.
B	Ertapenem	10 µg	≥22	-	19-21^	≤18	≤0.5	-	1^	≥2	**(37)** Breakpoints are based on a dosage regimen of 1 g administered every 24 h.

Table 2A. Enterobacterales (Continued)

Test/Report Group	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	
CARBAPENEMS (Continued)											
B	Imipenem	10 µg	≥23	-	20-22^	≤19	≤1	-	2^	≥4	**(38)** Breakpoints are based on a dosage regimen of 500 mg administered every 6 h or 1 g every 8 h. **See comment (13).**
B	Meropenem	10 µg	≥23	-	20-22^	≤19	≤1	-	2^	≥4	**(39)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
LIPOPEPTIDES											
(40) 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. **(41)** Several species are intrinsically resistant to the lipopeptides (colistin and polymyxin B). Refer to Appendix B.											
O	Colistin or polymyxin B		- -	- -	- -	- -	- -	- -	≤2 ≤2	≥4 ≥4	**(42)** Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see International Consensus Guidelines[8]). **(43)** Polymyxin B should be given with a loading dose and maximum recommended doses (see International Consensus Guidelines[8]). **(44)** When colistin or polymyxin B is given systemically, neither is likely to be effective for pneumonia. **(45)** 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)

Test/Report Group	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											
(46) 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.											
A	Gentamicin	10 µg	≥15	-	13-14^	≤12	≤4	-	8^	≥16	
A	Tobramycin	10 µg	≥15	-	13-14^	≤12	≤4	-	8^	≥16	
B	Amikacin	30 µg	≥17	-	15-16^	≤14	≤16	-	32^	≥64	
O	Kanamycin	30 µg	≥18	-	14-17^	≤13	≤16	-	32^	≥64	
O	Netilmicin	30 µg	≥15	-	13-14^	≤12	≤8	-	16^	≥32	
O	Streptomycin	10 µg	≥15	-	12-14^	≤11	-	-	-	-	
MACROLIDES											
B	Azithromycin	15 µg	≥13	-	-	≤12	≤16	-	-	≥32	**(47)** *S. enterica* ser. Typhi only: breakpoints are based on MIC distribution data and limited clinical data. **(48) Breakpoints are based on a dosage regimen of 500 mg administered daily.**
			≥16	**-**	**11-15**	**≤10**	**≤8**	**-**	**16**	**≥32**	**(49)** ***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 (48).**
TETRACYCLINES											
(50) 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.											
C	Tetracycline	30 µg	≥15	-	12-14	≤11	≤4	-	8	≥16	
O	Doxycycline	30 µg	≥14	-	11-13	≤10	≤4	-	8	≥16	
O	Minocycline	30 µg	≥16	-	13-15	≤12	≤4	-	8	≥16	

Table 2A. Enterobacterales (Continued)

Test/Report Group	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 Enterobacterales except *Salmonella* spp. (Please refer to Glossary I.)											
B	Ciprofloxacin	5 µg	≥ 26	-	22-25^	≤ 21	≤ 0.25	-	0.5^	≥ 1	**(51)** Breakpoints for ciprofloxacin are based on a dosage regimen of 400 mg IV or 500 mg orally administered every 12 h. **(52)** Breakpoints for levofloxacin are based on a dosage regimen of 750 mg administered every 24 h.
B	Levofloxacin	5 µg	≥ 21		17-20^	≤ 16	≤ 0.5	-	1^	≥ 2	
O	Cinoxacin	100 µg	≥ 19	-	15-18^	≤ 14	≤ 16	-	32^	≥ 64	See comment **(33)**.
O	Enoxacin	10 µg	≥ 18	-	15-17^	≤ 14	≤ 2	-	4^	≥ 8	See comment **(33)**.
O	Gatifloxacin	5 µg	≥ 18	-	15-17^	≤ 14	≤ 2	-	4^	≥ 8	
O	Gemifloxacin	5 µg	≥ 20	-	16-19	≤ 15	≤ 0.25	-	0.5	≥ 1	**(53)** For testing and reporting of *K. pneumoniae* only.
O	Grepafloxacin	5 µg	≥ 18	-	15-17	≤ 14	≤ 1	-	2	≥ 4	
O	Lomefloxacin	10 µg	≥ 22	-	19-21^	≤ 18	≤ 2	-	4^	≥ 8	
O	Nalidixic acid	30 µg	≥ 19	-	14-18	≤ 13	≤ 16	-	-	≥ 32	See comment **(33)**.
O	Norfloxacin	10 µg	≥ 17	-	13-16	≤ 12	≤ 4	-	8	≥ 16	See comment **(33)**.
O	Ofloxacin	5 µg	≥ 16	-	13-15^	≤ 12	≤ 2	-	4^	≥ 8	
Inv.	Fleroxacin	5 µg	≥ 19	-	16-18^	≤ 15	≤ 2	-	4^	≥ 8	
QUINOLONES AND FLUOROQUINOLONES for *Salmonella* spp. (Please refer to Glossary I.)											
(54) 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. **(55)** 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. **(56)** No single test detects resistance resulting from all possible fluoroquinolone resistance mechanisms that have been identified in *Salmonella* spp.										

Table 2A. Enterobacterales (Continued)

Test/Report Group	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.) (Continued)											
B	Ciprofloxacin	5 µg	≥ 31	-	21-30^	≤ 20	≤ 0.06	-	0.12-0.5 ^	≥ 1	**(57)** 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.
B	Levofloxacin	-	-	-	-	-	≤ 0.12	-	0.25-1^	≥ 2	
O	Ofloxacin	-	-	-	-	-	≤ 0.12	-	0.25-1^	≥ 2	
Inv.	Pefloxacin (surrogate test for ciprofloxacin)	5 µg	≥ 24	-	-	≤ 23	-	-	-	-	**(58)** 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 (56).
FOLATE PATHWAY ANTAGONISTS											
B	Trimethoprim-sulfamethoxazole	1.25/ 23.75 µg	≥ 16	-	11-15	≤ 10	≤ 2/38	-	-	≥ 4/76	See general comment (2).
U	Sulfonamides	250 or 300 µg	≥ 17	-	13-16	≤ 12	≤ 256	-	-	≥ 512	**(59)** Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
U	Trimethoprim	5 µg	≥ 16	-	11-15	≤ 10	≤ 8	-	-	≥ 16	
PHENICOLS											
C	Chloramphenicol	30 µg	≥ 18	-	13-17	≤ 12	≤ 8	-	16	≥ 32	**(60)** Not routinely reported on isolates from the urinary tract.
FOSFOMYCINS											
U	Fosfomycin	200 µg	≥ 16	-	13-15	≤ 12	≤ 64	-	128	≥ 256	**(61)** Disk diffusion and MIC breakpoints apply only to *E. coli* urinary tract isolates and should not be extrapolated to other species of Enterobacterales. **(62)** The 200-µg fosfomycin disk contains 50 µg of glucose-6-phosphate. **(63)** 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.

Table 2A. Enterobacterales (Continued)

Test/Report Group	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	
NITROFURANS											
U	Nitrofurantoin	300 µg	≥ 17	-	15-16	≤ 14	≤ 32	-	64	≥ 128	

Abbreviations: 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 ß-lactamase; I, intermediate; 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; UTI, urinary tract infection.

Symbol: ^, designation for agents that have the potential to concentrate in the urine.

دوره سی و دوم – زمستان 1397
انتروباکتریاسه از کشت ادرار
تعداد ازمایشگاهها 1754

Antibiotic	Group	Expected Result	No Lab	Correct Results%
Amikacin	B	S	602	74
Amoxi Clav	B	S	50	14
Ampicillin	A	R	489	96
Cefazolin	U	S	401	43
Cefipime	B	S	142	96
Ceftriaxone	B	S	625	91
Cephalexine	O	R	78	37
Cephalotin	O	R	115	44
Ciprofloxacine	B	S	1321	85
Fosfomycin	U	S	12	91
Gentamycin	A	S	1171	88

Antibiotic	Group	Expected Result	No Lab	Correct Results%
Imipenem	B	S	390	83
Nalidixic acid	O	S	524	3
Nitrofurantoin	U	R	1194	12
Norfloxacine	O	S	240	92
Ofloxacine	O	S	126	92
Piperacilin	O	R	33	84
Piperacilin sulbactam	B	S	23	86
Tobramycin	A	S	86	88
Trimetoprim	U	R	42	95
Trimetoprimsulsometaxazol	B	R	873	94

دوره سی و ششم – تابستان 1399
سالمونلا از نمونه گاستروانتریت

Antibiotic	Group	Expected Result	No Lab
Ampicilin	A	R	847
Ceftazidime	O	S	420
Cefixime	C	S	453
Chloramphenicol	C	R	238
Ciprofloxacin	B	I	1416
Imipenem	B	S	363
Meropenem	B	S	69
Trimetoprim sul	B	R	1109

Antibiotic	No Lab incorrect used
Nalidixic Acid	434
Nitrofurantoin	487
Gentamycin	849
Azitromycin	102

M100-2021

Zone Diameter and MIC Breakpoints for *Pseudomonas aeruginosa*

General Comments

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.

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.

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 infectious diseases practitioners, pharmacists, pharmacy and therapeutics committees, infection control committees, **and the antimicrobial stewardship team.**

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 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.

M100-2022

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 [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)

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.

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

Test/Report Group	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									
O	Piperacillin	100 µg	≥21	15-20^	≤14	≤16	32-64^	≥128	**(6)** Breakpoints for piperacillin (alone or with tazobactam) are based on a piperacillin dosage regimen of at least 3 g administered every 6 h.
β-LACTAM COMBINATION AGENTS									
A	Piperacillin-tazobactam	100/10 µg	≥21	15-20^	≤14	≤16/4	32/4-64/4^	≥128/4	**(7)** Breakpoints for piperacillin (alone or with tazobactam) are based on a piperacillin dosage regimen of at least 3 g administered every 6 h.
B	Ceftazidime-avibactam	30/20 µg	≥21	-	≤20	≤8/4	-	≥16/4	**(8)** Breakpoints are based on a dosage regimen of 2.5 g administered every 8 h over 2 h.
B	Ceftolozane-tazobactam	30/10 µg	≥21	17-20^	≤16	≤4/4	8/4^	≥16/4	**(9)** Breakpoints are based on a dosage regimen of 1.5 g administered every 8 h.
B	**Imipenem-relebactam**	**10/25 µg**	**≥23**	**20-22^**	**≤19**	**≤2/4**	**4/4^**	**≥8/4**	**(10) Breakpoints are based on a dosage regimen of 1.25 g administered every 6 h.** **(11) 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.**
O	Ticarcillin-clavulanate	75/10 µg	≥24	16-23^	≤15	≤16/2	32/2-64/2^	≥128/2	**(12)** 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.)									
A	Ceftazidime	30 µg	≥18	15-17^	≤14	≤8	16^	≥32	**(13)** Breakpoints are based on a dosage regimen of 1 g administered every 6 h or 2 g administered every 8 h.
B	Cefepime	30 µg	≥18	15-17^	≤14	≤8	16^	≥32	**(14)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h or 2 g administered every 12 h.
Inv.	Cefiderocol	30 µg	≥18	13-17^	≤12	≤4	8^	≥16	**(15)** Breakpoints are based on a dosage regimen of 2 g every 8 h administered over 3 h.
MONOBACTAMS									
B	Aztreonam	30 µg	≥22	16-21^	≤15	≤8	16^	≥32	**(16)** Breakpoints are based on a dosage regimen of 1 g administered every 6 h or 2 g administered every 8 h.

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
O	Piperacillin	100 µg	≥21	18-20	≤17	≤16	32-64	≥128	
β-LACTAM COMBINATION AGENTS									
(2) 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.									
A	Ampicillin-sulbactam	10/10 µg	≥15	12-14	≤11	≤8/4	16/8	≥32/16	
B	Piperacillin-tazobactam	100/10 µg	≥21	18-20	≤17	≤16/4	32/4-64/4	≥128/4	
O	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.)									
A	Ceftazidime	30 µg	≥18	15-17	≤14	≤8	16	≥32	
B	Cefepime	30 µg	≥18	15-17	≤14	≤8	16	≥32	
B	Cefotaxime	30 µg	≥23	15-22	≤14	≤8	16-32	≥64	
B	Ceftriaxone	30 µg	≥21	14-20	≤13	≤8	16-32	≥64	
B	Cefiderocol	30 µg	≥15	-	-	≤4	8	≥16	**(3)** 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.** **(4) For testing and reporting against *Acinetobacter baumannii* complex only.**
CARBAPENEMS									
A	Doripenem	10 µg	≥18	15-17	≤14	≤2	4	≥8	**(5)** Breakpoints for doripenem are based on a dosage regimen of 500 mg administered every 8 h.
A	Imipenem	10 µg	≥22	19-21	≤18	≤2	4	≥8	**(6)** Breakpoints for imipenem are based on a dosage regimen of 500 mg administered every 6 h.
A	Meropenem	10 µg	≥18	15-17	≤14	≤2	4	≥8	**(7)** Breakpoints for meropenem are based on a dosage regimen of 1 g administered every 8 h or 500 mg administered every 6 h.

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

Test/Report Group	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									
B	Doripenem	10 µg	≥19	16-18^	≤15	≤2	4^	≥8	**(17)** 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	**(18)** Breakpoints for imipenem are based on a dosage regimen of 1 g administered every 8 h or 500 mg administered every 6 h. **See comment (11).**
	Meropenem	10 µg	≥19	16-18^	≤15	≤2	4^	≥8	**(19)** Breakpoints for meropenem are based on a dosage regimen of 1 g administered every 8 h.
LIPOPEPTIDES									
(20) 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.									
O	Colistin or polymyxin B	- -	- -	- -	- -	- -	≤2 ≤2	≥4 ≥4	**(21)** Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see International Consensus Guidelines[4]). **(22)** Polymyxin B should be given with a loading dose and maximum recommended doses (see International Consensus Guidelines[4]). **(23)** When colistin or polymyxin B is given systemically, neither is likely to be effective for pneumonia. **(24)** 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)

Test/Report Group	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									
A	Gentamicin	10 µg	≥15	13–14^	≤12	≤4	8^	≥16	
A	Tobramycin	10 µg	≥15	13–14^	≤12	≤4	8^	≥16	
B	Amikacin	30 µg	≥17	15–16^	≤14	≤16	32^	≥64	
O	Netilmicin	30 µg	≥15	13–14^	≤12	≤8	16^	≥32	
FLUOROQUINOLONES									
B	Ciprofloxacin	5 µg	≥25	19–24^	≤18	≤ 0.5	1^	≥2	**(23)** Breakpoints are based on a dosage regimen of 400 mg IV administered every 8 h.
B	Levofloxacin	5 µg	≥22	15–21^	≤14	≤ 1	2^	≥4	**(24)** Breakpoints are based on a dosage regimen of 750 mg administered every 24 h.
O	Lomefloxacin	10 µg	≥22	19–21^	≤18	≤ 2	4^	≥8	**(25)** For testing and reporting of urinary tract isolates only.
O	**Norfloxacin**	**10 µg**	**≥17**	**13–16**	**≤12**	**≤ 4**	**8**	**≥16**	**See comment (25).**
O	Ofloxacin	5 µg	≥16	13–15^	≤12	≤ 2	4^	≥8	
O	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; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; **PK-PD, pharmacokinetic-pharmacodynamic**; QC, quality control; R, resistant; S, susceptible.

دوره سی و چهارم – پاییز 1398
تعداد ازمایشگاهها 1716
سودومونا از کشت زخم

Antibiotic	Group	Expected Result	No Lab	Correct Results%
Amikacin	B	R	1155	93
Cefepime	B	I	509	12.3
Ceftazidime	A	S	927	84
Ciprofloxacine	B	R	1399	96
Doripenem	B	R	2	0
Catifloxacine	O	R	4	75
Gentamycin	A	R	1411	94
Imipenem	B	R	917	88
Levofloxacin	B	R	174	98
Meropenem	B	R	265	98
Ofloxacin	O	R	137	96
Piperacilin	O	R	241	60
Piperacilin tazobactam	A	R	141	25
Tobramycin	A	R	332	96

Antibiotic	No Lab incorrect used
Ampicilin	165
Azitromycin	81
Ceazolin	126
Cefixim	322
Cefotaxim	262
Ceftizoxim	140
Cephalexin	78
Ceftriaxon	412
Colistin	69

Antibiotic	No Lab incorrect used
Nalidixic acid	221
Nitrofurantoin	338
Norflpxacin	215
Tetracyclin	108
Trimetoprim	404
Vancomycin	38
Penicilin	49
Erythromycin	28
Amoxicilin	57

دوره سی و هشتم – زمستان 1399
سودومونا از کشت ادرار

Antibiotic	Group	Expected Result	No Lab
Amikacin	B	S	1153
Azteronam	B	S	22
Cefepime	B	S	411
Ceftazidime	A	S	787
Ciprofloxacine	B	S	1498
Gentamycin	A	S	1452
Imipenem	B	S	867
Levofloxacin	B	S	219
Meropenem	B	S	280
Piperacilin tazobactam	A	S	138
Tobramycin	A	S	311

Antibiotic	No Lab incorrect used
Nalidixic acid	252
Nitrofurantoin	449
Norfloxacin	271
Trimetoprim	393

Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.

General Comments

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 . 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 as compared to the control

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

Most **oxacillin resistance is mediated by *mecA, encoding the PBP2a (also called PBP2'). Isolates that test positive for mecA or PBP2a should be reported as*** oxacillin resistant.

Oxacillin-resistant S. aureus and CoNS (MRS), are considered resistant to other β-lactam agents, ie, penicillins, β -lactam **combination agents, cephems** (with the exception of the cephalosporins with anti-MRSA activity), and carbapenems.

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).

General Comments

(1) 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.

(2) ***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]

Most methicillin (oxacillin) resistance is mediated by *mecA*, encoding PBP2a (also called PBP2′). **Testing 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 table below**).

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., excluding *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 **methicillin (oxacillin)-resistant staphylococci** (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.

Organism	Phenotypic Methods for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp.				
	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, testing for *mecA* or PBP2a should be considered, **because these are the most definitive tests for detection of methicillin (oxacillin) resistance** (see comment **[18]**). **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]

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

Test/Report Group	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 **(8)** 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. **(9)** Penicillin should be used to test the susceptibility of all staphylococci to penicillinase-labile penicillins (see Glossary I). Penicillin-resistant strains of staphylococci produce β-lactamase. Perform a test(s) to detect β-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 β-lactamase production may appear negative by β-lactamase tests. Consequently, for serious infections requiring penicillin therapy, laboratories should perform MIC tests and β-lactamase testing on all subsequent isolates from the same patient. PCR testing of the isolate for the *blaZ* β-lactamase gene maybe considered. See Tables 3D and 3E.												
A	Penicillin	All staphylococci	10 units	≥29	–	–	≤28	≤0.12		–	≥0.25	**(10)** For **methicillin** (oxacillin)-resistant staphylococci, report penicillin as resistant or do not report.
PENICILLINASE-STABLE PENICILLINS **(11)** 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. Isolates that test either *mecA* negative or PBP2a negative or cefoxitin susceptible should be reported as **methicillin** (oxacillin) susceptible. **(12)** 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: • β-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 β-lactam antimicrobial agents, with the exception of ceftaroline. Thus, susceptibility or resistance to a wide array of β-lactam antimicrobial agents may be deduced from testing only penicillin and either cefoxitin or oxacillin. Testing of other β-lactam agents, except ceftaroline, is not advised. See general comments (5) and (6). Additional explanation on the use of cefoxitin for prediction of *mecA*-mediated **methicillin** (oxacillin) resistance can be found in Subchapter 3.12 of M07[3] and Subchapter 3.9 of M02.[1]												

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

Test/ Report Group	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)												
A	Oxacillin	*S. aureus* and *S. lugdunensis*	–	–	–	–	–	≤2 (oxacillin)	–	–	≥4 (oxacillin)	**(13)** Oxacillin disk testing is not reliable for *S. aureus* and *S. lugdunensis*. **(14)** 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 (5) and (6) and comments (8), (11), and (12).
			30 µg cefoxitin (surrogate test for oxacillin)	≥22	–	–	≤21	≤4 (cefoxitin)	–	–	≥8 (cefoxitin)	
A	Oxacillin	*S. epidermidis*	1 µg oxacillin	≥18 (oxacillin)	–	–	≤17 (oxacillin)	≤0.25 (oxacillin)	–	–	≥0.5 (oxacillin)	See general comments (5) and (6) and comments (8), (11), and (12). **(15)** 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	≤0.25	–	–	≥0.5	**(16)** Neither cefoxitin MIC nor cefoxitin disk tests are reliable for detecting *mecA*-mediated resistance in *S. pseudintermedius* and *S. schleiferi*. See general comments (5) and (6) and comments (8), (11), and (12).

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

Test/ Report Group	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)												
A	Oxacillin	Other *Staphylococcus* spp., excluding *S. aureus* *S. lugdunensis* *S. epidermidis* *S. pseudintermedius* *S. schleiferi*	30 µg cefoxitin (surrogate test for oxacillin)	≥ 25 (cefoxitin)	–	–	≤ 24 (cefoxitin)	≤0.25 (oxacillin)	–	–	≥0.5 (oxacillin)	**(17) Oxacillin MIC breakpoints may overcall resistance, and some isolates for which the oxacillin MICs are 0.5–2 µg/mL may be *mecA* negative. Isolates from serious infections for which oxacillin MICs are 0.5–2 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 (5) and (6) and comments (8), (11), and (12).
CEPHEMS (PARENTERAL)												
B	Ceftaroline	*S. aureus*, including MRSA	30 µg	≥25	20–24		≤19	≤1	2–4	–	≥8	**(18)** The breakpoint for susceptible is based on a dosage regimen of 600 mg administered every 12 h. **(19)** The breakpoint for SDD is based on a dosage of 600 mg every 8 h administered over 2 h.

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

Test/Report Group	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 **(20)** 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.												
B	Vancomycin	*S. aureus*	–	–	–	–	–	≤2	–	4–8	≥16	**(21)** For *S. aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy. **(22)** Send any *S. aureus* for which the vancomycin is ≥8 µg/mL to a referral laboratory. See Appendix A. Also refer to Table 3F for *S. aureus*, Subchapter 3.12 in M07,[3] and Subchapter 3.9 in M02.[1]
		Staphylococcus spp. other than *S. aureus*	–	–	–	–	–	≤4	–	8–16	≥32	See comment (19). **(23)** 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[3] and Subchapter 3.9 in M02.[1]
LIPOGLYCOPEPTIDES												
C	Dalbavancin	*S. aureus*, including MRSA	–	–	–	–	–	≤0.25	–	–	–	
C	Oritavancin		–	–	–	–	–	≤0.12	–	–	–	
C	Telavancin		–	–	–	–	–	≤0.12	–	–	–	
Inv.	Teicoplanin	All staphylococci	–	–	–	–	–	≤8	–	16	≥32	
LIPOPEPTIDES												
B	Daptomycin	All staphylococci	–	–	–	–	–	≤1	–	–	–	**(24)** Daptomycin should not be reported for isolates from the respiratory tract.
AMINOGLYCOSIDES **(25)** For staphylococci that test susceptible, gentamicin is used only in combination with other active agents that test susceptible.												
C	Gentamicin	All staphylococci	10 µg	≥15	–	13–14	≤12	≤4	–	8	≥16	

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

Test/Report Group	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												
(21) 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.												
B	Vancomycin	*S. aureus*, **including MRSA**	-	-	-	-	-	≤2	-	4-8	≥16	**(22)** For *S. aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy. **(23)** 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	See comment (20). **(24)** 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												
C	Dalbavancin	*S. aureus*, including MRSA	-	-	-	-	-	≤0.25	-	-	-	**(25) 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.**
C	Oritavancin		-	-	-	-	-	≤0.12	-	-	-	**(26) Breakpoints are based on a dosage regimen of 1200 mg IV administered once.**

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

Test/ Report Group	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	
MACROLIDES **(26)** Not routinely reported on organisms isolated from the urinary tract.												
A	Azithromycin	All staphylococci	15 µg	≥18	–	14–17	≤13	≤2	–	4	≥8	
A	or clarithromycin		15 µg	≥18		14–17	≤13	≤2		4	≥8	
A	or erythromycin		15 µg	≥23		14–22	≤13	≤0.5		1–4	≥8	
O	Dirithromycin		15 µg	≥19	–	16–18	≤15	≤2	–	4	≥8	
TETRACYCLINES **(27)** 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.												
B	Tetracycline	All staphylococci	30 µg	≥19	–	15–18	≤14	≤4	–	8	≥16	
B	Doxycycline		30 µg	≥16	–	13–15	≤12	≤4	–	8	≥16	
B	Minocycline		30 µg	≥19	–	15–18	≤14	≤4	–	8	≥16	See comment (26).
FLUOROQUINOLONES **(28)** *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.												
C	Ciprofloxacin	All staphylococci	5 µg	≥21	–	16–20	≤15	≤1	–	2	≥4	
C	or Levofloxacin		5 µg	≥19	–	16–18	≤15	≤1	–	2	≥4	
C	Moxifloxacin		5 µg	≥24	–	21–23	≤20	≤0.5	–	1	≥2	
O	Enoxacin		10 µg	≥18	–	15–17	≤14	≤2	–	4	≥8	**(29)** For testing and reporting of urinary tract isolates only.
O	Gatifloxacin		5 µg	≥23	–	20–22	≤19	≤0.5	–	1	≥2	
O	Grepafloxacin		5 µg	≥18	–	15–17	≤14	≤1	–	2	≥4	
O	Lomefloxacin		10 µg	≥22	–	19–21	≤18	≤2	–	4	≥8	
O	**Norfloxacin**		**10 µg**	**≥17**	–	**13–16**	**≤12**	**≤4**	–	**8**	**≥16**	**See comment (29).**
O	Ofloxacin		5 µg	≥18	–	15–17	≤14	≤1	–	2	≥4	
O	Sparfloxacin		5 µg	≥19	–	16–18	≤15	≤0.5	–	1	≥2	
Inv.	Fleroxacin		5 µg	≥19	–	16–18	≤15	≤2	–	4	≥8	
NITROFURANTOINS												
U	Nitrofurantoin	All staphylococci	300 µg	≥17	–	15–16	≤14	≤32	–	64	≥128	

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

Test/ Report Group	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	
LINCOSAMIDES												
A	Clindamycin	All staphylococci	2 µg	≥21	–	15–20	≤14	≤0.5	–	1–2	≥4	**(30) 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 **3H**, Subchapter 3.9 in M02,[1] and Subchapter 3.12 in M07[3]). See comment (26).
FOLATE PATHWAY ANTAGONISTS												
A	Trimethoprim-sulfamethoxazole	All staphylococci	1.25/23.75 µg	≥16	–	11–15	≤10	≤2/38	–	–	≥4/76	
U	Sulfonamides	All staphylococci	250 or 300 µg	≥17	–	13–16	≤12	≤256	–	–	≥512	**(31)** Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
U	Trimethoprim	All staphylococci	5 µg	≥16	–	11–15	≤10	≤8	–	–	≥16	
PHENICOLS												
C	Chloramphenicol	All staphylococci	30 µg	≥18	–	13–17	≤12	≤8	–	16	≥32	See comment (26).
ANSAMYCINS												
B	Rifampin	All staphylococci	5 µg	≥20	–	17–19	≤16	≤1	–	2	≥4	**(32)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
STREPTOGRAMINS												
O	Quinupristin-dalfopristin	***S. aureus***	15 µg	≥19	–	16–18	≤15	≤1	–	2	≥4	**(33)** For reporting against methicillin **(oxacillin)**-susceptible *S. aureus*.

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

Test/ Report Group	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												
(35) *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.												
B	Linezolid	All staphylococci	30 µg	≥21	-	-	≤20	≤4	-	-	≥8	**(36)** 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.
B	Tedizolid	*S. aureus*, including MRSA	-	-	-	-	-	≤0.5	-	1	≥2	
PLEUROMUTILINS												
B	**Lefamulin**	***S. aureus***	**20 µg**	≥23	-	-	-	≤0.25	-	-	-	**(37) The breakpoints for susceptible are based on a dosage regimen of 150 mg IV or 600 mg orally administered every 12 h.** **(38) 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; 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; UTI, urinary tract infection.

دوره سی و سوم – تابستان 1398
تعداد ازمایشگاهها 1700
استافیلوکوک کواگولاز منفی کشت ادرار

Antibiotic	Group	Expected Result	No Lab	Correct Results%
Amikacin	B	S	271	85
Amoxi Clav	B	S	25	64
Ampicilin	A	R	249	67
Amp Sulbac	A	S	3	66
Azitromycin	A	S	243	83
Ceoxitin	A	S	323	96
Cephalexin	O	S	161	89
Cephalotin	O	S	130	95
Ciprofloxacin	B	S	1124	92
Claritromycin	A	S	6	100
Clindamycin	A	S	459	90
Doxycyclin	B	R	134	81
Erythromycin	A	S	529	80

Antibiotic	Group	Expected Result	No Lab	Correct Results%
Gentamycin	A	S	986	94
Levofloxacin	C	S	54	98
Linezolid	B	S	32	100
Nitrofurantoin	U	S	1170	97
Norfloxacin	O	S	259	95
Ofloxacin	O	S	126	93
Piperacilin	A	R	715	90
Piperacilin tazobactam	B	S	3	100
Sulfonamid	U	S	2	50
Trimetoprim	U	S	51	86
Trimetoprim sulfometaxazol	A	S	1172	86
Vancomycin	B	S	376	61

Antibiotic	No Lab incorrect used
Cefazolin	112
Cefixime	175
Cefotaxim	153
Ceftazidim	63
Ceftriaxon	230
Ceftizoxim	67
Doxycyclin	134
Imipenem	98
Kanamycin	12
Nalidixic acid	141
Rifampin	59
Tetracyclin	418

دوره سی و هفتم – پاییز 1399
استافیلوکوک کشت زخم

Antibiotic	No Lab incorrect used
Cefazolin	142
Cefixime	162
Cefotaxim	119
Ceftriaxon	230
Levofloxacin	109
Nalidixic acid	95
Nitrofurantoin	502
Norfloxacin	119
Ofloxacin	126
Vancomycin	466

Zone Diameter and MIC Breakpoints for *Enterococcus* spp.

General Comments

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.*

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

Intermediate ranges denoted with a "^" for the applicable antimicrobial agents in the drug groups in Tables 2 are based on the known ability of these agents to concentrate in the urine; some agents may also have the potential to concentrate at other anatomical sites (eg, epithelial lining).

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

Test/Report Group	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										
A	Penicillin	10 units	≥15	–	≤14	≤8	–	–	≥16	**(6)** 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*. **(7)** 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. **(8)** ***Rx:*** Combination therapy with ampicillin, 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 the *Enterococcus*. For strains with low-level penicillin or ampicillin resistance when combination therapy with a β-lactam is being considered, also see additional testing and reporting information in Table **3J**.[4] **(9)** Penicillin or ampicillin resistance among enterococci due to β-lactamase production has been reported very rarely. Penicillin or ampicillin resistance due to β-lactamase production is not reliably detected with routine disk or dilution methods but is detected using a direct, nitrocefin-based β-lactamase test. Because of the rarity of β-lactamase–positive enterococci, this test does not need to be performed routinely but can be used in selected cases. A positive β-lactamase test predicts resistance to penicillin as well as amino- and ureidopenicillins (see Glossary I).
A	Ampicillin	10 µg	≥17	–	≤16	≤8	–	–	≥16	

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

Test/Report Group	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	
GLYCOPEPTIDES										
B	Vancomycin	30 µg	≥17	15–16	≤14	≤4	–	8–16	≥32	**(10)** 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 3**G**. See general comment (4) and comment (**8**).
LIPOGLYCOPEPTIDES										
C	Dalbavancin	–	–	–	–	≤0.25	–	–	–	**(11)** For reporting against vancomycin-susceptible *E. faecalis*.
C	Oritavancin	–	–	–	–	≤0.12	–	–	–	See comment (11).
C	Telavancin	–	–	–	–	≤0.25	–	–	–	See comment (11).
Inv.	Teicoplanin	30 µg	≥14	11–13	≤10	≤8	–	16	≥32	
LIPOPEPTIDES										
B	Daptomycin ***E. faecium* only**	–	–	–	–	**–**	**≤ 4**	–	**≥ 8**	**(12)** Daptomycin should not be reported for isolates from the respiratory tract. **(13) 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.**
B	**Daptomycin *Enterococcus* spp. other than *E. faecium***	**–**	**–**	**–**	**–**	**≤2**	**–**	**4**	**≥ 8**	**(14) The breakpoint for susceptible is based on a dosage regimen of 6 mg/kg administered every 24 h.** **See comment (12).**
MACROLIDES										
O	Erythromycin	15 µg	≥23	14–22	≤13	≤0.5	–	1–4	≥8	**(15)** Not routinely reported on isolates from the urinary tract.

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

Test/Report Group	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	
TETRACYCLINES										
(16) 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.										
U	Tetracycline	30 µg	≥19	15–18	≤14	≤4	–	8	≥16	
O	Doxycycline	30 µg	≥16	13–15	≤12	≤4	–	8	≥16	
O	Minocycline	30 µg	≥19	15–18	≤14	≤4	–	8	≥16	
FLUOROQUINOLONES										
U	Ciprofloxacin	5 µg	≥21	16–20^	≤15	≤1	–	2^	≥4	
U	Levofloxacin	5 µg	≥17	14–16^	≤13	≤2		4^	≥8	
O	Gatifloxacin	5 µg	≥18	15–17^	≤14	≤2	–	4^	≥8	
O	**Norfloxacin**	**10 µg**	**≥17**	**13–16**	**≤12**	**≤4**	**–**	**8**	**≥16**	**(17) For testing and reporting of urinary tract isolates only.**
NITROFURANTOINS										
U	Nitrofurantoin	300 µg	≥17	15–16	≤14	≤32	–	64	≥128	
ANSAMYCINS										
O	Rifampin	5 µg	≥20	17–19	≤16	≤1	–	2	≥4	**(18) *Rx:*** Rifampin should not be used alone for antimicrobial therapy.
FOSFOYCINS										
U	Fosfomycin	200 µg	≥16	13–15	≤12	≤64	–	128	≥256	**(19)** For testing and reporting of *E. faecalis* urinary tract isolates only. **(20)** 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. **(21)** The 200-µg fosfomycin disk contains 50 µg glucose-6-phosphate.
PHENICOLS										
O	Chloramphenicol	30 µg	≥18	13–17	≤12	≤8	–	16	≥32	See comment (15).
STREPTOGRAMINS										
O	Quinupristin-dalfopristin	15 µg	≥19	16–18	≤15	≤1	–	2	≥4	**(22)** For reporting against vancomycin-resistant *Enterococcus faecium.*
OXAZOLIDINONES										
B	Linezolid	30 µg	≥23	21–22^	≤20	≤2	–	4^	≥8	
B	Tedizolid	–	–	–	–	≤0.5	–	–	–	**(23)** For reporting against *E. faecalis* only.

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; SDD, susceptible-dose dependent.

دوره سی و پنجم – زمستان 1398
تعداد ازمایشگاهها 777
انتروکوک از کشت ادرار

Antibiotic	Group	Expected Result	No Lab	Correct Results%
Ampicilin	A	R	424	90
Ciprofloxacin	U	R	618	87
Doxycyclin	O	R	59	83
Erythromycin	O	R	183	93
Levofloxacin	U	R	88	88
Linezolid	B	S	41	95
Nitrofurantoin	U	S	620	81
Penicilin	A	R	359	95
Rifampin	O	R	19	84
Vancomycin	B	R	429	87

Antibiotic	No Lab incorrect used
Amikacin	135
Amoxicilin	54
Azitromycin	47
Ceazolin	53
Cefixim	89
Cefotaxim	68
Ceftazidim	38
Ceftriaxon	107
Cephalotin	39
Chloramphenicol	40
Clindamycin	40
Gentamycin	367
Imipenem	69
Nalidixic acid	77
Norfloxacin	119

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®* 49226

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

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

General Comments

(1) 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.

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

(3) 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 cefmetazole, cefotetan, cefoxitin, and spectinomycin have a documented lower clinical cure rate (85% to 95%) compared with >95% for susceptible strains.

(4) 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.

Table 2F. *Neisseria gonorrhoeae* (Continued)

Test/Report Group	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									
O	Penicillin	10 units	≥47	27–46	≤26	≤0.06	0.12–1	≥2	See general comment (3). **(5)** A positive β-lactamase test predicts resistance to penicillin, ampicillin, and amoxicillin. **(6)** 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. **(7)** Gonococci that produce zones of inhibition of ≤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.)									
A	Ceftriaxone	30 µg	≥35	–	–	≤0.25	–	–	
O	Cefoxitin	30 µg	≥28	24–27	≤23	≤2	4	≥8	See general comment (2).
O	Cefuroxime	30 µg	≥31	26–30	≤25	≤1	2	≥4	See general comment (3).
O	Cefepime	30 µg	≥31	–	–	≤0.5	–	–	
O	Cefmetazole	30 µg	≥33	28–32	≤27	≤2	4	≥8	See general comment (2).
O	Cefotaxime	30 µg	≥31	–	–	≤0.5	–	–	
O	Cefotetan	30 µg	≥26	20–25	≤19	≤2	4	≥8	See general comment (2).
O	Ceftazidime	30 µg	≥31	–	–	≤0.5	–	–	
O	Ceftizoxime	30 µg	≥38	–	–	≤0.5	–	–	
CEPHEMS (ORAL)									
A	Cefixime	5 µg	≥31	–	–	≤0.25	–	–	
O	Cefpodoxime	10 µg	≥29	–	–	≤0.5	–	–	
Inv.	Cefetamet	10 µg	≥29	–	–	≤0.5	–	–	

Table 2F. *Neisseria gonorrhoeae* (Continued)

Test/Report Group	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	
TETRACYCLINES **(8)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.									
A	Tetracycline	30 µg	≥38	31–37	≤30	≤0.25	0.5–1	≥2	**(9)** Gonococci with 30-µg tetracycline disk zone diameters of ≤19 mm usually indicate a plasmid-mediated tetracycline-resistant *N. gonorrhoeae* isolate. Resistance in these strains should be confirmed by a dilution test (MIC ≥16 µg/mL).
FLUOROQUINOLONES See general comment (3).									
A	Ciprofloxacin	5 µg	≥41	28–40	≤27	≤0.06	0.12–0.5	≥1	
O	Enoxacin	10 µg	≥36	32–35	≤31	≤0.5	1	≥2	
O	Lomefloxacin	10 µg	≥38	27–37	≤26	≤0.12	0.25–1	≥2	
O	Ofloxacin	5 µg	≥31	25–30	≤24	≤0.25	0.5–1	≥2	
Inv.	Fleroxacin	5 µg	≥35	29–34	≤28	≤0.25	0.5	≥1	
AMINOCYCLITOLS									
O	Spectinomycin	100 µg	≥18	15–17	≤14	≤32	64	≥128	See general comment (2).

Abbreviations: ATCC®, American Type Culture Collection; I, intermediate; MHB, Mueller-Hinton broth; MIC, minimal inhibitory concentration; QC, quality control; NAD, nicotinamide adenine dinucleotide; R, resistant; S, susceptible.

Table 2F. *Neisseria gonorrhoeae* (Continued)

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Test/Report Group	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									
A	Azithromycin	–	–	–	–	≤1	–	–	**(8)** 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 **(9)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.									
A	Tetracycline	30 µg	≥38	31–37	≤30	≤0.25	0.5–1	≥2	**(10)** Gonococci with 30-µg tetracycline disk zone diameters of ≤19 mm usually indicate a plasmid-mediated tetracycline-resistant *N. gonorrhoeae* isolate. Resistance in these strains should be confirmed by a dilution test (MIC ≥16 µg/mL).
FLUOROQUINOLONES See general comment (3).									
A	Ciprofloxacin	5 µg	≥41	28–40	≤27	≤ 0.06	0.12–0.5	≥1	
AMINOCYCLITOLS									
O	Spectinomycin	100 µg	≥18	15–17	≤14	≤ 32	64	≥128	See general comment (2).

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.

Table 2F. *Neisseria gonorrhoeae* (Continued)

Test/Report Group	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									
A	Azithromycin M100 – 31 - 2021	**15 µg**	**≥ 30**	-	-	≤1	-	-	**(8)** 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									
(9) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.									
A	Tetracycline	30 µg	≥38	31-37	≤30	≤0.25	0.5-1	≥2	**(10)** Gonococci with 30-µg tetracycline disk zone diameters of ≤19 mm usually indicate a plasmid-mediated tetracycline-resistant *N. gonorrhoeae* isolate. Resistance in these strains should be confirmed by a dilution test (MIC ≥16 µg/mL).
FLUOROQUINOLONES									
See general comment (3).									
A	Ciprofloxacin	5 µg	≥41	28-40	≤27	≤ 0.06	0.12-0.5	≥1	
AMINOCYCLITOLS									
O	Spectinomycin	100 µg	≥18	15-17	≤14	≤ 32	64	≥128	See general comment (2).

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.

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% 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) 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.

(2) 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).

(3) 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. Their *in vitro* activity is best determined using an MIC method.

(4) For *S. pneumoniae* isolated from CSF, penicillin and cefotaxime, ceftriaxone, or meropenem should be tested by a reliable MIC method (such as that described in M07[1]) and reported routinely. Such isolates can also be tested against vancomycin using the MIC or disk diffusion method.

(5) 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.

Table 2G. *Streptococcus pneumoniae* (Continued)

Test/Report Group	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 **(5)** For nonmeningitis isolates, a penicillin MIC of ≤0.06 µg/mL (or oxacillin zone ≥20 mm) can predict susceptibility to the following β-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 (4).									
A	Penicillin	1 µg oxacillin	≥20	–	–	–	–	–	**(6)** Isolates of pneumococci with oxacillin zone sizes of ≥20 mm are susceptible (MIC ≤0.06 µg/mL) to penicillin. Penicillin and cefotaxime, ceftriaxone, or meropenem MICs should be determined for those isolates with oxacillin zone diameters of ≤19 mm, because zones of ≤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.
A	Penicillin parenteral (nonmeningitis)	–	–	–	–	≤2	4	≥8	**(7)** ***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. **(8)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
A	Penicillin parenteral (meningitis)	–	–	–	–	≤0.06	–	≥0.12	**(9)** ***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). **(10)** For CSF isolates, report only meningitis interpretations. See general comment (4).
A	Penicillin (oral penicillin V)	–	–	–	–	≤0.06	0.12–1	≥2	**(11)** Interpretations for oral penicillin may be reported for isolates other than those from CSF.

Table 2G. *Streptococcus pneumoniae* (Continued)

Test/Report Group	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)									
C	Amoxicillin (nonmeningitis)	–	–	–	–	≤2	4	≥8	
C	Amoxicillin-clavulanate (nonmeningitis)					≤2/1	4/2	≥8/4	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) See comment (5).									
O	Cefepime (meningitis)	–	–	–	–	≤0.5	1	≥2	**(12)** 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.
B	Cefepime (nonmeningitis)	–	–	–	–	≤1	2	≥4	**(13)** In the United States, only report interpretations for nonmeningitis and include the nonmeningitis notation on the report.
B	Cefotaxime (meningitis)	–	–	–	–	≤0.5	1	≥2	**(14)** For CSF isolates, report only meningitis interpretations. **(15) *Rx:*** Use of cefotaxime or ceftriaxone in meningitis requires therapy with maximum doses. See general comment (4).
B	Ceftriaxone (meningitis)	–	–	–	–	≤0.5	1	≥2	
B	Cefotaxime (nonmeningitis)	–	–	–	–	≤1	2	≥4	**(16)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
B	Ceftriaxone (nonmeningitis)	–	–	–	–	≤1	2	≥4	
C	Ceftaroline (nonmeningitis)	30 µg	≥26	–	–	≤0.5	–	–	**(17)** Breakpoints are based on a dosage regimen of 600 mg every 12 h.
C	Cefuroxime (parenteral)	–	–	–	–	≤0.5	1	≥2	
CEPHEMS (ORAL) See comment (5).									
C	Cefuroxime (oral)	–	–	–	–	≤1	2	≥4	
O	Cefaclor	–	–	–	–	≤1	2	≥4	
O	Cefdinir	–	–	–	–	≤0.5	1	≥2	
O	Cefpodoxime	–	–	–	–	≤0.5	1	≥2	
O	Cefprozil	–	–	–	–	≤2	4	≥8	
O	Loracarbef	–	–	–	–	≤2	4	≥8	

Table 2G. *Streptococcus pneumoniae* (Continued)

Test/Report Group	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 See comment (5).									
B	Meropenem	–	–	–	–	≤0.25	0.5	≥1	See general comment (4) and comment (6).
C	Ertapenem	–	–	–	–	≤1	2	≥4	
C	Imipenem	–	–	–	–	≤0.12	0.25–0.5	≥1	
O	Doripenem	–	–	–	–	≤1	–	–	
GLYCOPEPTIDES									
B	Vancomycin	30 µg	≥17	–	–	≤1	–	–	See general comment (4).
MACROLIDES **(18)** Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(19)** Not routinely reported for organisms isolated from the urinary tract.									
A	Erythromycin	15 µg	≥21	16–20	≤15	≤0.25	0.5	≥1	
O	Azithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
O	Clarithromycin	15 µg	≥21	17–20	≤16	≤0.25	0.5	≥1	
O	Dirithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
O	Telithromycin	15 µg	≥19	16–18	≤15	≤1	2	≥4	
TETRACYCLINES **(20)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.									
B	Tetracycline	30 µg	≥28	25–27	≤24	≤1	2	≥4	
B	Doxycycline	30 µg	≥28	25–27	≤24	≤0.25	0.5	≥1	
FLUOROQUINOLONES									
B	Gemifloxacin	5 µg	≥23	20–22	≤19	≤0.12	0.25	≥0.5	**(21)** *S. pneumoniae* isolates susceptible to levofloxacin are predictably susceptible to gemifloxacin and moxifloxacin. However, *S. pneumoniae* susceptible to gemifloxacin or moxifloxacin cannot be assumed to be susceptible to levofloxacin.
B	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
B	Moxifloxacin	5 µg	≥18	15–17	≤14	≤1	2	≥4	
O	Gatifloxacin	5 µg	≥21	18–20	≤17	≤1	2	≥4	
O	Ofloxacin	5 µg	≥16	13–15	≤12	≤2	4	≥8	
O	Sparfloxacin	5 µg	≥19	16–18	≤15	≤0.5	1	≥2	
FOLATE PATHWAY ANTAGONISTS									
A	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥19	16–18	≤15	≤0.5/9.5	1/19–2/38	≥4/76	
PHENICOLS									
C	Chloramphenicol	30 µg	≥21	–	≤20	≤4	–	≥8	See comment (19).
ANSAMYCINS									
C	Rifampin	5 µg	≥19	17–18	≤16	≤1	2	≥4	**(22) *Rx:*** Rifampin should not be used alone for antimicrobial therapy.

Table 2G. *Streptococcus pneumoniae* (Continued)

Test/Report Group	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									
B	Clindamycin	2 µg	≥19	16–18	≤15	≤0.25	0.5	≥1	**(23)** Inducible clindamycin resistance can be detected by disk diffusion using the D-zone test or by broth microdilution using the single-well test (containing both erythromycin and clindamycin) (see Table 3**G**, Subchapter 3.9 in M02,[2] and Subchapter 3.12 in M07[1]). See comment (19).
STREPTOGRAMINS									
O	Quinupristin-dalfopristin	15 µg	≥19	16–18	≤15	≤1	2	≥4	
OXAZOLIDINONES									
C	Linezolid	30 µg	≥21	–	–	≤2	–	–	

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

Table 2H-1. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. β-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®* 49619

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

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

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

General Comments

(1) 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. 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.

(2) For β-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).

(3) For this table, the β-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 β-hemolytic strains with group A, C, F, or G antigens (*S. anginosus* group, previously termed "*S. milleri*") are considered part of the viridans group, and breakpoints for the viridans group should be used (see Table 2H-2).

(4) Penicillin and ampicillin are drugs of choice for treatment of β-hemolytic streptococcal infections. Susceptibility testing of penicillins and other β-lactams approved by the US Food and Drug Administration for treatment of β-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 β-hemolytic streptococcus and have not been reported for *S. pyogenes*. If testing is performed, any β-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.)

Test/Report Group	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 **(6)** 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 β-hemolytic streptococci, penicillin is a surrogate for ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefazolin, cefepime, ceftaroline, cephradine, cephalothin, cefotaxime, ceftriaxone, ceftizoxime, imipenem, ertapenem, and meropenem. For group A β-hemolytic streptococci, penicillin is also a surrogate for cefaclor, cefdinir, cefprozil, ceftibuten, cefuroxime, and cefpodoxime.									
A	Penicillin or	10 units	≥24	–	–	≤0.12	–	–	See general comment (4).
A	ampicillin	10 µg	≥24	–	–	≤0.25	–	–	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) See comment (6).									
B	Cefepime or	30 µg	≥24	–	–	≤0.5	–	–	
B	cefotaxime or	30 µg	≥24	–	–	≤0.5	–	–	
B	ceftriaxone	30 µg	≥24	–	–	≤0.5	–	–	
C	Ceftaroline	30 µg	≥26	–	–	≤0.5	–	–	**(7)** Breakpoints are based on a dosage regimen of 600 mg administered every 12 h.
CARBAPENEMS See comment (6).									
O	Doripenem	–	–	–	–	≤0.12	–	–	
O	Ertapenem	–	–	–	–	≤1	–	–	
O	Meropenem	–	–	–	–	≤0.5	–	–	
GLYCOPEPTIDES									
B	Vancomycin	30 µg	≥17	–	–	≤1	–	–	
LIPOGLYCOPEPTIDES									
C	Dalbavancin	–	–	–	–	≤0.25	–	–	**(8)** For reporting against *S. pyogenes*, *S. agalactiae*, and *S. dysgalactiae*.
C	Oritavancin	–	–	–	–	≤0.25	–	–	
C	Telavancin	–	–	–	–	≤0.12	–	–	
LIPOPEPTIDES									
C	Daptomycin	–	–	–	–	≤1	–	–	**(9)** Daptomycin should not be reported for isolates from the respiratory tract.

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

Test/Report Group	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 **(10)** Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(11)** Not routinely reported on isolates from the urinary tract.									
A	Erythromycin	15 µg	≥21	16–20	≤15	≤0.25	0.5	≥1	**(12)** ***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 **3H**.
O	Azithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
O	Clarithromycin	15 µg	≥21	17–20	≤16	≤0.25	0.5	≥1	
O	Dirithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
TETRACYCLINES **(13)** 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.									
O	Tetracycline	30 µg	≥23	19–22	≤18	≤2	4	≥8	
FLUOROQUINOLONES									
C	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
O	Gatifloxacin	5 µg	≥21	18–20	≤17	≤1	2	≥4	
O	Grepafloxacin	5 µg	≥19	16–18	≤15	≤0.5	1	≥2	
O	Ofloxacin	5 µg	≥16	13–15	≤12	≤2	4	≥8	
O	Trovafloxacin	10 µg	≥19	16–18	≤15	≤1	2	≥4	
PHENICOLS									
C	Chloramphenicol	30 µg	≥21	18–20	≤17	≤4	8	≥16	See comment (11).

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

Test/Report Group	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									
A	Clindamycin	2 µg	≥19	16–18	≤15	≤0.25	0.5	≥1	See comments (11) and (12). **(14) 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 **3H**, Subchapter 3.9 in M02,[3] and Subchapter 3.12 in M07.[1]
STREPTOGRAMINS									
O	Quinupristin-dalfopristin	15 µg	≥19	16–18	≤15	≤1	2	≥4	**(15)** Report against *S. pyogenes*.
OXAZOLIDINONES									
C	Linezolid	30 µg	≥21	–	–	≤2	–	–	
C	Tedizolid	–	–	–	–	≤0.5	–	–	**(16)** For reporting against *S. pyogenes* and *S. agalactiae* only.

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

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®* 49619

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

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

General Comments

(1) 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.

(2) 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).

(3) 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 β-hemolytic strains with groups A, C, F, and G antigens. For detailed information on the species within the groups, please refer to recent literature.

(4) 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 boldface type is new or modified since the previous edition.

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

Test/Report Group	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									
A	Penicillin	–	–	–	–	≤0.12	0.25–2	≥4	**(5)** Viridans streptococci isolated from normally sterile body sites (eg, CSF, blood, bone) should be tested for penicillin susceptibility using an MIC method. **(6)** 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. **(7)** ***Rx:*** Penicillin- or ampicillin-intermediate isolates may necessitate combined therapy with an aminoglycoside for bactericidal action.
A	Ampicillin					≤0.25	0.5–4	≥8	
β-LACTAM COMBINATION AGENTS									
C	Ceftolozane-tazobactam	–	–	–	–	≤8/4	16/4	≥32/4	**(8)** Breakpoints are based on a dosage regimen of 1.5 g every 8 h.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
B	Cefepime	30 µg	≥24	22–23	≤21	≤1	2	≥4	
B	Cefotaxime	30 µg	≥28	26–27	≤25	≤1	2	≥4	
B	Ceftriaxone	30 µg	≥27	25–26	≤24	≤1	2	≥4	
CARBAPENEMS									
O	Doripenem	–	–	–	–	≤1	–	–	
O	Ertapenem	–	–	–	–	≤1	–	–	
O	Meropenem	–	–	–	–	≤0.5	–	–	
GLYCOPEPTIDES									
B	Vancomycin	30 µg	≥17	–	–	≤1	–	–	
LIPOGLYCOPEPTIDES									
C	**Dalbavancin**	**–**	**–**	**–**	**–**	**≤0.25**	**–**	**–**	**(9) For reporting against *S. pyogenes*, *S. agalactiae*, *S. dysgalactiae*, and *S. anginosus* group.**
C	Oritavancin	–	–	–	–	≤0.25	–	–	
C	Telavancin	–	–	–	–	≤0.06	–	–	
LIPOPEPTIDES									
O	Daptomycin	–	–	–	–	≤1	–	–	**(10)** Daptomycin should not be reported for isolates from the respiratory tract.

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

Test/Report Group	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 **(11)** Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(12)** Not routinely reported on isolates from the urinary tract.									
C	Erythromycin	15 µg	≥21	16–20	≤15	≤0.25	0.5	≥1	
O	Azithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
O	Clarithromycin	15 µg	≥21	17–20	≤16	≤0.25	0.5	≥1	
O	Dirithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
TETRACYCLINES **(13)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.									
O	Tetracycline	30 µg	≥23	19–22	≤18	≤2	4	≥8	
FLUOROQUINOLONES									
O	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
O	Ofloxacin	5 µg	≥16	13–15	≤12	≤2	4	≥8	
O	Gatifloxacin	5 µg	≥21	18–20	≤17	≤1	2	≥4	
O	Grepafloxacin	5 µg	≥19	16–18	≤15	≤0.5	1	≥2	
O	Trovafloxacin	10 µg	≥19	16–18	≤15	≤1	2	≥4	
PHENICOLS									
C	Chloramphenicol	30 µg	≥21	18–20	≤17	≤4	8	≥16	See comment (12).
LINCOSAMIDES									
C	Clindamycin	2 µg	≥19	16–18	≤15	≤0.25	0.5	≥1	See comment (12).
STREPTOGRAMINS									
O	Quinupristin-dalfopristin	15 µg	≥19	16–18	≤15	≤1	2	≥4	
OXAZOLIDINONES									
C	Linezolid	30 µg	≥21	–	–	≤2	–	–	
C	Tedizolid	–	–	–	–	≤0.25	–	–	**(14)** For reporting against *S. anginosus* group (includes *S. anginosus, S. intermedius,* and *S. constellatus*) only.

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.

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 taken into account 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®* 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.

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

General Comments

Important: For complete information on safety precautions, see *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Department of Health and Human Services; 2009. http://www.cdc.gov/biosafety/publications/bmbl5/. Accessed November 20, 2017.

(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, available at 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.

Test/Report Group	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									
C	Penicillin		–	–	–	≤0.06	0.12–0.25	≥0.5	????????
C	Ampicillin		–	–	–	≤0.12	0.25–1	≥2	
CEPHEMS									
C	Cefotaxime or	30 µg	≥34	–	–	≤0.12	–	–	
C	ceftriaxone	30 µg	≥34	–	–	≤0.12	–	–	
CARBAPENEMS									
C	Meropenem	10 µg	≥30	–	–	≤0.25	–	–	
MACROLIDES									
C	Azithromycin	15 µg	≥20	–	–	≤2	–	–	See general comment (6). **(7)** May be appropriate only for prophylaxis of meningococcal case contacts. These breakpoints do not apply to therapy of patients with invasive meningococcal disease.
TETRACYCLINES									
C	Minocycline	30 µg	≥26	–	–	≤2	–	–	See comment (7).
FLUOROQUINOLONES **(8)** For surveillance purposes, a nalidixic acid MIC ≥8 µg/mL or a zone ≤25 mm may correlate with diminished fluoroquinolone susceptibility.									
C	Ciprofloxacin	5 µg	≥35	33–34	≤32	≤0.03	0.06	≥0.12	See comment (7).
C	Levofloxacin	–	–	–	–	≤0.03	0.06	≥0.12	

Table 2I. *Neisseria meningitidis* (Continued)

Test/Report Group	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									
C	Sulfisoxazole	–	–	–	–	≤2	4	≥8	See comment (7).
C	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥30	26–29	≤25	≤0.12/2.4	0.25/4.75	≥ 0.5/9.5	**(9)** 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									
C	Chloramphenicol	30 µg	≥26	20–25	≤19	≤2	4	≥8	**(10)** Not routinely reported on isolates from the urinary tract.
ANSAMYCINS									
C	Rifampin	5 µg	≥25	20–24	≤19	≤0.5	1	≥2	See comment (7).

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.

ESBL

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*: 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.	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 twofold 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).
Reporting			For all confirmed ESBL-producing strains: If laboratories do not use current cephalosporin and aztreonam breakpoints, the test interpretation should be reported as resistant for all penicillins, cephalosporins, and aztreonam. 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). *E. coli* ATCC® 25922 (see acceptable QC ranges in Table 4A-1) *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	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). *E. coli* ATCC® 25922 = no growth (see acceptable QC ranges listed in Table 5A-1) *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	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be used for routine QC (eg, weekly or daily). **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. *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.	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be tested routinely (eg, weekly or daily). **Acceptable QC:** *E. coli* ATCC® 25922: < 3 twofold 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: ≥ 3 twofold 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; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK-PD, pharmacokinetic-pharmacodynamic; QC, quality control.

Tests for Carbapenemases in *Enterobacteriaceae and Pseudomonas aeruginosa*

Institutional infection control procedures or epidemiological investigations may necessitate identification of carbapenemase-producing *Enterobacteriaceae and P. aeruginosa*. ***Such testing is not currently recommended for routine use.***

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Table 3E. 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
Medium	MHA
Antimicrobial concentration	Standard disk content for the antimicrobials listed below: • Ampicillin 10 µg • Aztreonam 30 µg • Ceftazidime 30 µg • Ceftriaxone 30 µg • Tobramycin 10 µg • Trimethoprim-sulfamethoxazole 1.25/23.75 µg
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	16-18 hours
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 2A if the gram-negative bacillus tested is confirmed to be an Enterobacterales. If species is identified as another organism, do not interpret or report results.

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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
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-2. Enterobacterales (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Read Times, hours	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Comments
				S	SDD	I	R	
PENICILLINS								
A	Ampicillin	10 µg	8-10	-	-	-	-	(3) Results of ampicillin testing can be used to predict results for amoxicillin. (4) 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.
			16-18	≥ 17	-	14-16	≤ 13	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
B	Ceftriaxone	30 µg	8-10	≥ 23	-	20-22	≤ 19	(5) Breakpoints are based on a dosage regimen of 1 g administered every 24 h.
			16-18	≥ 23	-	20-22	≤ 19	
C	Ceftazidime	30 µg	8-10	≥ 21	-	18-20	≤ 17	(6) Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
			16-18	≥ 21	-	18-20	≤ 17	
MONOBACTAMS								
C	Aztreonam	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	
AMINOGLYCOSIDES								
A	Tobramycin	10 µg	8-10	≥ 15	-	13-14	≤ 12	
			16-18	≥ 15	-	13-14	≤ 12	
FOLATE PATHWAY ANTAGONISTS								
B	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-3. Zone Diameter Disk Diffusion Breakpoints for *Pseudomonas aeruginosa* Direct From Blood Culture

General Comments

(1) 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 infectious diseases practitioners, pharmacists, pharmacy and therapeutics committees, infection prevention committees, and the antimicrobial stewardship team.

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

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

Test/Report Group	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.)								
A	Ceftazidime	30 µg	8-10	-	-	-	-	(3) 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								
B	Meropenem	10 µg	8-10	-	-	-	-	(4) Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
			16-18	≥ 19	-	16-18	≤ 15	
AMINOGLYCOSIDES								
A	Tobramycin	10 µg	8-10	≥ 15	-	13-14	≤ 12	
			16-18	≥ 15	-	13-14	≤ 12	
FLUOROQUINOLONES								
B	Ciprofloxacin	5 µg	8-10	≥ 23	-	18-22	≤ 17	(5) 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.

Table 3E. Test for Detection of β-Lactamase Production in *Staphylococcus* spp.

Test	β-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") = β-lactamase positive (see Figure 1 below this table) Fuzzy zone edge ("beach") = β-lactamase negative (see Figure 2 below this table)	Nitrocefin-based test: conversion from yellow to red/pink = β-lactamase positive.
Additional testing and reporting	β-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. β-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.

Footnotes

The penicillin disk diffusion zone-edge test was shown to be more sensitive than nitrocefin-based tests for detection of ß-lactamase production in *S. aureus.*The penicillin zone-edge test is recommended if only one test is used for ß-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 ß-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)

For *S. lugdunensis, tests for ß-lactamase detection are not necessary because isolates producing a ß-lactamase will test penicillin resistant (MIC* >0.12 µg/mLand zone diameters <29 mm).

Table 3E. (Continued)

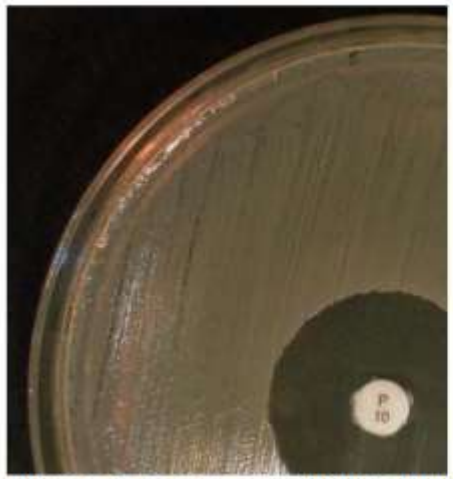


Figure 1. Positive Penicillin Disk Zone-Edge Test for β-Lactamase Detection. The zone edge is sharp or like a “cliff” indicating β-lactamase production.

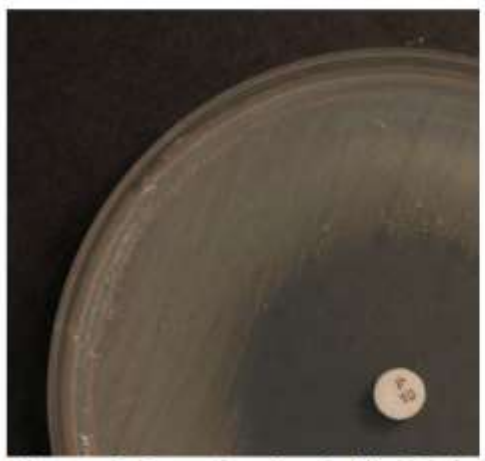


Figure 2. Negative Penicillin Disk Zone-Edge Test for β-Lactamase Detection. The zone edge is fuzzy or like a “beach,” indicating no β-lactamase production.

S. aureus with sharp zone edge and zone diameter ≥ 26 mm = Resistant

S. aureus with fuzzy zone edge and zone diameter ≥ 26 mm = Susceptible

Table 3F. Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp.

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin			Detecting *mecA*-Mediated Resistance Using Oxacillin			Detecting *mecA*-mediated Resistance Using Oxacillin Salt Agar
Test method	**Disk Diffusion**		**Broth Microdilution**	**Disk Diffusion**	**Broth Microdilution and Agar Dilution**		**Agar Dilution**
Organism group	*S. aureus* and *S. lugdunensis*	Other *Staphylococcus* spp. (excluding *S. pseudintermedius* and *S. schleiferi*)	***S. aureus* and *S. lugdunensis***	***S. epidermidis, S. pseudintermedius,* and *S. schleiferi***	***S. aureus* and *S. lugdunensis***	***Staphylococcus* spp. (excluding *S. aureus* and *S. lugdunensis*)**	*S. aureus*
Medium	MHA		CAMHB	**MHA**	**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	**1-µg oxacillin disk**	**2 µg/mL oxacillin**	**0.25 µg/mL oxacillin**	6 µg/mL oxacillin
Inoculum	Standard disk diffusion procedure		Standard broth microdilution Procedure	**Standard disk diffusion 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[a]		33 to 35°C; ambient air[a]	**33 to 35°C; ambient air[a]**	**33 to 35°C; ambient air[a]**		33 to 35°C; ambient air[a]
Incubation length	16–18 hours	24 hours (may be reported after 18 hours, if resistant)	16–20 hours	**16–18 hours**	**24 hours (may be reported after 18 hours, if resistant)**		24 hours; read with transmitted light
Results	≤ 21 mm = *mecA* positive ≥ 22 mm = *mecA* negative	≤ 24 mm = *mecA* positive ≥ 25 mm = *mecA* negative	**≥ 8** µg/mL =*mecA* positive ≤ 4 µg/mL = *mecA* negative	**≤ 17 mm = *mecA* positive** **≥ 18 mm = *mecA* negative**	**≥4 µg/mL = *mecA* positive** **≤ 2 µg/mL = *mecA* negative**	**≥ 0.5 µg/mL = *mecA* positive** **≤ 0.25 µg/mL = *mecA* negative**	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = oxacillin resistant

Table 3F. (Continued)

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin		Detecting *mecA*-Mediated Resistance Using Oxacillin		Detecting *mecA*-mediated Resistance Using Oxacillin Salt Agar
Additional testing and reporting	Cefoxitin is used as a surrogate for *mecA*-mediated **methicillin** (oxacillin) resistance. Isolates that test as *mecA* positive 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. .	Cefoxitin is used as a surrogate for *mecA*-mediated **methicillin** (oxacillin) resistance. Isolates that test as *mecA* positive should be reported as **methicillin** (oxacillin) (not cefoxitin) resistant; routine testing of other β-lactam agents, except **ceftaroline**, is not advised. Because of the rare occurrence of **methicillin** (oxacillin) resistance mechanisms other than *mecA*, isolates that test as *mecA* negative, but for which the oxacillin MICs are resistant (MIC ≥ 4 µg/mL), should be reported as **methicillin** (oxacillin) resistant.	**Isolates that test as *mecA* positive should be reported as methicillin or oxacillin (not cefoxitin) resistant; other β-lactam agents, except ceftaroline, should be reported as resistant or should not be reported.** **Because of the rare occurrence of methicillin (oxacillin)-resistance mechanisms other than *mecA*, isolates that test as *mecA* negative but for which the oxacillin MICs are resistant (MIC ≥ 4 µg/mL) should be reported as methicillin (oxacillin) resistant.**	**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 0.5–2 µg/mL may be *mecA* negative. Isolates from serious infections for which oxacillin MICs are 0.5–2 µg/mL may be tested for *mecA* or for PBP2a. Isolates that test *mecA* or PBP2a negative should be reported as methicillin (oxacillin) susceptible.**	**MRS** are resistant to all β-lactam agents with the exception of ceftaroline; other β-lactam agents should be reported as resistant or should not be reported

Table 3F. (Continued)

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin			Detecting *mecA*-Mediated Resistance Using Oxacillin			Detecting Oxacillin Resistance Using Oxacillin Salt Agar
Test method	**Disk Diffusion**		**Broth Microdilution**	**Disk Diffusion**	**Broth Microdilution and Agar Dilution**		**Agar Dilution**
Organism group	*S. aureus* and *S. lugdunensis*	Other *Staphylococcus* spp., excluding *S. pseudintermedius* *S. schleiferi*	*S. aureus* and *S. lugdunensis*	***S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi***	***S. aureus* and *S. lugdunensis***	***Staphylococcus* spp., excluding *S. aureus* and *S. lugdunensis*)**	*S. aureus*
QC recommendations – routine[b]	*S. aureus* ATCC® 25923 – *mecA* negative (cefoxitin zone 23–29 mm)		*S. aureus* ATCC® 29213 – *mecA* negative (cefoxitin MIC 1–4 µg/mL)	***S. aureus* ATCC® 25923 – *mecA* negative (oxacillin zone 18–24 mm)**	***S. aureus* ATCC® 29213 – *mecA* negative (oxacillin MIC 0.12–0.5 µg/mL)**		*S. aureus* ATCC®[c] 29213 – susceptible (with each test day)
QC recommendations – lot/shipment[d]			*S. aureus* ATCC® 43300 – *mecA* positive (MIC > 4 µg/mL)	***S. aureus* ATCC® 43300 – *mecA* positive (zone ≤ 24 mm)**	***S. aureus* ATCC® 43300 – *mecA* positive (MIC > 4 µg/mL)**		*S. aureus* ATCC® 43300 – resistant

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; **MRS, methicillin (oxacillin)-resistant staphylococci**; PBP2a, penicillin-binding protein 2a; QC, quality control.

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]	*S. aureus* ATCC®[f] 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[g]	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-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 β-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 3H. Test for Detecting Inducible Clindamycin Resistance in *Staphylococcus* spp., *Streptococcus pneumoniae,* and *Streptococcus* spp. β-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 β-hemolytic *Streptococcus* spp.	**All *Staphylococcus* spp.[c]**	*S. pneumoniae* and β-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 3H. (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 β-hemolytic *Streptococcus* spp.	**All *Staphylococcus* spp.**[c]	*S. pneumoniae* and β-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]			*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.

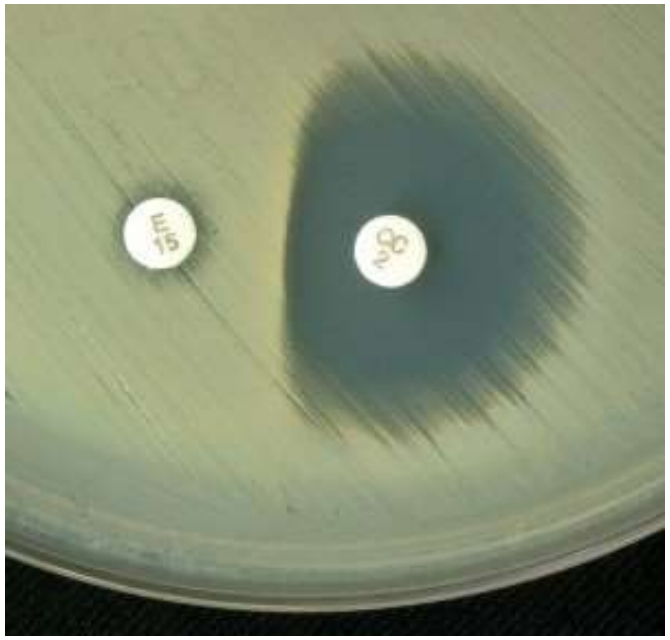
a. Antimicrobial susceptibility testing of β-hemolytic streptococci does not need to be performed routinely (see general comment [4] in Table 2H-1). When susceptibility testing is clinically indicated, **test** for **ICR in strains that are erythromycin resistant and clindamycin susceptible or intermediate**.

The “D” Test

- Is Staph aureus really susceptible to Clindamycin
- Why?? During therapy, S aureus isolates resistant to Erythromycin possess enzymes capable of inducing Clindamycin resistance
- Kirby Bauer zone around Clindamycin will be blunted to form a “D” if Clindamycin can be induced by Erythromycin to be resistant – so called **INDUCIBLE RESISTANCE**.
- Clindamycin should be reported as resistant by clindamycin induction and not used for therapy.

D test **positive** Inducible resistance

D test **negative** Clindamycin can be used for therapy



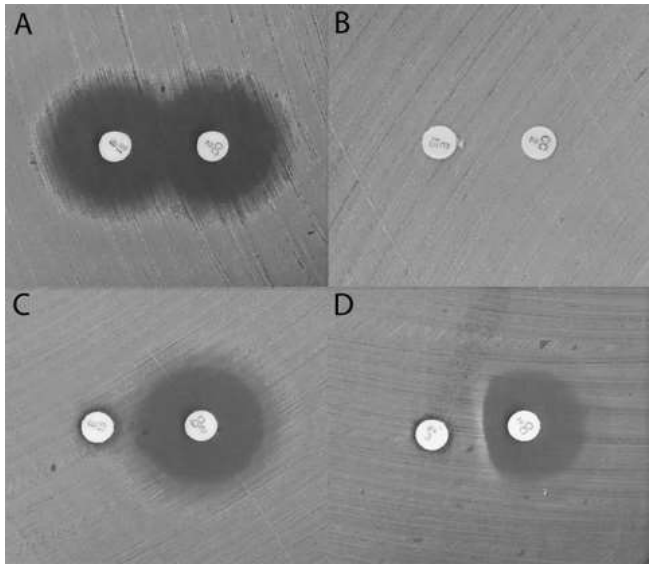


Table 3J. Test for Detecting High-Level Aminoglycoside Resistance in *Enterococcus* spp.[a] (Includes Disk Diffusion)

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
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 low levels of penicillin (MICs 16–64 µg/mL) or ampicillin (MICs 16–32 µg/mL) resistance 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.

Table 3G. 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

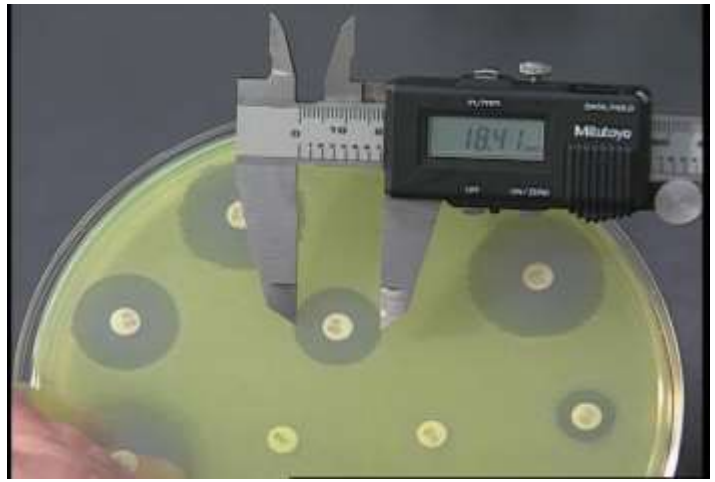
Abbreviations: ATCC®, American Type Culture Collection; BHI, brain heart infusion; MIC, minimal inhibitory concentration; QC, quality control.

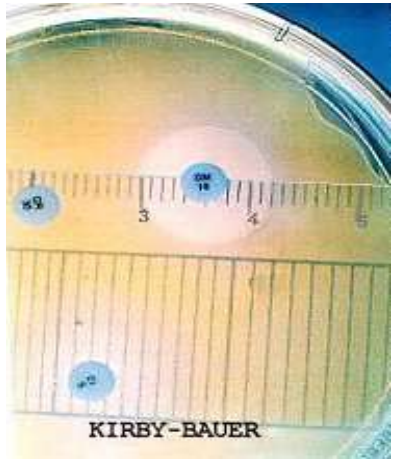
Reading guide

EUCAST disk diffusion method for antimicrobial susceptibility testing

Version 8.0

January 2021





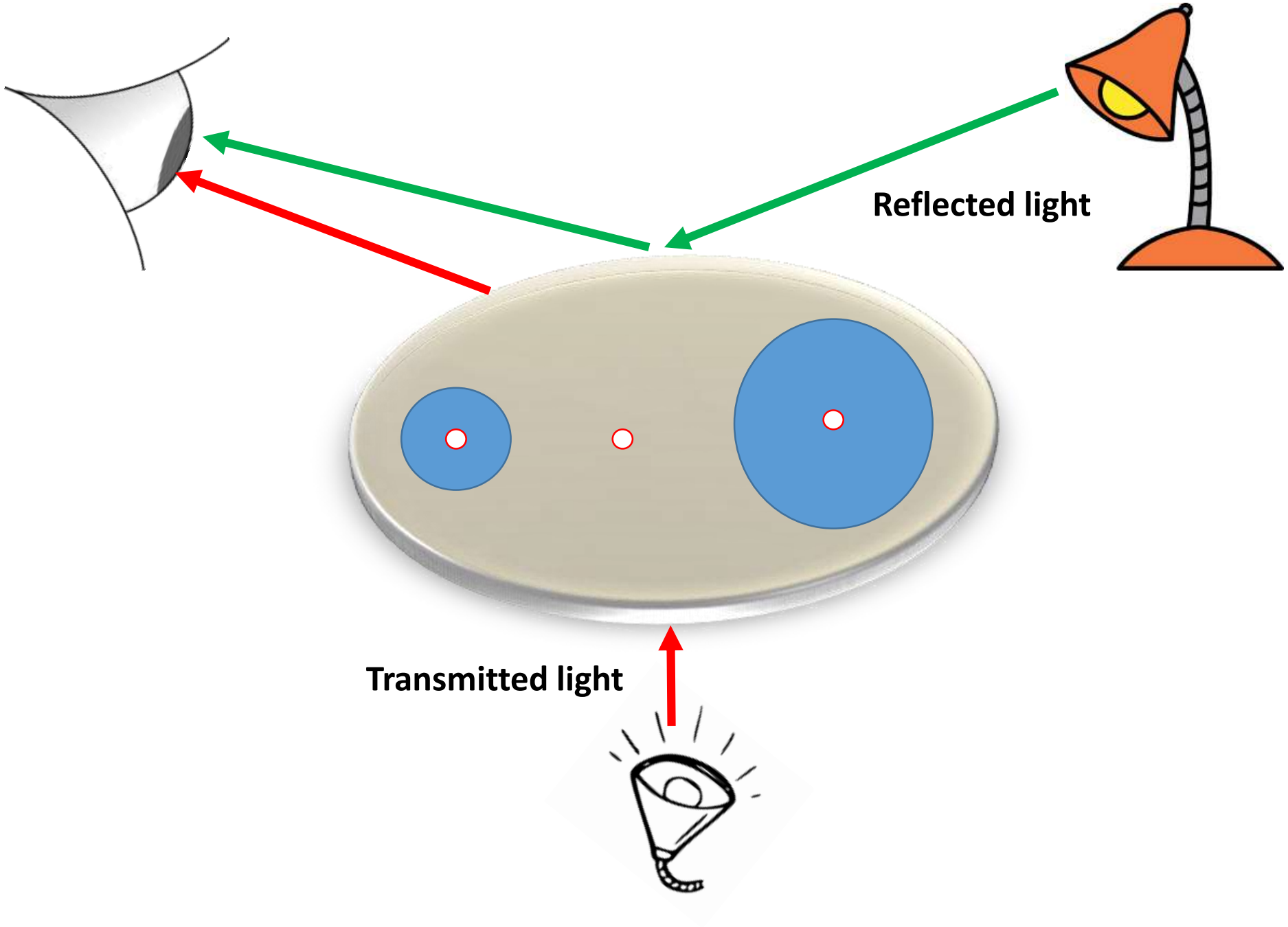
EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Reading guide

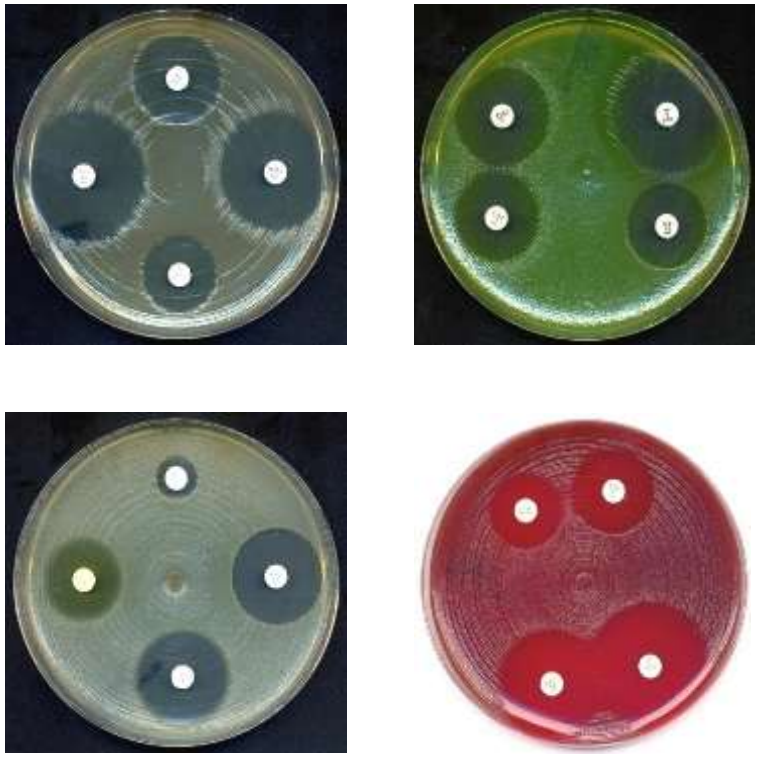
EUCAST disk diffusion method for antimicrobial susceptibility testing

Version 8.0
January 2021


Reflected light
Transmitted light

Examining purity of plate
Select the Colonies from Pure Isolates

The growth should be confluent and evenly spread over the plate



Plates should look like this..

..and NOT like this!

Reading Plates and Interpreting Results

* **16 to 18 hours** of incubation

* If the plate was satisfactorily streaked, and the inoculum was correct, the **zones of inhibition will be uniformly circular** The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc.

* Zones are measured using sliding calipers or a ruler, which is held on the **back of the inverted petri plate**.

* The petri plate is held a few inches above a **black, nonreflecting background** and illuminated with **reflected light**.

* If **blood** was added to the agar base (as with streptococci), the zones are measured from the **upper surface of the agar** illuminated with reflected light, with the **cover removed**.

* If the test organism is a ***Staphylococcus* or *Enterococcus* spp** , **24 hours of incubation** are required for **vancomycin and oxacillin**,

* **Transmitted light (plate held up to light)** is used to examine the **oxacillin and vancomycin** zones for light growth of methicillin- or vancomycin- resistant colonies, Any discernable growth within zone of inhibition is indicative of methicillin or vancomycin resistance.

Reading zones

• Read **MH plates from the back** against a dark background illuminated with reflected light.

• Read **MH-F plates from the** front with the lid removed illuminated with reflected light.

If cefoxitin is tested against Staphylococcus spp., read the zone diameters with reflected, not transmitted, light.

With glycopeptide susceptibility tests on *Enterococcus spp. resistant colonies* may not be visible until plates have been incubated for 24 h. However, plates may be examined after 16-20 h and any resistance reported, but plates of isolates appearing susceptible must be re-incubated and reread at 24 h.

Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored.

When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis.

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.

In case of double zones, or distinct colonies within zones, check for purity and repeat the test if necessary.

For *Stenotrophomonas maltophilia* with trimethoprim-sulfamethoxazole, an isolate showing any sign of inhibition zone = the susceptible breakpoint should be reported susceptible. Note that there may be substantial growth within zones. Read as no zone only if there is growth up to the disk and no sign of an inhibition zone.

For *Aeromonas spp. with trimethoprim-sulfamethoxazole, read the obvious* zone edge and disregard haze or growth within the inhibition zone. If there is an obvious inner zone edge, read the inhibition zone as the inner zone.

For Enterobacterales with ampicillin, ampicillin-sulbactam and amoxicillin clavulanic acid, Ignore growth that may appear as a thin film producing an inner zone on some batches of Mueller-Hinton agar.

For *Staphylococcus aureus with benzylpenicillin, examine the zone edge* closely from the front of the plate with the plate held up to light (transmitted light). Isolates with inhibition zone diameters = the susceptible breakpoint, but with sharp zone edges should be reported resistant.

When using cefoxitin for the detection of methicillin resistance in *Staphylococcus aureus, measure the obvious zone, and examine zones* carefully in good light to detect colonies within the zone of inhibition. These may be either a contaminating species or the expression of heterogeneous methicillin resistance.

For enterococci with vancomycin, examine the zone edge closely from the front of the plate with the plate held up to light (transmitted light). Fuzzy zone edges and colonies within zone indicate vancomycin resistance and should be investigated further. Isolates must not be reported susceptible before 24 h incubation.

Reading zones

- Zone edges should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye.

Examples:

E. coli
Ciprofloxacin

S. aureus
Erythromycin

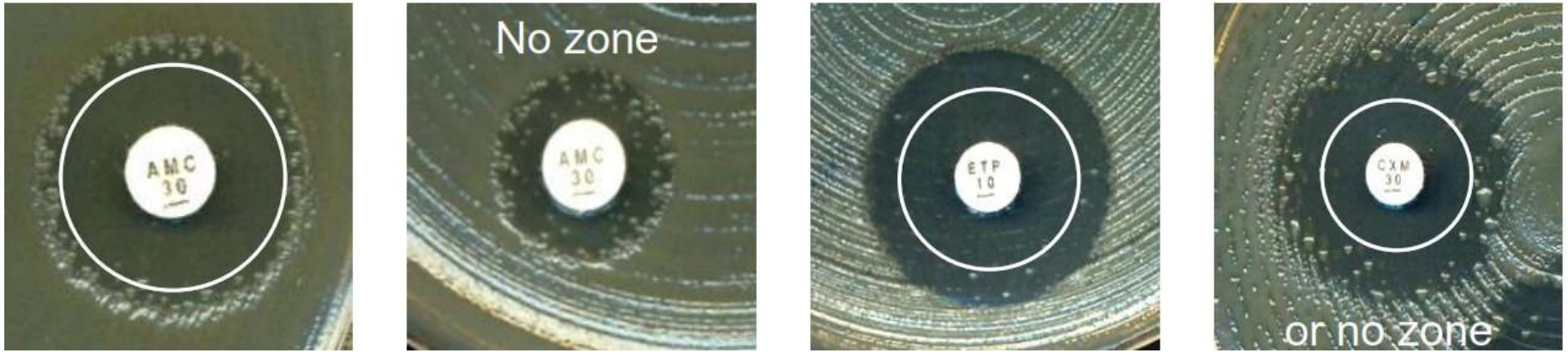
CoNS
Trimethoprim

S. pneumoniae
Rifampicin

Colonies within zone

- In case of distinct colonies within zones, check for purity and repeat the test if necessary.

- If cultures are pure, colonies within zones should be taken into account when measuring the diameter.



Reading of zones with colonies within the zone

Colonies within zone

- In case of distinct colonies within zones, check for purity and repeat the test if necessary.
- If cultures are pure, colonies within zones should be taken into account when measuring the diameter.

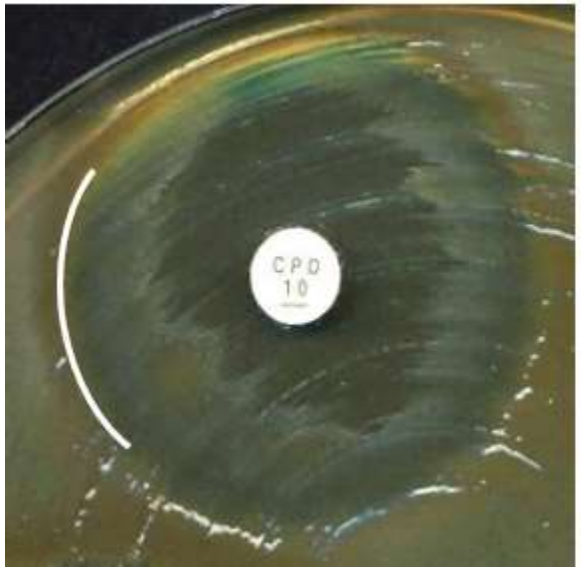
E. coli with ESBL

H. influenzae with PBP mutations

Reading of zones with colonies within the zone.

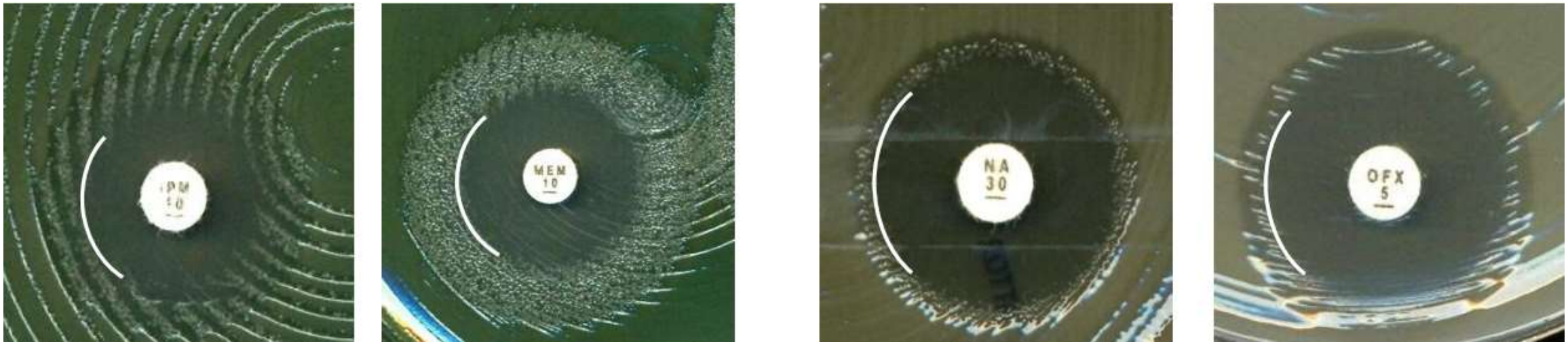
Swarming

- For *Proteus spp., ignore swarming and read* inhibition of growth.



Double zones

- In case of double zones, check for purity and repeat the test if necessary.
- If cultures are pure, read the inner zone.



Reading of double zones.

Fuzzy zone edges
Enterobacterales

Reading of zones with fuzzy zone edges for Enterobacterales.

Fuzzy zone edges

Staphylococci

Reading of zones with fuzzy zone edges for staphylococci.

ß-haemolysis

- Tilt the plate back and forth to better differentiate between haemolysis and growth.
- ß-haemolysis is usually free from growth.

S. pyogenes

Streptococcus group C

a-haemolysis

- Tilt the plate back and forth to better differentiate between haemolysis and growth.

There is usually growth in the whole area of a-haemolysis.

For some organisms, there is additional a-haemolysis without growth.
Tilt the plate to differentiate between haemolysis and growth.

Trimethoprim and trimethoprim-sulfamethoxazole

- Ignore haze or faint growth up to the disk within a zone with otherwise clear zone edge.

E. coli

CoNS

Moraxella

Haemophilus

Stenotrophomonas maltophilia *and* trimethoprim-sulfamethoxazole

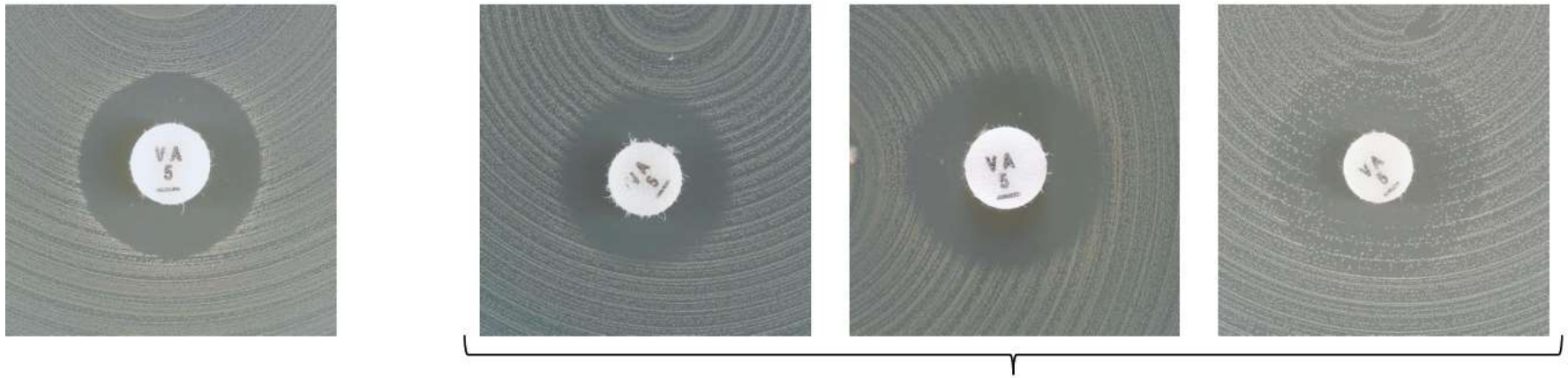
- An isolate showing any sign of inhibition zone = the susceptible breakpoint should be reported susceptible. Note that there may be substantial growth within zones.

Ignore growth and read an inhibition zone if any zone edge can be seen.
= Susceptible if zone diameter ≥ 16 mm

Growth up to the disk and no sign of inhibition zone = Resistant

Enterococci and vancomycin

– Vancomycin-susceptible enterococci exhibit sharp zone edges and do not exhibit colonies in the inhibition zone.
– If the zone edge is fuzzy, if colonies grow within the zone or if you are uncertain, investigate further even if the zone diameter is = 12 mm.



Quality Control
of
Antimicrobial Susceptibility Test

routine quality control strains

Organism	Culture collection numbers	Characteristics
E. coli	ATCC 25922; NCTC 12241 CIP 7624; DSM 1103 CCUG 17620; CECT 434	Susceptible, wild-type
E. coli	ATCC 35218; NCTC 11954 CIP 102181; DSM 5923 CCUG 30600; CECT 943	TEM-1 β-lactamase producer
P. aeruginosa	ATCC 27853; NCTC 12903 CIP 76110; DSM 1117 CCUG 17619; CECT 108	Susceptible, wild-type
S. aureus	ATCC 29213; NCTC 12973 CIP 103429; DSM 2569 CCUG 15915; CECT 794	Weak β-lactamase producer
E. faecalis	ATCC 29212; NCTC 12697 CIP 103214; DSM 2570 CCUG 9997; CECT 795	Susceptible, wild-type

routine quality control strains

Organism	Culture collection numbers	Characteristics
S. pneumoniae	ATCC 49619; NCTC 12977 CIP 104340; DSM 11967 CCUG 33638	Reduced susceptibility to benzylpenicillin
H. influenzae	ATCC 49766; NCTC 12975 CIP 103570; DSM 11970 CCUG 29539	Susceptible, wild-type
Campylobacter jejuni	ATCC 33560; NCTC 11351 CIP 702; DSM 4688 CCUG 11284	Susceptible, wild-type

strains for detection of specific resistance mechanisms (extended QC)

Organism	Culture collection numbers	Characteristics
K. pneumoniae	ATCC 700603; NCTC 13368 CCUG 45421; CECT 7787	ESBL producer (SHV-18)
S. aureus	NCTC 12493	Oxacillin hetero-resistant, *mecA* positive
E. faecalis	ATCC 51299; NCTC 13379 CIP 104676; DSM 12956 CCUG 34289	High-level aminoglycoside resistant (HLAR) and vancomycin resistant (*vanB* positive)
H. influenzae	ATCC 49247; NCTC 12699 CIP 104604; DSM 9999 CCUG 26214	Reduced susceptibility to β-lactam agents due to PBP mutations (BLNAR)

Table 4A-1. Disk Diffusion QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-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	18–26	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	30 μg	30–36	24–30	26–34
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	16–23	–	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	–	–

Table 4A-1. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[a,b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
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
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	–	–[j]
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, QC, quality control.

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®[a,b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d]	*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	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[e]	–	–	–	–
Cefepime	30 µg	31–37	25–31	23–29	**31–37**	23–29	6–15[f]	–	–	6–16[f]
Cefepime-enmetazobactam[e]	**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[e]	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[e]	–	–	–	–
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[e]	–	–	–	–
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[e,g]	10/25 µg	27–33	26–31	–	–	26–32	–	23–29	22–28	–
Meropenem[f]	10 µg	28–35	27–33	29–37	–	–	–	11–18[e]	6[e]	–

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]	*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	KPC-2 SHV	KPC-3 SHV-11 TEM-1	OXA-27
		Zone Diameter QC Ranges, mm								
Meropenem-vaborbactam[g]	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]

Frequency of Quality Control Testing

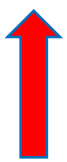
Monitor the overall performance of the test system using the QC limits by testing the appropriate QC strains **each day** the test is performed or, if satisfactory performance is documented test the QC strains **weekly**.

The weekly QC testing option is not applicable when disk diffusion tests are performed less than once a week. QC testing should be performed each test day for disk diffusion tests performed less than once a week.

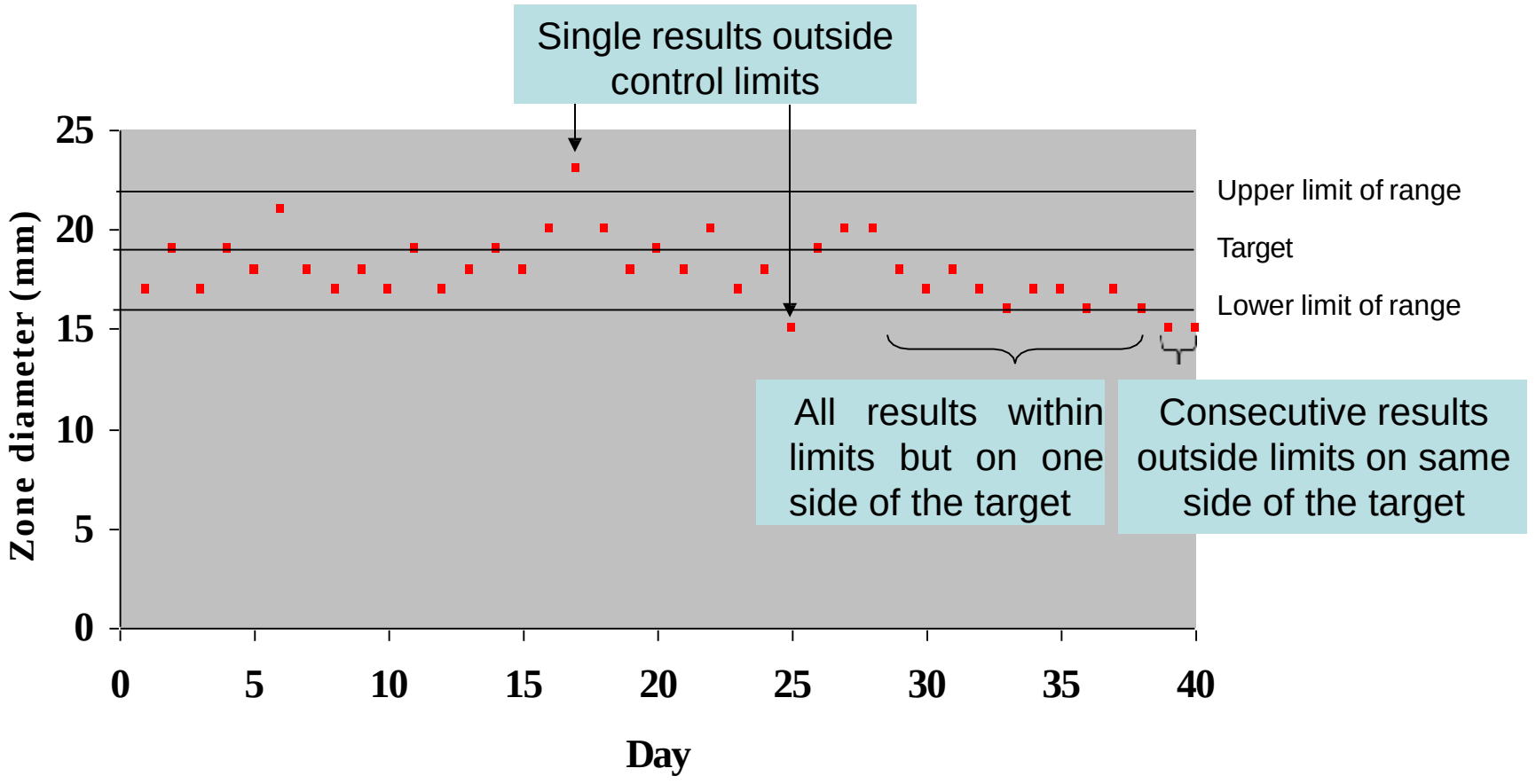
Daily Quality Control Testing

A laboratory can perform QC testing daily. Daily (vs weekly) QC testing must be performed each day patient isolates are tested if disk diffusion tests are performed less than once a week.

"Daily QC testing" or testing on "consecutive test days" means testing of QC strains each day disk diffusion tests are performed on patient isolates. It does not refer to calendar days.



Monitoring test performance



Daily Testing

Performance is satisfactory for daily QC testing when no more than three out of 30 results obtained on consecutive test days for each antimicrobial agent/organism combination are outside the acceptable limit

Performance Criteria for Reducing Quality Control Frequency to Weekly

Two plans are available to demonstrate satisfactory performance with daily QC testing before going to weekly QC testing. These include: 1) the 20- or 30-day plan or 2) the 15-replicate (3 × 5 day) plan.

The 20- or 30-Day Plan

- Test all applicable QC strains for 20 or 30 consecutive test days and document results.
- Follow recommended actions as described in Appendix A.
- If no more than one out of 20 or three out of 30 zone diameter measurements for each antimicrobial agent/organism combination are outside the acceptable zone diameter QC range listed in M100[1] Tables 4A and 4B, it is acceptable to go to weekly QC testing.
- If completion of the 20- or 30-day plan is unsuccessful, take corrective action as appropriate, and continue daily QC testing.

- If a laboratory is routinely testing QC strains each day of use and desires to convert to a weekly QC plan, it is acceptable to retrospectively analyze QC data from consecutive tests available during the previous two years, providing no aspects of the test system have changed.

The 15-Replicate (3 × 5 Day) Plan

- Test three replicates of each applicable QC strain using individual inoculum preparations for five consecutive test days and document results.
- Follow recommended actions as described in Appendix A and Table 5, below.
- Upon successful completion of the 15-replicate (3 × 5 day) plan, it is acceptable to go to weekly QC testing.
- If completion of the 15-replicate (3 × 5 day) plan is unsuccessful, take corrective action as appropriate, and continue daily QC testing.

Table 5. 15-Replicate (3 × 5 Day) Plan: Acceptance Criteria and Recommended Action*

Number Out of Range With Initial Testing (Based on 15 Replicates)	Conclusion From Initial Testing (Based on 15 Replicates)	Number Out of Range After Repeat Testing (Based on All 30 Replicates)	Conclusion After Repeat Testing
0–1	Plan is successful. Convert to weekly QC testing.	N/A	N/A
2–3	Test another 3 replicates for 5 days.	2–3	Plan is successful. Convert to weekly QC testing.
≥4	Plan fails. Investigate and take corrective action as appropriate. Continue QC each test day.	≥4	Plan fails. Investigate and take corrective action as appropriate. Continue QC each test day.

*Assess each QC strain/antimicrobial agent combination separately.
Abbreviations: N/A, not applicable; QC, quality control.

Implementing Weekly Quality Control Testing

- Weekly QC testing may be implemented once satisfactory performance with daily QC testing has been documented (see Subchapters 4.7.2.1 and 4.7.2.2).

- Perform QC testing once per week and whenever any reagent component of the test (eg, a new lot of agar or a new lot of disks from the same or a different manufacturer) is changed.

- If any of the weekly QC results are out of range, take corrective action.

Out-of-Range Results With Quality Control Strains and Corrective Action

Out-of-range QC results can be categorized into those that are 1) random, 2) identifiable, or 3) system related.

QC ranges are established to include ≥95% of results obtained from routine testing of QC strains. A small number of (random) out-of-range QC results may be obtained even when the test method is performed correctly and materials are maintained according to recommended protocols. Such occurrences are due to chance.

Out-of-range results with QC strains due to random or identifiable errors can usually be resolved by a single repeat of the QC test. However, out-of-range QC results that are due to a problem with the test system usually do not correct when the QC test is repeated and may indicate a serious problem that can adversely affect patient results. Every out-of-range QC result must be investigated.

Daily or Weekly Quality Control Testing – Out-of-Range Result Due to Identifiable Error

If the reason for an out-of-range result can be identified and easily corrected, correct the problem, document the reason, and retest the QC strain on the day the error is observed. If the repeated result is within range, no further corrective action is required.

Out-of-Control Result Due to Identifiable Error

- QC strain
  - Use of the wrong QC strain
  - Improper storage
  - Inadequate maintenance (eg, use of the same working culture for > 1 month)
  - Contamination
  - Nonviability
  - Changes in the organism (eg, mutation, loss of plasmid)

- Testing supplies
  - Improper storage or shipping conditions
  - Contamination
  - Use of a defective agar plate (eg, too thick or too thin)
  - Use of damaged (eg, cracked) plates
  - Use of expired materials

- Testing process
  - Use of the wrong incubation temperature or conditions
  - Inoculum suspensions incorrectly prepared or adjusted
  - Inoculum prepared from a plate incubated for the incorrect length of time
  - Inoculum prepared from differential or selective media containing anti-infective agents or other growth-inhibiting compounds
  - Use of wrong disk, ancillary supplies
  - Improper disk placement (eg, inadequate contact with the agar)
  - Incorrect reading or interpretation of test results
  - Transcription error

- Equipment
  - Not functioning properly or out of calibration (eg, pipettes)

Out-of-Control Result With No Error Identified

Immediate Corrective Action

• Test the out-of-control antimicrobial agent/organism combination on the day the error is observed and/or as soon as a new working culture or subculture is available. Monitor for five consecutive test days. Document all results.

– If all five zone diameter measurements for the antimicrobial agent/organism combination are within the acceptable ranges, no additional corrective action is necessary.

– If any of the five zone diameter measurements are still outside the acceptable range, additional corrective action is required

• Daily control tests must be continued until final resolution of the problem is achieved.

Additional Corrective Action

When immediate corrective action does not resolve the problem, the problem is likely due to a system error rather than a random error.

If necessary, obtain a new QC strain (either from freezer storage or a reliable source) and new lots of materials (including new turbidity standards), possibly from different manufacturers. It is also helpful to exchange QC strains and materials with another laboratory using the same method in order to determine the root cause of unexplained system problems.

If a problem is identified and corrected, documentation of satisfactory performance for another five days is required to return to weekly QC testing. If a problem is not identified, but results go back into control without any specific corrective action, documentation of satisfactory performance for another 20 or 30 consecutive test days is required in order to return to weekly QC testing

Weekly Quality Control Testing – Out-of-Range Result Not Due to Identifiable Error

If the reason for the out-of-range result with the QC strain cannot be identified, perform corrective action, as follows, to determine if the error is random.

- Test the out-of-range antimicrobial agent/organism combination on the day the error is observed or as soon as an F2 or F3 subculture of the QC strain is available.

- If the repeat results are in range, evaluate all QC results available for the antimicrobial agent/organism combination when using the same lot numbers of materials that were used when the out-of-range QC result was observed. If five acceptable QC results are available, no additional days of QC testing are needed. The following tables illustrate two scenarios that might be encountered and suggested actions:

Ampicillin *E. coli* ATCC® 25922; acceptable range: 15 to 22 mm

Week	Day	Lot Number (Disks)	Lot Number (MHA)	Result	Action
1	1	3564	16481	18	
2	1	3564	16481	19	
3	1	3564	16481	18	
4	1	3564	16481	19	
5	1	3564	16481	14	Out of range. Repeat QC same day.
5	2	3564	16481	17	In range. Five acceptable in-range QC tests for *E. coli* ATCC® 25922 with ampicillin disks lot 3564 and MHA lot 16481. Resume weekly QC testing.

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

Conclusion: Random QC error.

Ampicillin *E. coli* ATCC® 25922; acceptable range: 15 to 22 mm

Week	Day	Lot Number (Disks)	Lot Number (MHA)	Result	Action
1	1	9661	16922	18	
2	1	9661	16922	19	
3	1	9661	16922	14	Out of range. Repeat QC same day.
3	2	9661	16922	18	In range. Three acceptable in-range QC tests for *E. coli* ATCC® 25922 with ampicillin disks lot 9661 and MHA lot 16922. Repeat QC 2 more consecutive days.
3	3	9661	16922	18	In range.
3	4	9661	16922	17	In range. Five acceptable in-range QC tests for *E. coli* ATCC® 25922 with ampicillin disks lot 9661 and MHA lot 16922. Resume weekly QC testing.

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

Conclusion: Random QC error.

Reporting Patient Results When Out-of-Control Tests Occur

Whenever an out-of-control result occurs or corrective action is necessary, careful assessment of whether to report patient test results should be made on an individual patient basis, taking into account if the source of the error, when known, is likely to have affected relevant patient test results. Considerations may include, but are not limited to:

- Size and direction of error (eg, slightly or significantly increased zone size, slightly or significantly decreased zone size).

- Is the patient result close to the interpretive breakpoint?

- Results with other QC organisms.

- Results with other antimicrobial agents.

- Is the QC strain/antimicrobial agent an indicator for a procedural or storage issue (eg, inoculum dependent, heat labile)?

4.10 Confirmation of Results When Testing Patient Isolates

Multiple test parameters are monitored by following the QC recommendations described in this standard. However, acceptable results derived from testing QC strains do not guarantee accurate results when testing patient isolates. It is important to review all of the results obtained from all drugs tested on a patient's isolate before reporting the results. This should include ensuring that:

- The antimicrobial susceptibility results are consistent with the identification of the isolate.

- The results from individual antimicrobial agents within a specific drug class follow the established hierarchy of activity rules (eg, third-generation cephalosporins are more active than first- or second-generation cephalosporins against *Enterobacteriaceae*).

- The isolate is susceptible to those antimicrobial agents for which resistance has not been documented (eg, vancomycin and *Streptococcus* spp.) and for which only "susceptible" interpretive criteria exist in M100.[1]

Unusual or inconsistent results should be confirmed by checking for:

- Previous results on the patient (eg, did the patient previously have the same isolate with an unusual antibiogram?)

- Previous QC performance (eg, is there a similar trend or observation with recent QC testing?)

- Problems with the testing supplies, process, or equipment (see Subchapter 4.8.1 and M100[1] Table 4D, Troubleshooting Guide)

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.

	Required QC Frequency			
Test Modification	**1 Day**	**5 Days**	**15-Replicate Plan or 20- or 30-Day Plan**	**Comments**
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.

Selection of Safe Antibiotic for Pregnancy

Group B : No Risk in Controlled animal studies

All Cephalosporines
All Erythromycines except Erythromycin Estolate
Azithromycin but not Claritromycin
All Penicilines
Clindamycin
Nitrofurantoin before 36 weeks
Trimetoprime sulfamethoxazole (two trimester only)

Group C : Small Risk in controlled animal Studies

Imipenem
All Fluoroquinolones (cartilage damage risk)
Claritromycin
Trimetoprime (may be use a part of SXT in second trimester)
Vancomycine
Chloramphenicol
Gentamycine

Group D : Strong evidence of Risk to Human Fetus

Amikacin
Kanamycin
Streptomycin
Tobramycin
Trimetoprime sulfamethoxazole (Third trimester)
All Tetracyclines
Nitrofurantoin (Third trimester) : hemolytic aenemia , related to immature liver and G6PD deficiency

Antibiotic Selection for Children

Penicilines (AMX , PG)

Beta Lactamase inhibitor (AMX- Clavulonic , Augmentin)

Cephalosporines

Azitromycin , Erythromycin

Trimethoprim sulfamethoxazole

Cephalexine

References

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European Society of Clinical Microbiology and Infectious Diseases

Antimicrobial susceptibility testing

EUCAST disk diffusion method

Version 9.0

January 2021

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European Society of Clinical Microbiology and Infectious Diseases

Reading guide

EUCAST disk diffusion method for antimicrobial susceptibility testing

Version 8.0
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Thank you for your attention