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CLSI Subcommittee on Antimicrobial Susceptibility Testing

CLSI AST News Update

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This biannual CLSI AST News Update highlights current issues related to antimicrobial susceptibility testing (AST) and reporting.

CLSI and the AST Subcommittee Meetings

1. Content from the Summer 2022, Winter 2023, and Summer 2023 meetings can be found **here.**
2. Save the date for the next meetings:
   - May 12–June 3, 2025 | Dallas, TX
   - January 22–27, 2026 | Tempe, AZ
   - May 30–June 2, 2026 | Denver, CO

Inside This Issue:

What does the CLSI AST Subcommittee do?

The first edition of the CLSI AST News Update (Vol 1, Issue 1, Spring 2016) described details about the organization and operation of the CLSI AST Subcommittee.

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Featured Article

Updates to Susceptibility Breakpoints for *Stenotrophomonas maltophilia*

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Introduction

S. maltophilia is a motile gram-negative bacillus, frequently identified as a nosocomial pathogen in individuals with indwelling devices, long-term hospitalization or intensive care unit (ICU) stay, chronic respiratory disease, or an immunocompromising condition.[1,2] *S. maltophilia* has undergone several naming/classification changes since its first discovery.[2] The CLSI Subcommittee on Antimicrobial Susceptibility Testing formed an *ad hoc* working group (AHWG) in January 2022 to evaluate the existing *S. maltophilia* breakpoints for ceftazidime, levofloxacin, minocycline, and trimethoprim-sulfamethoxazole (SXT). At that time, the U.S. Food and Drug Administration (FDA) recognized a breakpoint only for ceftazidime, whereas CLSI had existing breakpoints for the aforementioned agents and also cefiderocol, chloramphenicol, and ticarcillin-clavulanate. The recognized European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints included only cefiderocol and SXT at that time.[3] Chloramphenicol, cefiderocol, and ticarcillin-clavulanate were not reviewed by the *S. maltophilia* AHWG; thus, there have been no changes to CLSI breakpoints for these agents. The updated breakpoints described in the summary below were first published in the 34th edition of CLSI M100 (Table 1).[4]

Ceftazidime

Ceftazidime breakpoints for *S. maltophilia* were removed due to a lack of data supporting the previous breakpoint, along with the uncertainty as to whether the species identification of the organisms utilized in the clinical studies leading to FDA approval were actually *S. maltophilia* because of nomenclature changes. Notably, the package insert for ceftazidime never listed *S. maltophilia* or the previous names assigned to this species as an organism for which there were sufficient data to predict activity.

Contemporary microbiologic *S. maltophilia* data were reviewed when reassessing the breakpoints. Surveillance studies reported the frequent production of both L1 and L2 β-lactamases among clinical *S. maltophilia* isolates.[1] The L1 β-lactamases (aka class B3 β-lactamases) are metallo-β-lactamases [MBLs] capable of hydrolyzing carbapenems and other β-lactams with the exception of aztreonam and L2 β-lactamases are class A cephalosporinases that confer resistance to extended-spectrum cephalosporins (e.g., ceftazidime) and aztreonam but are still inhibited by serine-β-lactamase inhibitors such as clavulanic acid and avibactam.[1,5] In one study evaluating clinical strains of *S. maltophilia* collected over a 10-year period across the United States, L1 β-lactamases were detected in 100/130 (77%) isolates, and L2 β-lactamases were found in 116/130 (89%) isolates.[1] Therefore, in isolates with L1/L2 β-lactamases, reduced ceftazidime activity is expected.

Contemporary data presented at CLSI indicated that neither reference broth microdilution (BMD) nor reference agar dilution minimum inhibitory concentrations (MICs) were reproducible. One large multicenter study reported poor accuracy when testing ceftazidime and *S. maltophilia* using three commercial antimicrobial susceptibility test (AST) systems, with 11.1 - 41.8% very major error rates when compared to reference BMD interpreted according to the former EUCAST pharmacokinetic/pharmacodynamic (PK/PD) reference breakpoints of ≤4 µg/mL, susceptible; 8 µg/mL, intermediate; and >8 µg/mL, resistant.[6]

Several PK/PD models demonstrated ceftazidime monotherapy to be insufficient for treatment of *S. maltophilia*.[7-9] Garrison et al. used a simulated dosing regimen (1g q8h) that was not corrected for protein binding and noted *S. maltophilia* regrowth to baseline after treatment of both susceptible and resistant isolates.[7] Additional data supporting removal of the ceftazidime breakpoints include an MIC distribution supporting an epidemiologic cutoff value (ECV) of 64 µg/mL, which is several dilutions higher than the ≤8 µg/mL clinical breakpoint previously set by CLSI for ceftazidime.[4] Data used for these studies were composed of contemporary isolates from International Health Management Associates (IHMA) (n = 5826) and JMI Laboratories (n = 2107).[10,11]

Furthermore, the AHWG found a lack of high-quality clinical outcome studies comparing ceftazidime and other antimicrobials for treatment of *S. maltophilia*. Sparse data have been published for clinical outcomes, including only a limited number of reports of successful treatment with ceftazidime monotherapy in patients without removable foci of infection (e.g., indwelling devices or lines) or surgical intervention.[12,13] Due to the lack of data supporting a breakpoint, as well as the lack of FDA-approved indications for *S. maltophilia*, ceftazidime breakpoints were removed from CLSI M100 in 2024.[4] Importantly, this change was recognized by the FDA in May 2024, and ceftazidime breakpoints for *S. maltophilia* were subsequently removed from the FDA susceptibility test interpretive criteria (STIC) website.[14]

Updates to Susceptibility Breakpoints for *Stenotrophomonas maltophilia* (*Continued*)

Levofloxacin

Several small PK/PD animal studies of levofloxacin and *S. maltophilia* were evaluated, including neutropenic murine pneumonia models and a neutropenic murine thigh model.[15,16] Using a single clinical *S. maltophilia* isolate and a levofloxacin dose of 10 mg/kg q24h for 5 days, Imoto et al. demonstrated a significantly longer mouse survival time ($P = 0.0006$) as compared to saline control in a hemorrhagic pneumonia model.[15] Similar results were demonstrated by Nakamura et al. when using two clinical isolates and three different levofloxacin doses (10, 30, 100mg/kg).[16] Although promising results were seen with these two studies, neither used human simulated dosing of 750 mg daily.[17]

Due to limited PK/PD studies using human simulated regimens and limited clinical outcome data, there was insufficient evidence for a levofloxacin breakpoint update. However, a change was made to CLSI M100[4] with the addition of a comment stating that levofloxacin should not be used as monotherapy against *S. maltophilia*. This aligns with the Infectious Diseases Society of America (ISDA) "Guidance on the Treatment of Antimicrobial Resistant Gram-Negative Infections," which suggests that, if a clinician wishes to use levofloxacin for therapy of *S. maltophilia* infection, it should be used in combination with other antimicrobials rather than as monotherapy.[18]

Minocycline

New PK/PD data contributed significantly to the decision to lower the breakpoints for minocycline with *S. maltophilia*. Two studies were evaluated to support this decision. Fratoni et al. conducted dose fractionation studies in the neutropenic murine thigh infection model and determined the PD index needed for stasis and 1 $\log_{10}$ reduction in colony forming units (CFU) was a free area under the curve (*f*AUC)/MIC of 9.6 and 23.6, respectively.[19] Monte Carlo simulations using minocycline at 200 mg IV q12h achieved the 1 $\log_{10}$ kill threshold (*f*AUC/MIC ≥23.6) with probability of target attainment (PTA) of 93% for isolates at MICs of 0.5 µg/mL and 51.7% at 1 µg/mL. When using the stasis threshold (*f*AUC/MIC ≥9.6), PTA was 97% at 1 µg/mL. In contrast, the PTA at the previous breakpoint of 4 µg/mL was only 0.1%. A Monte Carlo simulation from another group using minocycline dosing of 100 mg IV q12 h demonstrated ≥94.4% PTA at <4 µg/mL however, they used a lower target *f*AUC/MIC >8.75 that was extrapolated from gram-positive bacteria.[20]

Updated MIC distribution data for minocycline obtained from testing isolates at IHMA (n = 942) and JMI Laboratories (n = 1977) showed a modal MIC of 0.5 µg/mL, though an ECV could not be calculated from data available at the time.

Additional support for the breakpoint revision included four retrospective observational clinical outcome studies that showed that rates of minocycline failure were similar to those of SXT, however noting limitations that these studies included primarily respiratory isolates and many polymicrobial samples.[21-24] Jacobson et al. reported a significantly lower clinical failure rate for patients (n=93) treated with minocycline for *S. maltophilia* when the MIC was <4 µg/mL compared to those when infections were caused by isolates with MICs of 4 µg/mL (2.6% versus 29.4%, P=0.004).[24] Ultimately, the susceptible breakpoint was lowered to ≤1 µg/mL, with the breakpoint being based on a dose of 200 mg q12h.

Minocycline DD breakpoints were also revised using data generated in a multi-center disk correlation study coordinated by the CLSI AST subcommittee (data unpublished).[25] The DD breakpoints were established using three media manufacturers and two disk manufacturers tested across three separate laboratories.

Trimethoprim-sulfamethoxazole

Existing data evaluating PK/PD and clinical outcomes data for SXT against *S. maltophilia*, some of which are summarized below, were reviewed but ultimately the breakpoints were not changed.[16,26-28]

A neutropenic rabbit model of *S. maltophilia* pneumonia demonstrated no significant reduction in lung weight, a marker of organism-mediated pulmonary injury, when compared to untreated control animals following an SXT dosing regimen of 5 mg/kg IV q12.[26] However, lung weights of cefiderocol-treated rabbits were significantly decreased when compared to untreated control animals. Data from this study were difficult to translate to human experience since plasma concentrations were not obtained and it was therefore unclear how closely the regimen simulates human dosing. Furthermore, PK/PD modeling studies show that clinically equivalent AUC exposures achieved only a 0.5 $\log_{10}$ reduction in CFU at best.[27,28]

Nys et al. conducted a retrospective study of 45 patients who received SXT for monomicrobial *S. maltophilia* infection.[29] Of these patients, 38/45 (84.4%) experienced microbiological cure and 3/45 (6.7%) harbored SXT-resistant isolates. Although the Nys et al. study provided positive evidence supporting use of SXT, Junco et al. reported no significant difference in clinical failure rates between SXT (77/217 [35.5%] patients) and minocycline (12/39 [30.8%] patients).[21] Thus, clinical outcome studies show variable results.

Updates to Susceptibility Breakpoints for *Stenotrophomonas maltophilia* (*Continued*)

Due to a lack of evidence that the current breakpoints are inappropriate, the SXT breakpoints remained unchanged and a comment was added that SXT should not be used alone for antimicrobial therapy in order to align with the IDSA "Guidance on the Treatment of Antimicrobial Resistant Gram-Negative Infections".[18]

See the table below for an excerpt of current zone diameters and MIC breakpoints for *S. maltophilia* from the 35th edition of CLSI M100.

Conclusions

In summary, *S. maltophilia* breakpoints for ceftazidime were removed, minocycline breakpoints were lowered, and comments warning against the use of monotherapy for levofloxacin and for SXT were added. Although CLSI generally refrains from making treatment-specific recommendations, the SXT and levofloxacin monotherapy comments align with the IDSA treatment guidance for *S. maltophilia*, which states that a "standard of care" regimen is not available and suggests that combination therapy with at least two active agents should be used.[18] Specifically, the guidance suggests the use of either: (1) two of the following agents: cefiderocol, minocycline, TMP-SMX, or levofloxacin or (2) the combination of ceftazidime-avibactam and aztreonam.

As with any new breakpoint changes, clinical laboratories should try to implement as soon as possible. It is important for laboratories to discuss and collaborate with infectious diseases clinicians and antimicrobial stewardship teams to ensure that implementation is streamlined per institutional practices.

Table. CLSI M100 35th Edition Breakpoints for *Stenotrophomonas maltophilia*, Including Modifications Made in CLSI M100 34th Edition (2024) for Levofloxacin and Trimethoprim-Sulfamethoxazole[a]

Antimicrobial Agent	CLSI M100 35th Edition (2025)					
	MIC (µg/mL)			Zone (mm)		
	S	I	R	S	I	R
Cefiderocol	≤1	-	-	≥15	-	-
Chloramphenicol	≤8	16	≥32	-	-	-
Levofloxacin[b]	≤2	4	≥8	≥17	14-16	≤13
Minocycline	≤1	2	≥4	≥26	21-25	≤20
Ticarcillin-clavulanate	≤16/2	32/2-64/2	≥128/2	-	-	-
Trimethoprim-sulfamethoxazole[c]	≤2/38	-	≥4/76	≥16	11-15	≤10

[a] Ceftazidime breakpoints removed from CLSI M100 34th edition
[b] Comment added "Rx: Levofloxacin should not be used alone for antimicrobial therapy"
[c] Comment added "Rx: Trimethoprim-sulfamethoxazole should not be used alone for antimicrobial therapy"
Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible

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Updates to Susceptibility Breakpoints for *Stenotrophomonas maltophilia* (*Continued*)

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Updates to Susceptibility Breakpoints for *Stenotrophomonas maltophilia* (*Continued*)

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Case Study

Cefepime Reporting Strategies for Carbapenemase-producing Isolates of Enterobacterales

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Clinical Case

A 34-year-old patient with quadriplegia was admitted with tachypnea and fever. A diagnostic evaluation for pneumonia was performed, including a molecular pneumonia panel and respiratory cultures on expectorated sputum. The molecular pneumonia panel detected *Klebsiella oxytoca* and bla_{KPC}. In response to these results, the patient was started on ceftazidime-avibactam. Respiratory cultures revealed heavy growth of *K. oxytoca* and normal flora the following day. Results of antimicrobial susceptibility testing (AST) for the *K. oxytoca* are shown in Table 1.

Table 1. *Klebsiella oxytoca* Antimicrobial Susceptibility Results

Antimicrobial Agent	MIC (µg/mL)	Interpretation
Ampicillin	>32	R
Ampicillin-sulbactam	>32	R
Cefazolin	>64	R
Cefepime	2	S
Ceftazidime	16	R
Ceftazidime-avibactam	1	S
Ceftriaxone	>64	R
Ciprofloxacin	≤0.25	S
Gentamicin	≤1	S
Imipenem	8	R
Levofloxacin	0.25	S
Meropenem	>16	R
Piperacillin-tazobactam	64	R
Trimethoprim-sulfamethoxazole	>8	R

Abbreviations: MIC, minimal inhibitory concentration; S, susceptible; R, resistant

Test Results Assessment and Troubleshooting

AST results for ceftriaxone, ceftazidime, meropenem and imipenem (all resistant) were consistent with the bla_{KPC} gene detected by the molecular panel. However, the cefepime MIC of 2 µg/mL (S), was unexpected for a carbapenemase-producing isolate. This result could be due to testing errors or a true phenotype, so troubleshooting was performed.

Purity plates were re-examined, which showed no evidence of a mixed culture. Next, a phenotypic test for carbapenemase production (CarbaNP) was performed on the *K. oxytoca* isolate. This test was positive, indicating active expression of the bla_{KPC} gene. A disk diffusion test was also performed for cefepime, to rule out random error in the original AST; this revealed a susceptible zone of growth inhibition. Together, these suggested no testing errors had occurred.

CLSI M100 Appendix G, Table G3 provides the following guidance for cefepime susceptible or susceptible dose-dependent carbapenemase-producing isolates of Enterobacterales[6]:

1. Suppress the cefepime result, or
2. Report cefepime as resistant.

Cefepime Reporting Strategies for Carbapenemase-producing Isolates of Enterobacterales (*Continued*)

These options had been discussed previously with the hospital antimicrobial stewardship team. Since the *bla*$_{KPC}$ gene was endemic in the local population, the stewardship team had decided the safest course of action after laboratory troubleshooting was to report cefepime as resistant. In keeping with this policy, the laboratory released the final report in Table 2.

Table 2. *Klebsiella oxytoca* Antimicrobial Susceptibility Final Report

Antimicrobial Agent	MIC (µg/mL)	Interpretation
Ampicillin	>32	**R**
Ampicillin-sulbactam	>32	**R**
Cefazolin	>64	**R**
Cefepime*	-	**R**
Ceftazidime	16	**R**
Ceftazidime-avibactam	1	**S**
Ceftriaxone	>64	**R**
Ciprofloxacin	≤0.25	S
Gentamicin	≤1	S
Imipenem	8	**R**
Levofloxacin	0.25	S
Meropenem	>16	**R**
Piperacillin-tazobactam	64	**R**
Trimethoprim-sulfamethoxazole	>8	R

**Cefepime MIC was suppressed from the report to avoid confusion of a susceptible MIC but a resistant interpretation.*
Abbreviations: MIC, minimal inhibitory concentration; S, susceptible; R, resistant

Discussion Points

Approximately 15% and 30% of KPC-producing Enterobacterales isolates test susceptible or susceptible dose-dependent to cefepime, despite carbapenem resistance.[1,2] In 2021, CLSI M100, CLSI broadly recommended phenotypic and genotypic tests be repeated for cases where a carbapenemase target was detected but third or fourth generation cephalosporins tested as S or SDD (Table 3).[3] In 2024, CLSI M100, additional guidance was provided for cefepime because of the frequency of cefepime susceptible MICs in carbapenem-resistant isolates.[4] Animal model data on the efficacy of cefepime in carbapenemase-producing and non-carbapenemase-producing Enterobacterales with a range of cefepime MICs were presented to CLSI in 2023.[7] These data showed 1-log bacterial reduction was not possible for carbapenemase (including KPC)-producing Enterobacterales treated with cefepime, even if cefepime MIC was ≤2 µg/mL. In contrast, non-carbapenemase producing carbapenem-resistant isolates achieved bacterial killing in this model, if cefepime MICs were S or SDD. These data indicated that cefepime should be avoided for treatment of serious infections caused by Enterobacterales isolates with known carbapenemase activity.[7] These data supported current cefepime breakpoints and confirmed that cefepime should not be reported as susceptible for carbapenemase-producing Enterobacterales. It must be highlighted that most of these data are based on studies investigating KPC-producing Enterobacterales in animal models alone.

Cefepime Reporting Strategies for Carbapenemase-producing Isolates of Enterobacterales (*Continued*)

Table 3. Previous and Current Recommendations in CLSI M100 for Reporting 3rd- and 4th-Generation Cephalosporins for Carbapenemase-Producing Enterobacterales [extracted from CLSI M100 Appendix H Table H3 (31st, 32nd and 33rd editions) or Appendix G Table G3 (34th edition)][3-6]

CLSI M100 Edition	Phenotypic AST	Comment in CLSI M100
31st, 32nd, 33rd (2021-2023)	S or SDD to 3rd- or 4th-generation cephalosporins	Current clinical and laboratory evidence is insufficient to conclude whether cephalosporin therapy of carbapenemase-carrying strains with an MIC in the S/SDD range will be effective.
34th (2024)	S to 3rd-generation cephalosporins	Current clinical and laboratory evidence is insufficient to conclude whether cephalosporin therapy of carbapenemase-carrying strains with an MIC in the S range will be effective
	S or SDD to cefepime	Suppress cefepime or report as "R"

Abbreviations: MIC, minimal inhibitory concentration; S, susceptible; SDD, susceptible dose dependent; R, resistant

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Hot Topic

Rezafungin, a New Second-Generation Echinocandin

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Rezafungin is a novel systemic antifungal agent of the echinocandin class, which was approved by the US Food and Drug Administration (FDA) in March 2023 for the treatment of candidemia and invasive candidiasis in adults with limited treatment options. The echinocandins are members of a class of systemic antifungal agents that directly target the fungal cell wall.[1,2] They are semi-synthetic lipopeptides developed from fermentation products of certain fungi such as *Aspergillus nidulans*.[3] Currently, four echinocandins have been developed for clinical use: first-generation echinocandins caspofungin, micafungin, and anidulafungin, and, most recently, the second-generation rezafungin. All four inhibit β-1,3-D-glucan synthase that catalyzes the biosynthesis of β-1,3-D-glucan, a key component of the cell wall of most fungi.[4] Since mammalian cells do not contain this enzyme, direct human cell toxicity is minimal.[5] Although the echinocandins exhibit similar activity against a wide spectrum of fungal pathogens, differences in pharmacokinetics and pharmacodynamics should be considered when treating patients with serious fungal infections. Each of the echinocandins differs in its kinetics of hepatic metabolism, tissue distribution, half-life and drug-drug interaction profiles that leads to different dosing strategies. Of note, the echinocandins that are currently approved for humans have limited oral bioavailability and therefore, must be administered by intravenous infusion.

Echinocandins are highly active (i.e., fungicidal) against a range of *Candida* species including isolates that are resistant to triazoles (e.g., fluconazole, voriconazole) and those that form biofilms.[6,7] They have modest activity (i.e., fungistatic) against *Aspergillus* spp., thermally dimorphic fungi, and melanized fungi.[8-10] Their activity is weak against *Mucorales, Fusarium* spp., *Scedosporium* spp., *Cryptococcus* spp., and *Trichosporon* spp. due to lack of β-1,3-D-glucan in the cell wall of these organisms.[5]

Rezafungin

Rezafungin (formerly known as biafungin, CD101, and SP3025) possesses chemical and biological properties that are improved over those of the first-generation echinocandins. It is more stable both *in vitro* and *in vivo* than the other echinocandins, with higher solubility in aqueous solutions. It has a long half-life of ~80 hours following first dose (~3-fold longer than other echinocandins) and slower clearance (~7-fold slower) after intravenous injection, which enables once-weekly dosing as opposed to the first-generation echinocandins that require dosing once-daily. Tissue penetration is high, and the safety profile of rezafungin is good.

Rezafungin shows potent *in vitro* and *in vivo* activity against *Candida* spp. including *Candida auris* and other *Candida* species resistant to first-generation echinocandins.[11] Most *C. auris* isolates demonstrate susceptibility to rezafungin. Studies also show that rezafungin may be used to treat infections due to *Candida* isolates with FKS mutations (i.e., resistant to first-generation echinocandins) when administered in higher than usual doses.[12]

Despite not being FDA-approved for mold infections, it has been used experimentally for *Aspergillus* spp., including *A. fumigatus* isolates harboring *CYP51A* mutations that lead to azole resistance.[13]

Susceptibility Testing and Breakpoints for Rezafungin

CLSI standard methods can be used for disk diffusion and minimal inhibitory concentration (MIC) testing of rezafungin for yeasts.[14] Currently, rezafungin disks (5 µg, FDA-approved) are commercially available from Hardy Diagnostics and Oxoid Limited (Part of Thermo Fisher Scientific). The YeastOne AST plates from ThermoFisher Scientific (YO4IVD: FDA-cleared panel and YO11: Research Use Only) can be also used for rezafungin susceptibility testing (in the dilution range of 0.008-8µg/mL) using the Sensititre platform. Verification/validation should be performed before rezafungin testing has been clinically implemented, ideally with a set of isolates that have been tested by reference broth microdilution. Currently, FDA publishes rezafungin breakpoints (MIC and disk diffusion) for four *Candida* species: *C. albicans, C. glabrata, C. parapsilosis* and *C. tropicalis* (Table 1). However, in 2022, CLSI published rezafungin MIC breakpoints for seven Candida species: *C. auris, C. dubliniensis,* and *C. krusei,* in addition to the 4 species listed above.[15] The FDA and CLSI breakpoints for rezafungin are shown in Table 1. CLSI has not yet approved disk diffusion breakpoints for rezafungin. The only species for which the FDA and CLSI MIC breakpoints are the same is *C. parapsilosis*. Since nonsusceptible isolates have only been rarely recovered at this point, only a susceptible breakpoint exists. If a *C. auris* tests nonsusceptible to rezafungin, the laboratory should confirm identification of the isolate, repeat the MIC test using reference broth microdilution, if possible (or perform an MIC test if disk diffusion testing was initially performed), and, if your results reproduce, consider sending to a public health laboratory for confirmation.

Rezafungin, a New Second-Generation Echinocandin (*Continued*)

Table 1. Rezafungin Breakpoints for Various *Candida* Species After 24-hour Incubation.

Fungi	FDA Breakpoints						CLSI Breakpoints					
	MIC (µg/mL)			DD (mm)			MIC (µg/mL)			DD (mm)		
	S	I	R	S	I	R	S	I	R	S	I	R
Candida albicans	≤0.12	-	-	≥13	-	-	≤ 0.25	-	-	-	-	-
Candida auris	-	-	-	-	-	-	≤ 0.5	-	-	-	-	-
Candida dubliniensis	-	-	-	-	-	-	≤ 0.12	-	-	-	-	-
Candida (Nakaseomyces) glabrata	≤0.12	-	-	≥ 15	-	-	≤ 0.5	-	-	-	-	-
Candida krusei (Pichia kudriavzevii)	-	-	-	-	-	-	≤ 0.25	-	-	-	-	-
Candida parapsilosis	≤2	-	-	≥9	-	-	≤2	-	-	-	-	-
Candida tropicalis	≤0.12	-	-	≥ 14	-	-	≤ 0.25	-	-	-	-	-

Abbreviations: DD, disk diffusion; FDA, Food and Drug Administration; I, intermediate; MIC, minimal inhibitory concentration; S, susceptible; R, resistant.

In summary, rezafungin is an intravenous echinocandin antifungal agent approved by the FDA for once-weekly treatment of candidemia and invasive candidiasis in adults who have limited or no alternative treatment options. Rezafungin is the only antifungal agent for which a clinical breakpoint has been set by CLSI for *C. auris* and also offers a promising treatment option for several *Candida* spp. when other echinocandins test resistant.

Rezafungin, a New Second-Generation Echinocandin (*Continued*)

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Practical Tips

Changes in Methodology and Breakpoints for *Staphylococcus* spp. Linezolid and Tedizolid Disk Diffusion Tests

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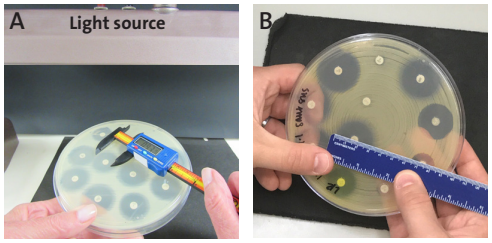
Tedizolid disk diffusion breakpoints for staphylococci, beta-hemolytic streptococci and viridans streptococci were added to CLSI M100 in 2024. In addition, as related to disk diffusion testing, changes were made to oxazolidinone quality control ranges, linezolid breakpoints, and zone measurement guidance for *Staphylococcus* spp. using reflected light as shown in Table 1[1] and Figure 1.

Table 1. *Staphylococcus* spp. Disk Diffusion Quality Control (QC) and Breakpoint Revisions

Antimicrobial Agent	Disk Content	*Staphylococcus aureus* ATCC® 25923 QC Ranges (mm)		Zone Diameter Breakpoints (mm)	
		CLSI M100-Ed33	CLSI M100-Ed34	CLSI M100-Ed33	CLSI M100-Ed34
Linezolid	30 µg	25-32[a]	24-30[b]	≥21 S, ≤20 R[a]	≥26 S, 23-25 I, ≤22 R[b]
Tedizolid*	2 µg	18-24[a]	19-25[b]	–	≥19 S, 16-18 I, ≤15 R[b]

Abbreviations: I, Intermediate; R, Resistant; S, Susceptible
*Tedizolid breakpoints apply only to *S. aureus*, including methicillin-resistant *S. aureus* (MRSA)
[a] Read using transmitted light
[b] Read using reflected light



- Invert the Petri plate and hold it a few inches above a black background that does not reflect light. Use a light source above the plate to read the zones.
- Measure complete zones of inhibition from the back of the inverted Petri plate (Figures 3A and 3B).

Figure 3. Measuring Zones of Inhibition Using Reflected Light and Translucent Media

Figure 1. The figure above is from the CLSI M02 Disk Diffusion Reading Guide.

How did these revisions and additions for oxazolidinones and staphylococci come about?

Following the introduction of tedizolid MIC breakpoints to CLSI M100 in 2016, the Methods Development and Standardization Working Group of CLSI's AST Subcommittee began work to establish tedizolid disk diffusion quality control ranges and zone diameter breakpoints. This revealed issues with previous zone diameter breakpoints for testing staphylococci with linezolid, which was used as an in-class control agent for testing tedizolid. In addition, *Staphylococcus aureus* ATCC® 25923 yielded a higher number of reproducible results for both linezolid and tedizolid when zones were measured with reflected light as opposed to transmitted light. Reading with transmitted light had been the previous CLSI recommendation.[3]

Why was transmitted light previously used for testing linezolid with staphylococci?

Oxazolidinone resistance remains uncommon among staphylococci. Because resistant isolates were rarely encountered when CLSI linezolid MIC and disk diffusion breakpoints were first established in 2006, only a susceptible breakpoint was assigned (≤4 µg/mL and ≥21 mm Susceptible). Disk diffusion zones were initially measured using reflected light.[4] However, a 2007 publication reported that among a small set of non-susceptible isolates (n=15) submitted to the Centers for Disease Control and Prevention (CDC), 53.3% were incorrectly categorized as susceptible when tested by disk diffusion. However, these errors were reduced by measuring zones using transmitted light that better detected faint or pinpoint growth within the zones of inhibition.[5] Subsequently, in 2008 a comment

Changes in Methodology and Breakpoints for *Staphylococcus* spp. Linezolid and Tedizolid Disk Diffusion Tests (*Continued*)

was added to CLSI M100 Table 2C stating that, "when testing linezolid, disk diffusion zones should be examined using transmitted light. Organisms with non-susceptible results should be confirmed using an MIC method." In 2010, resistant breakpoints of ≥8 µg/mL and ≤20 mm were established.[6] However, an evaluation of linezolid resistance-enriched datasets by CLSI in the past few years demonstrated that the zone diameter breakpoints were still resulting in a >15% very major error rate for *S. aureus*, despite use of transmitted light.

What impact do these changes have?

While MIC breakpoints for linezolid remain ≤4 µg/mL "S" and ≥8 ug/mL "R" with no intermediate MIC interpretive category, the introduction of an intermediate category for disk diffusion only and revision of the "S" and "R" zone diameter breakpoints have significantly improved the accuracy of disk diffusion results as compared to MIC testing by reducing the number of very major errors to ≤1.5%.

Use of reflected light when reading *Staphylococcus* spp. zone diameters harmonizes with the methodology used for enterococci when testing linezolid and when reading results for all other antimicrobial agents, simplifying the reading process and reducing the likelihood that methodological errors will occur.

Lastly, disk breakpoints for tedizolid have been established for the first time, enabling many more laboratories to perform tedizolid testing in-house, decreasing laboratory costs and improving turnaround time.

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Recent Developments

Mycobacterium chelonae Extended Incubation Needed for Clarithromycin Antimicrobial Susceptibility Testing

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CLSI M24 provides a standard method for antimicrobial susceptibility testing (AST) of rapidly growing mycobacteria (RGM), including *M. chelonae*.[1] Some RGM species, such as most *M. abscessus* subsp. *abscessus* and subsp. *bolletii*, but not subsp. *massiliense*, are known to possess a functional erythromycin methylase resistance (*erm*) gene that can confer inducible resistance to macrolides. As a result, CLSI M24 indicates that *erm* sequencing or extended incubation of clarithromycin phenotypic AST out to 14 days should be used to provide an indication of macrolide resistance due to the presence of this gene.[1] At the time of publication of CLSI M24 in 2018, there was no evidence of an inducible *erm* gene in *M. chelonae* and therefore there was no recommendation in CLSI M24 to perform extended 14-day incubation of *M. chelonae* isolates.

However, in 2023, Brown-Elliott et. al., published an article in the *Journal of Clinical Microbiology* providing evidence that *M. chelonae* does possess a novel *erm*, *erm*(55), which can cause high-level inducible macrolide resistance in clinical isolates.[2] *Erm*(55) has been found to be either putatively carried on a plasmid, chromosome, or transposon. While the chromosomal and transposon *erm*(55) is constitutive and detected after 3 days of incubation, the plasmid-borne *erm*(55) is inducible, requiring up to 14 days of incubation to detect resistance and this *erm* was detected in 3.8% of clinical isolates in this two-center study. As a result, extended incubation of *M. chelonae* isolates for 14 days for clarithromycin or sequencing of *erm*(55) is required to detect inducible macrolide resistance in this *Mycobacterium* species. CLSI M24 is under consideration for an update at this time and this information will be included in any update but in the meantime, laboratories should perform extended 14-day incubation of *M. chelonae* isolates, like that already performed for *M. abscessus*, in order to detect inducible macrolide resistance. The same 2023 study also provided evidence of *erm*(55) in other RGM species. Therefore, the recommendation for extended incubation or sequencing for *erm*(55) should also be strongly considered for all RGM or at least those isolates with a 3-4 day clarithromycin MIC of ≥ 0.5µg/mL. The mobility of plasmids and transposons with this *erm*(55) resistance mechanism is disturbing. Moreover, the potential for spread among genera as has been shown, creates more therapeutic challenges for these already difficult to treat diseases caused by nontuberculous mycobacteria (NTM).

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