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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.

Inside This Issue:

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2. Save the date for the next meetings!
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What does the CLSI AST Subcommittee do?

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Featured Article

The Important and Challenging Role of Antifungal Testing to Guide Therapy of Severe Mold Infections

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Epidemiology

Over recent decades, the population at risk for invasive mold infections has increased substantially.[1] This rise is primarily driven by the growing number of immunocompromised patients due to underlying malignancies, organ transplantation, and immunosuppressive therapies, as well as patients in intensive care settings.[2] Concurrently, there has been an expansion in the diversity of fungi causing human disease. Although an estimated 1–5 million fungal species exist,[3] only a small portion (±700 species) are implicated in invasive and chronic human infections, though many of these are associated with high morbidity and mortality. Among these, species of *Aspergillus, Fusarium, Scedosporium, Lomentospora, Histoplasma capsulatum, Coccidioides* spp. and members of the order *Mucorales* comprise the majority of severe invasive infections.

Pathogenesis, Clinical Manifestations, and Diagnostic Challenges

Fungal infections most commonly occur through inhalation of airborne spores, leading to respiratory tract colonization or disease. In immunocompetent individuals, infection is often self-limited or asymptomatic; however, in immunocompromised hosts, infection may progress to angioinvasion, tissue necrosis, and dissemination to multiple organ systems, frequently resulting in life-threatening disease.[4] Some fungal diseases have the potential for human-to-human transmission, contributing to emerging global infectious disease trends. An example is dermatophytosis, a highly prevalent superficial fungal infection caused by *Trichophyton* spp., with infections caused by several emerging strains often presenting as widespread, pruritic, and inflammatory lesions involving the body, groin, or face.[5]

Early identification of mold pathogens as a cause of infection remains challenging.[6] Clinical and radiographic findings are often nonspecific in early disease. If invasive fungal infection is not initially considered in the differential diagnosis, appropriate therapy may be delayed or omitted.[7] Unlike many bacterial pathogens, molds require specialized culture conditions, prolonged incubation, and often complementary histopathological or molecular methods for accurate identification. These limitations frequently contribute to delays in initiation of targeted antifungal therapy, which is associated with poorer clinical outcomes.

Antifungal Therapy and Drug Classes

Selection of appropriate empirical and definitive antifungal therapy is fundamental to the management of invasive fungal infections but is complicated by the broad spectrum of potential mold pathogens and their frequent heterogeneous and often intrinsic antifungal resistance profiles (e.g., voriconazole resistance of *Mucorales*). Therapeutic options are limited, and antifungal agents available are often associated with toxicity, drug-drug interactions, and the emergence of resistance. Currently available systemic antifungal agents span several mechanistic classes (Table 1). In addition, several novel agents targeting essential fungal pathways are in clinical development.[8]

Table 1. Classes of Systemic Antifungal Agents and Representative Drugs

Class	Representative Drugs
Polyenes	Amphotericin B
Azoles	Ketoconazole; Fluconazole; Itraconazole; Voriconazole; Posaconazole; Isavuconazole; Oteseconazole
Echinocandins	Caspofungin; Micafungin; Anidulafungin; Rezafungin
Triterpenoid	Ibrexafungerp
Nucleoside analogues	Flucytosine
Microtubule inhibitors	Griseofulvin
Allylamines	Terbinafine
Orotomide (DHODH inhibitor)	Olorofim (F901318) – investigational
GPI-anchor biosynthesis inhibitor (Gwt1)	Fosmanogepix (prodrug of manogepix) – investigational

The Important and Challenging Role of Antifungal Testing to Guide Therapy of Severe Mold Infections (*Continued*)

Antifungal Susceptibility Testing (AFST) for Molds

Standardized reference broth microdilution methods for antifungal susceptibility testing of filamentous fungi (i.e., molds) have been developed by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The CLSI documents that describe testing procedures, breakpoints (BPs), and epidemiological cutoff values (ECVs) are listed in Table 2. At present, CLSI clinical breakpoints are available only for *Aspergillus fumigatus* with voriconazole and isavuconazole (Table 3). For all other drug-organism combinations addressed in CLSI documents, assessment of antifungal activity relies on the application of ECVs, which have not been correlated with clinical outcome.

Table 2. CLSI Documents for Antifungal Susceptibility Testing of Filamentous Fungi

CLSI Document	Title	Description
M38-3rd Ed. (2017)[9]	Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi	Describes details of broth microdilution testing of molds
M38M51S-Ed4 (2026)[10]	Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi	Lists breakpoints for *Aspergillus fumigatus* with voriconazole and isavuconazole
M57S-Ed4 (2022)[11]	Epidemiological Cutoff Values for Antifungal Susceptibility Testing	Provides ECVs and QC tables

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

Table 3. CLSI Clinical Breakpoints for *Aspergillus fumigatus*[a]

Antifungal Agent	MIC (µg/mL)[a]		
	Susceptible (S)	Intermediate (I)	Resistant (R)
Voriconazole	≤ 0.5	1	≥ 2
Isavuconazole	1	2	≥ 4

Abbreviation: MIC, minimal inhibitory concentration.
[a] CLSI M38M51S[10]

Unlike yeasts, molds often require extended incubation times that vary by organism. AFST of *Aspergillus* and *Fusarium* spp. typically requires 48 hours, *Scedosporium* spp. up to 72 hours, and dermatophytes up to 96 hours. In contrast, rapidly growing *Mucorales* usually yield results within 24 hours.

Figure 1 presents examples of mold susceptibility testing reports generated in the clinical microbiology laboratory at the National Institutes of Health Clinical Center. Importantly, the correlation between *in vitro* susceptibility and clinical outcome is less predictable for molds than for bacteria and yeasts. Therapeutic response is strongly influenced by host factors, including immune status, infection site, and pharmacokinetic/pharmacodynamic exposure. As a result, isolates with low MICs may still be associated with treatment failure in severely immunocompromised patients, whereas clinical improvement may occur despite elevated MICs in the context of immune recovery or surgical intervention.[12, 13]

The Important and Challenging Role of Antifungal Testing to Guide Therapy of Severe Mold Infections (*Continued*)

A.

Fungal isolate ID: *Aspergillus fumigatus*

Source: *Bronchoalveolar lavage*

Methodology: CLSI M38

Isolate	*Isolate# 01*	
Antibiotics	MIC	Interp
Amphotericin B	2	NI D1
Isavuconazole	2	I
Itraconazole	>16	NI D1
Micafungin	<=0.015	NI D1
Olorofim	0.06	NI D2
Posaconazole	1	NI D1
Terbinafine	1	NI D1
Voriconazole	2	R

-----DRUG COMMENTS----------

D1 : **There are no standardized susceptibility testing breakpoints for this drug.**

D2 : **Olorofim is not currently approved by the FDA for routine clinical use in humans, and is currently in clinical trials. Olorofim in vitro susceptibility testing is performed by established CLSI broth dilution methods. Clinical breakpoints for susceptibility and resistance have not been established, so the results should be interpreted with caution.**

S=Susceptible R=Resistant NI=No Interpretation NS=Nonsusceptible

All numeric results are in micrograms/mL.

B.

Fungal isolate ID: *Rhizopus arrhizus*

Source: *Biopsy lung*

Methodology: CLSI M38

Isolate	*Isolate# 01*	
Antibiotics	MIC	Interp
Amphotericin B	0.25	NI
Isavuconazole	>16	NI
Manogepix	>4	NI D1
Posaconazole	2	NI
Terbinafine	>2	NI

-----DRUG COMMENTS----------

D1 : **Fosmanogepix, the prodrug of the active moiety of manogepix, is not currently approved by the FDA for routine clinical use in humans and is currently in clinical trials. Manogepix in vitro susceptibilty testing is performed by established CLSI broth dilution methods. Clinical breakpoints for susceptibility and resistance have not been established, so the results should be interpreted with caution.**

NI=No Interpretation NS=Nonsusceptible

All numeric results are in micrograms/mL.

C.

Fungal isolate ID: *Trichophyton rubrum*

Source: *Skin scraping*

Methodology: CLSI M38

Isolate	*Isolate# 01*	
Antibiotics	MIC	Interp
Isavuconazole	<=0.03	NI
Itraconazole	<=0.03	NI
Posaconazole	<=0.03	NI
Terbinafine	0.008	NI
Voriconazole	<=0.03	NI

NI=No Interpretation

All numeric results are in micrograms/mL.

Abbreviations: I, intermediate; interp, interpretation; MIC, minimal inhibitory concentration; NI, no interpretation; R, resistant.

Figure 1. Examples of Susceptibility Testing Reports from a Clinical Microbiology Laboratory for *Aspergillus fumigatus* (A), *Rhizopus arrhizus* (B), and *Trichophyton rubrum* (C).

The Important and Challenging Role of Antifungal Testing to Guide Therapy of Severe Mold Infections (*Continued*)

Emerging Resistance and Clinical Use of AFST

Antifungal resistance in molds has been most clearly established in clinically relevant species such as *A. fumigatus* and dermatophytes (i.e., *Trichophyton*). Other opportunistic molds demonstrate intrinsic resistance to certain antifungal classes rather than acquired resistance (e.g., *Scedosporium*, *Lomentospora*). In contrast, endemic dimorphic fungi such as *Histoplasma capsulatum*, which commonly infect immunocompetent individuals, have not yet shown emergence of antifungal resistance.[14]

Azoles have been the cornerstone of first-line therapy for invasive aspergillosis. However, the recent emergence of azole-resistant *Aspergillus fumigatus* has become a significant global public health concern. Resistance is associated with high mortality. The prevalence of resistance is likely underestimated due to limited diagnostic and surveillance capacity.[15] Resistance arises through both patient-related and environmental mechanisms. In clinical settings, prolonged azole exposure can select for resistant isolates, whereas in the environment, widespread use of agricultural azole fungicides exerts strong selective pressure. The detection of resistant strains in azole-naïve patients further supports the role of environmental reservoirs in resistance acquisition, emphasizing the importance of enhanced surveillance and performance of AFST.

Antifungal-resistant dermatophyte infections have also recently emerged as an important and expanding global health concern. A 2024 survey of U.S. infectious diseases specialists demonstrated that only 65% were aware of antifungal-resistant dermatophytes, and only 39% knew how to access susceptibility testing, highlighting significant gaps in clinician awareness of diagnostic resources.[16] The terbinafine-resistant dermatophyte *Trichophyton indotineae* represents a major clinical challenge. Additional resistant strains, including *Trichophyton mentagrophytes* genotype VII and terbinafine-resistant *T. rubrum*, have been increasingly reported in the United States since 2023.[17] Resistance in *T. indotineae* is thought to be driven in part by inappropriate use of topical corticosteroid–antifungal combinations and suboptimal or incomplete antifungal therapy.

Given reduced susceptibility to terbinafine, first-line oral therapy for dermatophytosis increasingly requires alternative systemic agents such as itraconazole. Optimal outcomes depend on clinician familiarity with resistant dermatophyte infections and, when available, susceptibility testing to guide therapy.

Currently, AFST is often performed selectively, particularly in cases of treatment failure, suspected resistance, or for infections caused by uncommon or intrinsically resistant molds, rather than as a universal first-line diagnostic tool. Additionally, unlike susceptibility testing for bacterial isolates, AFST for molds is performed by only a small number of centers and reference laboratories.

Public Health Surveillance and Screening for Antifungal Resistance

The Centers for Disease Control and Prevention (CDC), in collaboration with a network of public health laboratories, is strengthening surveillance for antifungal resistance through targeted national programs. Within the CDC's Antimicrobial Resistance Lab Network (ARLN), regional public health laboratories, including the Maryland State Public Health Laboratory and the Tennessee State Public Health Laboratory, perform nationwide screening for azole-resistant *A. fumigatus* using a rapid phenotypic screening panel,[18] with confirmatory testing of presumptive resistant isolates using the CLSI M38[9] reference broth microdilution method. Figure 2 demonstrates results with the screening panel (Match Biolabs, Rockville, MD, USA; www.matchbiolabs.com) for azole-susceptible and azole-resistant *A. fumigatus*. Some clinical laboratories are using this panel for initial screening of antifungal resistance in *A. fumigatus*. The Wadsworth Center Mycology Laboratory at the New York State Department of Health in Albany, NY serves as the ARLN reference laboratory for dermatophyte identification and AFST, including testing of *Trichophyton, Epidermophyton,* and *Microsporum* species. The laboratory also performs whole-genome sequencing (WGS) to characterize antifungal resistance mechanisms and monitor transmission dynamics.

The Important and Challenging Role of Antifungal Testing to Guide Therapy of Severe Mold Infections (*Continued*)

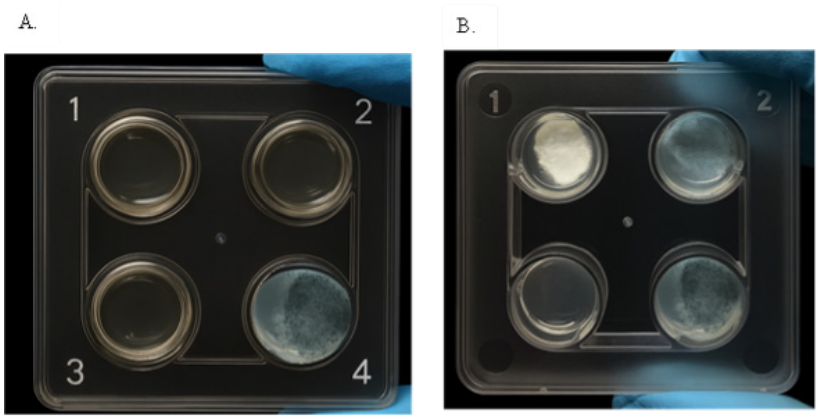

Figure 2. Demonstration of Azole Susceptibility in *Aspergillus fumigatus* Using a Rapid Phenotypic Screening Panel. Wells: 1, itraconazole (4 µg/mL); 2, voriconazole (2 µg/mL); 3, posaconazole (0.5 µg/mL); 4, growth control. (A) *Aspergillus fumigatus* susceptible to itraconazole, voriconazole, and posaconazole. (B) *Aspergillus fumigatus* resistant to itraconazole and voriconazole, but susceptible to posaconazole. Courtesy of Match Biolabs, Rockville, MD.

Molecular approaches such as targeted PCR and sequencing assays, including WGS, are increasingly being developed to detect resistance-associated mutations in molds, including CYP51A mutations associated with azole resistance in *A. fumigatus*, as well as squalene epoxidase mutations in dermatophytes associated with resistance to terbinafine. These methods are expected to play an increasing role in identifying novel resistance mechanisms and tracking transmission and are likely to become integrated into clinical workflows in the near future. In addition, innovative technologies such as matrix-assisted laser desorption/time-of flight mass spectrometry (MALDI-TOF MS)-based susceptibility testing, flow cytometry, and other rapid phenotypic or hybrid genotypic-phenotypic approaches are under development for detection of antifungal resistance in molds.[20]

Conclusion

Invasive mold infections have been increasingly recognized in vulnerable patient populations and are associated with substantial morbidity and mortality. Their incidence is rising, driven by increasing numbers of at-risk patients and the expanding use of molecular diagnostic methods that enhance detection of previously unrecognized fungal pathogens. Despite advances in diagnostics and therapeutics, timely identification of the causative organism, the limited number of antifungal drug classes, emerging and geographically expanding resistance (particularly in *A. fumigatus* and dermatophytes), and limited access to AFST are challenges which complicate clinical management. Integration of microbiological, clinical, and public health approaches is critical to address the growing burden of antifungal resistance and highlights the need to expand laboratory capacity, standardize testing methodologies, and improve diagnostic accessibility to support timely detection and management of antifungal resistance.

Key Learning Points

- Invasive mold infections are increasing globally driven by expanding immunocompromised populations and improved detection of diverse fungal pathogens.
- Antifungal therapy is constrained by limited drug classes, toxicity, and emerging resistance, making pathogen- and susceptibility-guided treatment essential.
- Clinical outcomes in mold infections are strongly influenced by host factors, and *in vitro* MIC values do not always predict therapeutic success.
- Azole-resistant *A. fumigatus* and terbinafine-resistant dermatophytes are increasing global threats with significant clinical impact.
- Simplified screening assays, such as those used by the CDC ARLN, provide an effective first-line approach for presumptive detection of azole resistance in *A. fumigatus*.
- Expanding molecular and genomic tools alongside rapid phenotypic and hybrid technologies are expected to improve antifungal resistance detection, surveillance, and turnaround times in routine clinical practice.

The Important and Challenging Role of Antifungal Testing to Guide Therapy of Severe Mold Infections (*Continued*)

References

1 Denning DW. Global incidence and mortality of severe fungal disease. *Lancet Infect Dis.* 2024;24(7):e428-e438.

2 Martinson ML, Lapham J. Prevalence of immunosuppression among US adults. *JAMA.* 2024;331(10):880-882.

3 Hawksworth DL, Lücking R. Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiol Spectr.* 2017 Jul;5(4):10.1128/microbiolspec.funk-0052-2016.

4 Little J, Rauseo AM, Zuniga-Moya JC, et al. Clinical mycology today: emerging challenges and opportunities. *Open Forum Infect Dis.* 2024;11(7):ofae363.

5 Gu D, Hatch M, Ghannoum M, et al. Treatment-resistant dermatophytosis: a representative case highlighting an emerging public health threat. *JAAD Case Rep.* 2020;6(11):1153-1155.

6 Fang W, Wu J, Cheng M, et al. Diagnosis of invasive fungal infections: challenges and recent developments. *J Biomed Sci.* 2023;30(1):42.

7 Schelenz S, Abdolrasouli A, Armstrong-James D, et al. British Society for Medical Mycology best practice recommendations for the diagnosis of serious fungal diseases: 2025 update. *Lancet Infect Dis.* 2025 Nov 10:S1473-3099(25)00550-X.

8 Hoenigl M, Arastehfar A, Arendrup MC, et al. Novel antifungals and treatment approaches to tackle resistance and improve outcomes of invasive fungal disease. *Clin Microbiol Rev.* 2024;37(2):e0007423.

9 CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi.* CLSI standard M38. Clinical and Laboratory Standards Institute; 2017.

10 CLSI. *Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi.* 4th ed. CLSI supplement M38M51S. Clinical and Laboratory Standards Institute; 2026.

11 CLSI. *Epidemiological Cutoff Values for Antifungal Susceptibility Testing.* 4th ed. CLSI supplement M57S. Clinical and Laboratory Standards Institute; 2022.

12 Naveen KV, Saravanakumar K, Sathiyaseelan A, et al. Human fungal infection, immune response, and clinical challenge-a perspective during COVID-19 pandemic. *Appl Biochem Biotechnol.* 2022;194(9):4244-4257.

13 Paterson PJ, Seaton S, Prentice HG, et al. Treatment failure in invasive aspergillosis: susceptibility of deep tissue isolates following treatment with amphotericin B. *J Antimicrob Chemother.* 2003;52(5):873-6.

14 Verweij PE, Alastruey-Izquierdo A, Amilon K, et al. Closing the gap on antifungal resistance. *Nat Med.* 2026 Apr 15.

15 Zubovskaia A. Azole-resistant *Aspergillus fumigatus:* epidemiology, diagnosis, and treatment considerations. *J Fungi (Basel).* 2025;11(10):731.

16 Gold JAW, Benedict K, Lockhart SR, et al. Recognition of antifungal-resistant dermatophytosis by infectious diseases specialists, United States. *Emerg Infect Dis.* 2024;30(9):1978-1980.

17 Gold JAW, Lipner SR. The rise of antifungal-resistant dermatophyte infections: what dermatologists need to know. *Cutis.* 2025;115(5):151-154.

18 Arendrup MC, Verweij PE, Mouton JW, et al. Multicentre validation of 4-well azole agar plates as a screening method for detection of clinically relevant azole-resistant *Aspergillus fumigatus. J Antimicrob Chemother.* 2017 Dec 1;72(12):3325-3333.

19 Centers for Disease Control and Prevention. AR Lab Network testing services. https://www.cdc.gov/antimicrobial-resistance-laboratory-networks/php/about/testing-services.html. Accessed 15 June 2026.

20 Knoll MA, Ulmer H, Lass-Flörl C. Rapid antifungal susceptibility testing of yeasts and molds by MALDI-TOF MS: a systematic review and meta-analysis. *J Fungi (Basel).* 2021 Jan 18;7(1):63.

Case Study

Successful Treatment of a Metallo-Beta-Lactamase (MBL)-Producing Enterobacterales with Aztreonam-Avibactam

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Case Study

A patient with a history of recent travel to Bangladesh was admitted to the hospital in December 2025 with fever and left unilateral costovertebral angle tenderness. Urinalysis revealed nitrite production and pyuria. A diagnosis of pyelonephritis was made. Blood cultures were performed, which revealed gram-negative bacteria. A rapid molecular test performed on the blood culture broth detected *Escherichia coli*, and both CTX-M and NDM beta-lactamase genes. Shortly after these results became available, therapy was switched from piperacillin-tazobactam to cefiderocol. Per the laboratory's protocols for Enterobacterales isolates positive for metallo-beta-lactamases (MBL), a cefiderocol disk diffusion test was performed in addition to routine antimicrobial susceptibility testing (AST).

The patient remained febrile for an additional 48 hours, with AST revealing resistance to all routinely tested antimicrobials and an "intermediate" result for cefiderocol. Accordingly, treatment was adjusted to aztreonam-avibactam. Because the laboratory had not yet completed a verification study to perform in-house testing of aztreonam-avibactam, the isolate was sent to the laboratory's local Antimicrobial Resistance Laboratory Network (ARLN) laboratory.[1] The aztreonam-avibactam susceptibility testing was performed with reference broth microdilution method (rBMD) by the ARLN laboratory and the MIC result reported was 0.25/4 µg/mL (susceptible). These additional results were added to the patient's report, as shown in Table 1. The patient was treated with aztreonam-avibactam for 7 days, with clinical resolution and subsequent discharge from the hospital.

Table 1. Diagnostic Methods Used by Laboratories to Detect *C. auris*

Antimicrobial Agent	MIC (µg/mL)	Interpretive Category
Amikacin	>32	R
Aztreonam	>64	R
Cefepime	>16	R
Cefiderocol[a]	-	I
Ceftazidime-avibactam	>64/4	R
Ceftriaxone	>32	R
Ciprofloxacin	>2	R
Ertapenem	>8	R
Gentamicin	>8	R
Meropenem	>8	R
Meropenem-vaborbactam	>16/8	R
Piperacillin-tazobactam	>64/4	R
Trimethoprim-sulfamethoxazole	>2/38	R
Aztreonam-avibactam[b]	0.25/4	S

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible.
[a] Testing was performed by the disk diffusion method, with a zone diameter of 13 mm (intermediate).
[b] Performed by the Antimicrobial Resistance Laboratory Network (ARLN) Regional Laboratory.

Successful Treatment of a Metallo-Beta-Lactamase (MBL)-Producing Enterobacterales with Aztreonam-Avibactam (*Continued*)

This case demonstrates the challenges with treatment of MBL-producing isolates of Enterobacterales. MBL enzymes hydrolyze penicillins, cephalosporins and carbapenems, but not monobactams (aztreonam). Although cefiderocol is also not efficiently hydrolyzed by MBLs, resistance can occur due to other mechanisms. The most common MBLs found in clinical isolates globally are the New Delhi metallo-beta-lactamase (NDM), the Verona-integron-mediated (VIM) enzyme and imipenemase (IMP). Aztreonam can be hydrolyzed by other beta-lactamases that are co-expressed by ~85% of MBL producers,[2] including extended-spectrum beta-lactamases (ESBLs), carbapenemases such as KPC or OXA-48, or AmpC enzymes. In the present case, aztreonam resistance was driven by the CTX-M ESBL. While currently available beta-lactamase inhibitors do not inhibit MBL enzymes, avibactam does inhibit the activity of class A (including CTX-M), class C and some class D beta-lactamases. As such, the combination of aztreonam with avibactam is an excellent treatment option for MBL-producing bacterial infections largely based on experience with ceftazidime-avibactam plus aztreonam because this has been the only form of avibactam available.[3] The Infectious Diseases Society of America (IDSA) recommends this treatment (aztreonam plus ceftazidime-avibactam) for infections due to MBL-producing Enterobacterales.[4] However, several challenges exist with administration of aztreonam plus ceftazidime-avibactam, including the limited clinical trial experience reported in the literature and lack of data on optimal dosing.[5] In February 2025, the US FDA approved the combination, aztreonam-avibactam, for the treatment of complicated intra-abdominal infections. The IDSA recommendation will likely be updated with the next version of their guidelines to reflect the availability of aztreonam-avibactam. In 2026, CLSI published aztreonam-avibactam breakpoints for Enterobacterales in the CLSI M100 36th ed.[6]

Resistance to aztreonam-avibactam has been documented. In *E. coli*, resistance usually results from a combination of a modified penicillin binding protein (PBP)-3 target protein to which aztreonam has less affinity and the production of AmpC enzymes, particularly CMY-42, which are not completely inhibited by avibactam.[7] A recent international study showed only 78.1% susceptibility to aztreonam-avibactam among 594 MBL-producing *E. coli* studied.[8] Of these, 70% were recovered from patients in India. Notably, recent data from the ARLN laboratory found that only 28/40 (70%) MBL-producing *E. coli* submitted from 2019-2025 were susceptible to aztreonam-avibactam,[9] reinforcing the importance of susceptibility testing when this combination is used to treat infections.

References

1. ARLN Expanded AST. Accessed 30 March 2026. https://www.cdc.gov/antimicrobial-resistance-laboratory-networks/php/about/testing-services.html
2. Rossolini GM, Stone G, Kantecki M, et al. *In vitro* activity of aztreonam/avibactam against isolates of Enterobacterales collected globally from ATLAS in 2019. *J Glob Antimicrob Resist*. 2022; 30(2022): 214-221.
3. Falcone M, Giordano C, Leonildi A, et al. Clinical features and outcomes of infections caused by metallo-β-lactamase-producing Enterobacterales: A 3-year prospective study from an endemic area. *Clin Infect Dis*. 2024 May 15;78(5):1111-1119. doi: 10.1093/cid/ciad725. Erratum in: Clin Infect Dis. 2024 Aug 16;79(2):580. doi: 10.1093/cid/ciae311. PMID: 38036465.
4. Tamma PD, Heil EL, Justo JA, et al. Infectious Diseases Society of America 2024 guidance on the treatment of antimicrobial-resistant gram-negative infections. *Clin Infect Dis*. 2024; doi:10.1093/cid/ciae403.
5. Shields RK, Doi Y. Aztreonam combination therapy: an answer to metallo-beta-lactamase-producing gram-negative bacteria? *Clin Infect Dis*. 2020; 71(4): 1099-1101.
6. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 36th ed. CLSI supplement M100. Clinical and Laboratory Institute; 2026.
7. Helsens N, Sadek M, Le Terrier C, et al. Reduced susceptibility to aztreonam-avibactam conferred by acquired AmpC-type beta-lactamases in PBP3-modified *Escherichia coli*. *Eur J Clin Microbiol Infect Dis*. 2024; doi:10.1007/s10096-024-04769-z.
8. Lee YL, Wang WY, Huang YT, et al. *In vitro* activity of aztreonam-avibactam and distribution of carbapenemase genes in ceftazidime-avibactam-resistant carbapenem-resistant Enterobacterales: data from the global Antimicrobial Testing Leadership and Surveillance, 2019-2023. *Antimicrob Agents Chemother*. 2026: 70(4):e0154925.
9. Eklund E, Maruka T, Kruczinski K, et al. High *in vitro* activity of aztreonam/avibactam against NDM-producing Enterobacterales with emerging resistance in *Escherichia coli* in the United States Mid-Atlantic region. *Microbiol Spectr*. 2026; 14:e03971-25.

Practical Tips

Practical Tips for Laboratory Testing of Aztreonam-Avibactam for Metallo-Beta-Lactamase (MBL)-Producing Enterobacterales

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This article covers several practical FAQs to consider when you encounter a metallo-beta-lactamase (MBL)-producing isolate of Enterobacterales in your laboratory. As seen in the accompanying case study, testing aztreonam-avibactam may be essential for appropriate clinical management of patients.

1. When should I perform susceptibility testing of aztreonam-avibactam?

Laboratories should develop a testing plan in conjunction with their antimicrobial stewardship team and other relevant institutional stakeholders. Aztreonam-avibactam is appropriate to test against isolates of Enterobacterales that are known to harbor an MBL, including NDM, IMP or VIM.

A statement in CLSI M100 Table 2A-1 strongly recommends that laboratories perform carbapenemase testing for Enterobacterales that are resistant to at least one carbapenem (ertapenem, imipenem, meropenem).[1] For *Proteus, Providencia*, and *Morganella* spp., carbapenemase testing should be considered if isolates are resistant to carbapenems other than imipenem. Carbapenemase testing should identify and ideally differentiate the presence of specific carbapenemase types (e.g., KPC, NDM, OXA-48, VIM, IMP).

Carbapenem-resistant Enterobacterales that are resistant to ceftazidime-avibactam, meropenem-vaborbactam and/or imipenem-relebactam may warrant testing for aztreonam-avibactam, even if a carbapenemase test is not performed. Some hospitals may opt to use aztreonam-avibactam to treat carbapenemase-producing Enterobacterales, such as those that express KPC genes but are negative for MBLs. For this reason, close collaboration with the pharmacy and the antimicrobial stewardship team is imperative when determining testing and reporting protocols. In CLSI M100 Table 1A-1, CLSI recommends testing aztreonam-avibactam routinely in institutions that serve patients at high risk for multidrug-resistant organisms.[1] However, aztreonam-avibactam should only be reported following cascade reporting rules. For many institutions, testing aztreonam-avibactam on reflex, following documentation of an MBL-producing isolate of Enterobacterales, is likely appropriate.

2. What testing options are currently available for aztreonam-avibactam?

510(k) premarket notifications[2] have been awarded for the following devices which are considered FDA-cleared for testing aztreonam-avibactam (Table 1).

Table 1. FDA-Cleared Devices for Testing Aztreonam-Avibactam

Vendor	Test
Hardy Diagnostics	HardyDisk™ Aztreonam-avibactam 30/20 µg
Thermo Fisher Scientific	Oxoid™ Aztreonam-avibactam disk 30/20 µg
bioMérieux	Etest® Aztreonam-avibactam
Liofilchem™	MTS™ Aztreonam-avibactam
Thermo Fisher Scientific	Sensititre Aztreonam-avibactam MIC test

Laboratories should consult with their local vendors on the availability of these tests. In addition, the drug manufacturer, AbbVie, has an online resource that provides an updated list of FDA-cleared tests for aztreonam-avibactam.[3]

Laboratories that elect not to test aztreonam-avibactam in-house should consult with their contracted reference laboratories to determine the availability of testing. As in the case study, another option for obtaining aztreonam-avibactam results is through the Antibiotic Resistance Laboratory Network (ARLN).[4] Laboratories should contact their local public health laboratory to determine how to utilize this resource.

Practical Tips for Laboratory Testing of Aztreonam-Avibactam for Metallo-Beta-Lactamase (MBL)-Producing Enterobacterales (*Continued*)

3. Where can I get isolates to help with verification of the aztreonam-avibactam tests?

A specific panel of isolates for verification of aztreonam-avibactam tests is not yet available from the CDC & FDA Antimicrobial Resistance (AR) Bank. However, over 160 isolates deposited in the AR Bank have aztreonam-avibactam results available and laboratories may select from these for their aztreonam-avibactam verification.[5] A set of 30 isolates is available for purchase from Laboratory Specialists for aztreonam-avibactam verification.[6] Isolates from the AR Bank and Laboratory Specialists are provided with reference broth microdilution MIC results, which can be used as the comparator for laboratories verifying aztreonam-avibactam tests.

4. What quality control should be performed when testing aztreonam-avibactam?

Quality control ranges for select QC strains are available in CLSI M100 36th ed. for disk diffusion and MIC testing.[1] The preferred quality control strain is *Klebsiella pneumoniae* ATCC® 700603™. Assessing this isolate's beta-lactamase integrity should be done when the strain is first subcultured from a frozen or lyophilized stock by testing aztreonam or another antimicrobial agent. Refer to CLSI M100 Table 4A-2 or 5A-2 for guidance on which antimicrobial agents may be tested.[1] When using commercial tests, laboratories should always follow the quality control recommendations put forth by the manufacturer.

5. What breakpoints should be used for aztreonam-avibactam?

CLSI published disk diffusion and MIC breakpoints in CLSI M100 36th ed. for the Enterobacterales (excluding *Salmonella* and *Shigella* spp.).[1] These breakpoints as shown below in Table 2 are also recognized by the U.S. FDA.

Table 2. CLSI and FDA Breakpoints for Enterobacterales

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints (mm)			Interpretive Categories and MIC Breakpoints (µg/mL)		
		S	I[a]	R	S	I	R
Aztreonam-avibactam	30/20 µg	≥25	22-24	≤21	≤4/4	8/4	≥16/4

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible.
[a] Confirmatory MIC testing is indicated for isolates with zones of 22-24 mm to avoid overcalling susceptible results as intermediate.

6. Do I need to be aware of any issues with aztreonam-avibactam disk diffusion tests?

During review of the aztreonam-avibactam disk diffusion versus MIC test results, CLSI noted that isolates with intermediate zones of 22-24 mm may demonstrate MICs in the susceptible range.[1] Specifically, 28 of 32 (87.5%) of isolates with a susceptible MIC of 4/4 µg/mL demonstrated a disk diffusion result of 22-24 mm (Intermediate). As such, isolates with disk diffusion results in the intermediate category should be tested by an MIC method as noted in the footnote to Table 2 above. Few data are currently available on the performance of commercial tests, but laboratories should review FDA decision summaries[2] for performance information on tests they are considering implementing.

7. Can I use the aztreonam-ceftazidime-avibactam broth disk elution (BDE) test published in CLSI M100 to evaluate aztreonam-avibactam susceptibility?

Ceftazidime-avibactam-aztreonam BDE results agreed with aztreonam-avibactam results when a limited number of isolates (n=61) was tested during the validation of BDE. While the BDE test is still an approved method in CLSI M100 36th ed.,[1] it was introduced prior to establishment of aztreonam-avibactam breakpoints. In addition, the concentration of aztreonam tested by the BDE method does not align with approved breakpoints. Use of FDA-cleared tests for aztreonam-avibactam is preferable.[7]

8. If my hospital pharmacy does not have access to clinical formulations of aztreonam-avibactam, but would like to use ceftazidime-avibactam plus aztreonam, do I need to test the triple combination?

In general, aztreonam-avibactam MICs should predict the activity of ceftazidime-avibactam plus aztreonam. However, interpretation of these results should be undertaken with caution, as the 2026 CLSI breakpoints for aztreonam-avibactam are based on a dose of 2 g (1.5 g aztreonam plus 0.5 g avibactam) IV q 6h over 3h,[1] while dosing regimens for ceftazidime-avibactam plus aztreonam are poorly standardized.

Practical Tips for Laboratory Testing of Aztreonam-Avibactam for Metallo-Beta-Lactamase (MBL)-Producing Enterobacterales (*Continued*)

9. Is aztreonam-avibactam active against MBL-producing bacteria other than Enterobacterales?

There are no breakpoints from CLSI or FDA for bacteria other than the Enterobacterales, however, the following information is available:

- Enterobacterales breakpoints in CLSI M100[1] do not apply to *Salmonella* or *Shigella*. *Salmonella* isolates have been documented to harbor MBLs, but there are no *in vivo* or *in vitro* data at this time to demonstrate efficacy with aztreonam-avibactam.
- *Pseudomonas aeruginosa* may harbor MBLs, but *in vitro* data show limited activity of aztreonam-avibactam against *P. aeruginosa*.[8]
- *Acinetobacter baumannii* are intrinsically resistant to aztreonam, so treatment with aztreonam-avibactam is not effective.
- *Stenotrophomonas maltophilia* harbor an L1 MBL and an L2 class A beta-lactamase. As such, aztreonam-avibactam is an attractive option for treatment of *S. maltophilia.* The Infectious Diseases Society of America (IDSA) recommends use of the ceftazidime-avibactam plus aztreonam combination for treatment of *S. maltophilia* infections.[9] *In vitro,* aztreonam-avibactam was shown to inhibit 97.8% of 1839 *S. maltophilia* at an MIC of ≤8/4 µg/mL.[10] A murine neutropenic thigh infection model was used to assess *in vivo* efficacy of humanized doses of aztreonam-avibactam against *S. maltophilia*.[11] This study showed efficacy for most isolates with MICs ≤4/4 µg/mL. At this time, CLSI has not yet set breakpoints for *S. maltophilia* and aztreonam-avibactam, but this may be reviewed soon. Testing may be indicated, but there are limited data that document performance of currently available MIC methods, and disk diffusion correlates have yet to be established.

References

1. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 36th ed. CLSI supplement M100. Clinical and Laboratory Institute; 2026.
2. U.S. Food & Drug Administration. Devices@FDA. Accessed 30 March 2026. www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm
3. EMBLAVEO™ Susceptibility Testing. Accessed 30 March 2026. https://www.emblaveo.com/susceptibility-testing.
4. ARLN Expanded AST. Accessed 30 March 2026. https://www.cdc.gov/antimicrobial-resistance-laboratory-networks/php/about/testing-services.html
5. CDC & FDA Antimicrobial Resistance Isolate Bank. Atlanta (GA): CDC. Accessed 30 March 2026. https://wwwn.cdc.gov/arisolatebank/
6. Laboratory Specialists, Inc. Accessed 30 March 2026. www.labspec.org/reference-laboratory-mic-testing-of-new-antimicrobial-agents/
7. Hamze H, Stefanovic A, Gowland L, et al. Evaluation of aztreonam/avibactam gradient test strip and aztreonam-ceftazidime/avibactam broth disk elution method for susceptibility testing of metallo-beta-lactamase positive carbapenemase-producing bacteria. *Diagn Microbiol Infect Dis.* 2026; 114(3): 117216.
8. Mauri C, Maraolo AE, Di Bella S, et al. The revival of aztreonam in combination with avibactam against metallo-beta-lactamase-producing gram-negatives: A systematic review of *in vitro* studies and clinical cases. *Antibiotics (Basel).* 2021; 10(8):1012.
9. Tamma PD, Heil EL, Justo JA, et al. Infectious Diseases Society of America 2024 guidance on the treatment of antimicrobial-resistant gram-negative infections. *Clin Infect Dis.* 2024; doi:10.1093/cid/ciae403.
10. Sader HS, Duncan LR, Arends SJR, et al. Antimicrobial activity of aztreonam-avibactam and comparator agents when tested against a large collection of contemporary *Stenotrophomonas maltophilia* isolates from medical centers worldwide. *Antimicrob Agents Chemother.* 2020; 64(11): e01433-20.
11. Fu Y, Fouad A, Roenfanz HF, et al. Efficacy of human-simulated aztreonam-avibactam against *Stenotrophomonas maltophilia* in the neutropenic murine thigh infection model. *J Antimicrob Chemother.* 2025; 80(12): 3431-3437.

Practical Tips

Disk Diffusion Testing Approaches and Validation of Updated Breakpoints

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FDA and Clearance of Disks

Background

Recent antimicrobial susceptibility testing (AST) breakpoint updates have brought important progress and ongoing challenges for laboratories and AST manufacturers. As CLSI continues to update MIC breakpoints, corresponding disk diffusion (DD) breakpoints have also been revised and published. A list of these updates can be found in the front of CLSI M100 36th ed.[1]

In January 2025, the FDA released substantial updates to the Susceptibility Test Interpretive Criteria (STIC) website.[2] With these updates the FDA formally recognized many additional CLSI breakpoints, resulting in broad alignment between FDA-recognized and current CLSI breakpoints. This shift has been widely welcomed by clinical laboratories, as it reduces long-standing discrepancies between CLSI standards and FDA recognition. The CLSI Breakpoint Implementation Toolkit (BIT) Part B now provides a consolidated list of CLSI MIC breakpoints alongside their FDA recognition status, serving as a key reference for laboratories navigating breakpoint implementation.[3]

Despite the progress with breakpoint harmonization, significant challenges remain for disk manufacturers. Unlike MIC-based devices, there is still no clear FDA guidance on how manufacturers can update DD breakpoints for disks that were previously FDA-cleared. In 2024, FDA introduced Predetermined Change Control Plan (PCCP) guidance that applies to FDA clearance of updated breakpoints for MIC tests only.[4] When a manufacturer submits a 510(k) for a new drug for an MIC method, a protocol for how MIC breakpoints will be updated in the future is also submitted as part of the 510(k) packet. This protocol, once approved by FDA, can then also be applied to other drugs previously FDA-cleared for the MIC method. For a 510(k) submission for a new drug disk, FDA expects the manufacturer to submit a protocol for updating breakpoints for the new disk should DD breakpoints be updated in the future. However, this protocol cannot be applied retroactively to disks that were previously FDA-cleared, as can be done for MIC tests.[4] This is likely because disks cleared prior to about 2017 were able to gain FDA-clearance with a simple labeling 510(k) submission and did not require submission of DD test performance data as part of the packet. Because no DD test performance data were required to clear disks previously, it is unclear how the manufacturer can assess performance of an older disk when the breakpoint is updated. As a result, it is assumed that disk manufacturers must conduct new DD test performance studies and submit new 510(k) applications to update disk breakpoint labeling. However, this has never been confirmed as no disk 510(k) re-submissions have been made by manufacturers due to the lack of FDA guidance and substantial costs associated with such studies.

These regulatory hurdles have downstream implications for clinical laboratories. Under Centers for Medicare & Medicaid Services (CMS) requirements, laboratories must verify or validate diagnostic tests before patient use, and any change to breakpoints triggers the need for re-verification or re-validation. Together, these factors continue to complicate timely adoption of updated DD breakpoints, even as regulatory alignment on MIC breakpoints improves.

Disk Diffusion Testing Approaches and Validation of Updated Breakpoints (*Continued*)

CLSI Process for Updating Disk Diffusion Breakpoints

The procedure for updating CLSI MIC and DD breakpoints is described in CLSI M23.[5] Disk diffusion breakpoints are determined after the MIC breakpoints have been set. In brief, this involves:

- Performing DD testing concurrent with reference broth microdilution (rBMD) testing against a robust set of bacteria appropriate for the antimicrobial agent/combination (e.g., testing 100 isolates of a species). For updating breakpoints, new and previously collected data from multiple laboratories are often used.
- Testing isolates by rBMD in parallel with DD testing using disks from three manufacturers. All testing is performed from one inoculum.
- Preparing scattergrams that demonstrate the MIC value and corresponding zone size of inhibition for each isolate tested. (Figure 1)
- Determining the zone diameter values that correlate best with updated MIC breakpoints to minimize the numbers of discrepancies due to very major (false susceptible), major (false resistant) or minor errors (one result is intermediate and the other is susceptible or resistant) results.

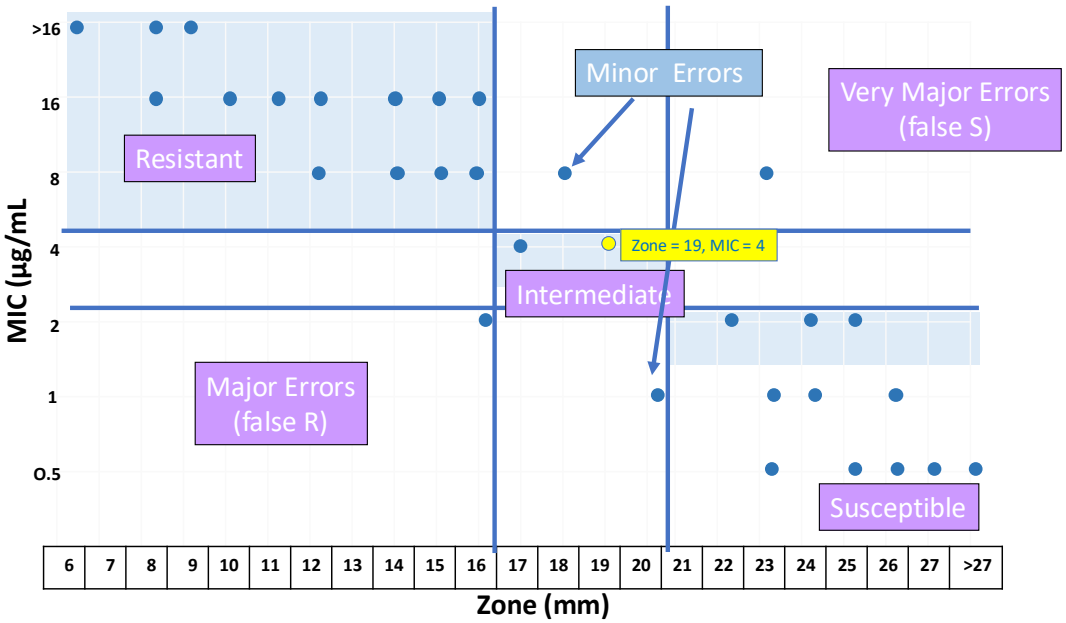

Figure 1. Scattergram for Hypothetical Drug X for *Pseudomonas aeruginosa*. Extracted from CLSI M100 Course.[6] Note the yellow dot that shows an isolate with a zone of 19 mm and an MIC of 4 µg/mL. Horizontal lines mark the MIC breakpoints (µg/mL) at ≤2 Susceptible, 4 Intermediate, ≥8 Resistant. Vertical lines mark the corresponding DD breakpoints (mm) at ≥21 Susceptible, 17-20 Intermediate, ≤16 Resistant.

Approach to Disk Diffusion Validation

Since FDA has not cleared commercial disks for any of the "updated" CLSI breakpoints, a validation study is needed prior to implementing these DD breakpoints in the clinical laboratory.

A validation is done to establish performance characteristics of a test which is not FDA-cleared or if it differs from the FDA-cleared testing recommendations (e.g., different breakpoints). In contrast, a verification is performed on FDA-cleared tests to verify that the system is performing within the manufacturer's specifications.

Disk Diffusion Testing Approaches and Validation of Updated Breakpoints (*Continued*)

Because updated DD breakpoints published in CLSI M100[1] were validated through studies that meet CLSI M23 requirements,[5] the purpose of each laboratory's validation is to determine whether acceptable test performance using these breakpoints is achieved by their laboratory with the particular brand(s) of disks and agar used. This can be done by testing representative isolates and resolving discrepancies that occur during their study.

To determine how much testing needs to be done for a validation, several factors need to be considered. For example:

- Have there been changes to CLSI QC ranges or to recommendations for measuring zone diameters? Multiple changes indicate that testing of more isolates may be helpful.
- Are there new or multiple types of resistance that need to be detected using the disk? For example, were the disk studies performed many years ago when resistant strains were not circulating? Or does the patient population served by the laboratory harbor high levels of multidrug-resistant isolates? If so, this may require more testing so that isolates with various mechanisms of resistance can be included.

To highlight potential approaches to DD validation, here are examples for validating updated meropenem and cefazolin urine DD breakpoints. The final decision on the extent of testing is made by the laboratory director who must consider risks associated with breakpoint modification. Records should be maintained as per the laboratory's quality assurance policies.

Scenario #1 – meropenem disks

The laboratory has been using a commercial automated MIC system for testing meropenem, which has been FDA-cleared and verified with the current (updated) meropenem breakpoints. The laboratory decided to implement meropenem DD as a backup since they have seen increasing numbers of carbapenem-resistant organisms and a few instances where confirmation of results with a second method would be helpful.

The laboratory notes that CLSI updated (and FDA subsequently recognized) the MIC and DD breakpoints for meropenem for Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter* spp.[1,2]

Scenario #1 - validation plan

1. Test 10 isolates of a variety of species of Enterobacterales and 5 isolates each of *P. aeruginosa*, and *Acinetobacter* spp. using meropenem disks.
2. Select from clinical isolates with a variety of meropenem MIC susceptibility results.
3. Compare interpretations (S, I, R) obtained from DD testing with interpretations from MIC testing using the laboratory's commercial AST system for each isolate.
4. If discrepancies are noted:
   a. Repeat testing by both test (i.e., DD) and comparator (i.e., MIC) testing using the same inoculum suspension or source plate, as appropriate.
   b. If discrepancies persist, the director must determine if additional testing is warranted using the same or alternative disk and medium sources and/or if the test does not perform satisfactorily to be used in their laboratory.

Scenario #2 – cefazolin disks

The laboratory, which uses DD as its primary AST method, was recently asked to review oral agents reported on outpatient urine isolates as the institution will soon see a substantial increase in outpatient urine cultures due to acquisition of a new clinic. They have not been reporting cefazolin using urine breakpoints but now wish to do so.

As background, CLSI added breakpoints in 2014 for when cefazolin is used as a surrogate test to predict results of oral cephalosporins used for uncomplicated urinary tract infections (UTIs) due to *E. coli, Klebsiella pneumoniae* and *Proteus mirabilis* as shown in Table 1.

Disk Diffusion Testing Approaches and Validation of Updated Breakpoints (*Continued*)

Table 1. CLSI Cefazolin Urine Breakpoints[1]

Antimicrobial Agent	Disk Content	Zone (mm)			MIC (µg/mL)		
		S	I	R	S	I	R
Cefazolin (U)[a] (surrogate test for oral cephalosporins and uncomplicated UTIs)	30 µg	≥15	-	≤14	≤16	-	≥32

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible; UTI, urinary tract infection.
[a] Report only on organisms isolated from the urinary tract.

However, FDA does not recognize CLSI cefazolin urine breakpoints as indicated by the following notation on the FDA STIC website for cefazolin dated 2/12/25:[2]

"Exceptions or additions to the recognized standards:

For the bacteria listed below, susceptibility test interpretive criteria are not recognized at this time: Enterobacterales (uncomplicated UTI only)"

Scenario #2 – validation plan

1. Test 5 isolates each of *E. coli, K. pneumoniae* and *P. mirabilis* using cefazolin disks.
   - **a.** Select from clinical isolates with a variety of cefazolin MIC susceptibility results and send to a reference lab that has validated the updated cefazolin DD and/or MIC urine breakpoints, OR
   - **b.** Select isolates from CDC & FDA Antimicrobial Resistance (AR) Bank[7] or other reliable isolate resource.
   - **c.** For both a. and b., ensure cefazolin MIC results can been interpreted using the cefazolin urine breakpoints (Table 1) with the MIC method selected.

   Note: As of May 2026, the highest concentration of cefazolin tested for isolates in the CDC & FDA AR Bank was 8 µg/mL. Therefore "resistant" disk diffusion results cannot be validated with these isolates. Although there is an ongoing effort to compile a panel of CDC & FDA AR Bank isolates which have been tested for cefazolin at concentrations of 16 µg/mL, at this time an alternative source of isolates with "resistant" disk diffusion results is needed (ideally, 5 isolates).
2. Compare interpretations (S, R) obtained from DD testing with interpretations from MIC testing or from a validated cefazolin DD test (e.g., at a reference laboratory).
3. If discrepancies are noted:
   - **a.** Repeat DD testing and comparator test as appropriate depending on how the validation was performed.
   - **b.** If discrepancies persist, the director must determine if additional testing is warranted using the same or alternative disk and medium sources and/or if the test does not perform satisfactorily to be used in the laboratory.

   Note: For DD testing, there may be differences in results based on different Mueller-Hinton agar sources, which should be considered during troubleshooting.

Discussion

There are currently no formal standards or guidelines for validation of DD tests. However, CLSI has been working on such a guideline which is expected to be published in late 2026. CLSI published the "Breakpoint Implementation Toolkit" in 2023 for validation of updated MIC breakpoints and although specific guidelines for DD tests are not included in the toolkit as of yet, some of the recommendations in this resource can be applied to validation of DD tests. In addition, verification or validation of any laboratory test must always take into consideration accrediting agency requirements.

Disk Diffusion Testing Approaches and Validation of Updated Breakpoints (*Continued*)

References

1. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 36th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2026.
2. FDA-Recognized Antimicrobial Susceptibility Test Interpretive Criteria. Accessed 30 March 2026. https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria
3. CLSI Breakpoint Implementation Toolkit (BIT). CLSI. Accessed 30 March 2026 https://clsi.org/resources/breakpoint-implementation-toolkit/
4. FDA – Predetermined Change Control Plans for Medical Devices. Draft Guidance for Industry and FDA Staff; 2024. Accessed 30 March 2026 https://www.fda.gov/regulatory-information/search-fda-guidance-documents/predetermined-change-control-plans-medical-devices
5. CLSI. *Development of* In Vitro *Susceptibility Test Methods, Breakpoints, and Quality Control Parameters.* 6th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute; 2023.
6. CLSI M100 Course. Accessed 30 March 2026. https://clsi.org/shop/education/courses/m100-course/
7. CDC & FDA Antimicrobial Resistance Isolate Bank. Atlanta (GA): CDC. Accessed 30 March 2026. https://wwwn.cdc.gov/arisolatebank/

Hot Topic

Emerging Trends in Carbapenemase-Producing Carbapenem-Resistant Enterobacterales in the United States, 2019-2023

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Infections with carbapenemase-producing carbapenem-resistant Enterobacterales (CP-CRE) represent a significant and evolving threat to patient safety and public health. These infections are associated with high mortality rates and limited treatment options.[1] Furthermore, some carbapenemase genes can be carried on mobile genetic elements, facilitating horizontal spread between and within bacterial species.[2] In the United States, the five most common carbapenemase genes circulating among healthcare-associated gram-negative bacilli are *Klebsiella pneumoniae* carbapenemase (bla_{KPC}), New Delhi metallo-β-lactamase (MBL) (bla_{NDM}), oxacillinase-48-like carbapenemases ($bla_{OXA\text{-}48\text{-}like}$), Verona integron-encoded MBL (bla_{VIM}), and the imipenemase metallo-β-lactamase (bla_{IMP}).[3]

In December 2025, CDC published a report describing CP-CRE trends using data reported to CDC's Antimicrobial Resistance Laboratory Network (AR Lab Network).[4] Rankin et al., developed an open cohort of states in the U.S. with state-mandated submission of CR-*Klebsiella* spp., CR-*Escherichia coli* and CR-*Enterobacter* spp.[4] The cohort started July 1st, 2020, and included at least 36 consecutive months of data from January 2019 through December 2023. CP-CRE was defined as the presence of ≥1 carbapenemase (KPC, NDM, VIM, IMP or OXA-48-like) using molecular or immunochromatographic methods, or carbapenemase production positivity without the presence of one of the above carbapenemases. Annual aggregate and state-specific incidence rates were estimated for CP-CRE overall and by carbapenemase using isolate counts as the numerator and 2010 U.S. Census Bureau population estimates as the denominator. Age-adjusted rates used the 2010 United States population estimates as the standard population.

In the Rankin et al. paper, the number of states in the open cohort increased from 24 to 29 between 2019 to 2021, remained stable through 2023, and 35% of the U.S. population was represented. Overall, 37,784 CRE isolates were tested and 14,182 (38%) harbored a carbapenemase. The unadjusted annual CRE incidence was 18% higher in 2023 than in 2019. The age-adjusted incidence of CP-CRE and NDM-CRE increased 69% and 461%, respectively, between 2019 and 2023. The increase in NDM-CRE was observed across 48% of states within the cohort and across all genera, particularly *E. coli*. OXA-48-like incidence increased slightly, and the incidence of VIM and IMP both remained relatively low and stable over the study period. The incidence of KPC-CRE decreased from 2019 to 2020 and then increased again, with rates in 2023 reaching levels similar to those observed in 2019.

This report underscores two major concerns. Firstly, the epidemiology of CP-CRE has changed from 2019 to 2023, with a notable increase in NDM, a carbapenemase associated with high resistance and limited treatment options. KPC was by far the most common carbapenemase detected in the U.S. in 2019 but its trend varied and by 2023, its incidence rate was similar to that of NDM. Secondly, this report highlights the critical importance of comprehensive carbapenemase mechanism testing to appropriately guide treatment decisions, support molecular surveillance and epidemiology and inform infection prevention strategies.

In 2025, CLSI recommended phenotypic and/or molecular testing for any Enterobacterales isolate resistant to any carbapenem tested, with caveats noted for specific species.[5] However, not all clinical laboratories are able to perform testing in-house due to capacity and resource challenges.[6] Since 2017, the AR Lab Network has supported all state public health jurisdictions across the country by providing carbapenemase mechanism testing, thereby helping bridge gaps in laboratory capacity for carbapenemase testing. In the Fall of 2026, the AR Lab Network will expand antimicrobial susceptibility testing to align with the most current Infectious Diseases Society of America (IDSA)-recommended antimicrobials, which includes aztreonam-avibactam specifically for NDM-CRE isolates, representing another important step toward mitigating the threat of highly resistant organisms.[7]

Given testing capability across the AR Lab Network, laboratories should consider sending suspected isolates to their state public health laboratory for carbapenemase testing and mechanism characterization, if carbapenemase mechanism or phenotypic testing is unavailable in their own laboratory.

Emerging Trends in Carbapenemase-Producing Carbapenem-Resistant Enterobacterales in the United States, 2019-2023 (*Continued*)

References

1 Schwaber M.J, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of carbapenem-resistant *Klebsiella pneumoniae* acquisition among hospitalized adults and effect of acquisition on mortality. *Antimicrob Agents Chemother.* 2008, 52:1028–1033.

2 CDC. *Antibiotic resistance threats in the United States, 2019.* U.S. Department of Health and Human Services, CDC, Atlanta, GA (2019).

3 CDC. Antimicrobial Resistance & Patient Safety Portal. https://arpsp.cdc.gov/profile/arln/cre.

4 Rankin D.A, Stahl A, Sabour S, Khan M.A, Armstrong T, Huang J.Y, Baggs J, Walters M.S. Changes in carbapenemase-producing carbapenem-resistant Enterobacterales, 2019 to 2023. *Annals of Internal Medicine.* Volume 178, Issue 12, December 2025, pages 1677-1828.

5 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 35th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, 2025.

6 Shugart A, Walters M.S, Weiner L.M, Lonsway D, Kallen A.J. Hospital microbiology laboratory practices for Enterobacteriaceae: Centers for Disease control and prevention national healthcare safety network (NHSN) annual survey, 2015 and 2016. *Infect Control Hosp Epidemiol.* 39 (9) (2018), pages. 1115-1117.

7 Tamma P.D, Heil, E.L, Justo J.A, Mathers A.J, Satlin M.J, Bonomo R.A. Infectious Diseases Society of America 2024 Guidance on the Treatment of Antimicrobial-Resistant Gram-Negative Infections. *Clinical Infectious Diseases.* ciae403, https://doi.org/10.1093/cid/ciae403.

Next Up!

What's Coming and What's Under Consideration by the CLSI AST Subcommittee

Review of current breakpoints

- Oral cephalosporins
- *Acinetobacter* spp. - cephalosporins
- *Neisseria gonorrhoeae* – cefixime and ceftriaxone disk diffusion
- Several breakpoints for other non-fermenters

Quality Control

- Ongoing monitoring of current QC ranges to determine if adjustments are needed
- QC strains for newer drugs
- Harmonization of QC with EUCAST

Methods

- Piperacillin-tazobactam disk content
- Cefiderocol disk content
- Use of early growth from agar plates for inoculum preparation
- Colony counts (turbidity vs numbers of organisms in inoculum) for QC
- Alternative methods when CLSI M07 reference method doesn't work for a new antimicrobial agent

Other

- *Pseudomonas aeruginosa* – when to perform carbapenemase testing
- Intrinsic resistance definition; additions/revisions to CLSI M100 Appendix B
- Designation and inclusion of investigational drugs in CLSI M100
- Additional educational materials and "tools" for:
  - Optimizing QC
  - Updating breakpoints in user's laboratory (Update 2023 BIT)
- Updating CLSI M100 Course to CLSI M100 36th edition

Documents expected soon

- CLSI M52 2nd edition (verification) - projected Fall 2026
- New! CLSI M68 1st edition (validation of AST breakpoints) - projected Fall 2026
- CLSI M45 4th edition (fastidious/uncommon bacteria) - projected January 2027
- CLSI M100 37th edition - projected January 2027