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CLSI Subcommittee on Antimicrobial Susceptibility Testing

CLSI AST News Update

Janet A. Hindler, MCLS, MT(ASCP), F(AAM), Editor
Audrey N. Schuetz, MD, MPH, D(ABMM), Editor

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

Inside This Issue:

M100 Update:
CLSI M100-Ed33: Updated Aminoglycoside Breakpoints for Enterobacterales and *Pseudomonas aeruginosa* 2

Case Study:
Aminoglycoside Case Study 6

News Update:
The Latest on Testing Cefiderocol 8

Case Study:
New CLSI Intrinsic Resistance Guidance for Fungi 10

CLSI and the AST Subcommittee Meetings

1. Content from the Winter 2021, Summer 2021, and Winter 2022 meetings can be found **here.**
2. Save the date for the next meetings:
   - January 18-23, 2024 | Tempe, Arizona
   - March 10-14, 2024 | Atlanta, Georgia
   - June 21-25, 2024 | Chicago, Illinois

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.

- You can access that Newsletter **here.**
- To learn more about upcoming or past meetings, click **here.**
- CLSI posts meeting minutes and summaries for public access **here.**
- For a quick overview, you can check out a "New Attendee Orientation" video presentation **here.**

Interested in becoming a CLSI volunteer? Learn more here.

Please remember that CLSI AST Subcommittee welcomes suggestions from you about any aspect of CLSI documents, educational materials, or this News Update.

New! CLSI M100-Ed33: Updated Aminoglycoside Breakpoints for Enterobacterales and *Pseudomonas aeruginosa*

Romney M. Humphries, Vanderbilt University Medical Center, Nashville, Tennessee, USA

Major changes to the aminoglycoside (gentamicin, tobramycin, and amikacin) breakpoints were published in CLSI M100-Ed33 (see Table 1).

Table 1. Status of Breakpoint Revisions for Aminoglycosides in CLSI M100-Ed33

Aminoglycoside	Organism/Organism Group	
	Enterobacterales	*P. aeruginosa*
Gentamicin	Lowered	Deleted
Tobramycin	Lowered	Lowered
Amikacin	Lowered	Changed to urine only
Plazomicin	Added	-

No changes were made to aminoglycoside breakpoints for *Acinetobacter* spp. or "Other Non-Enterobacterales."

Background and Reasons for the Changes

Aminoglycosides inhibit protein synthesis by binding to the aminoacyl site of the 16S ribosomal RNA. This antimicrobial class has activity against both gram-positive and gram-negative bacteria, as well as many *Mycobacterium* spp. and some parasites. Aminoglycosides have no activity against anaerobic bacteria and are inactive against *Burkholderia, Stenotrophomonas, Streptococcus,* and *Enterococcus* (with the exception of use in combination therapy to attain synergy for the enterococci). Although aminoglycosides may appear active against *Salmonella* and *Shigella in vitro,* they are ineffective against these genera clinically. Today, the most common use of the aminoglycosides is to treat serious infections caused by aerobic gram-negative bacilli, either alone or as part of combination therapy.

Resistance to the aminoglycosides in gram-negative bacteria occurs by three primary pathways:

1. Inactivation of the aminoglycoside by the bacterium's production of aminoglycoside-modifying enzymes that acetylate, phosphorylate, or adenylate the drugs
2. Alteration of the bacterial ribosomal target site through methylation
3. Decreasing the cell wall permeability to the aminoglycosides, particularly for *P. aeruginosa*

The newest aminoglycoside, plazomicin (released in 2018), was engineered to overcome the action of aminoglycoside-modifying enzymes, and breakpoints for plazomicin and Enterobacterales only are published for the first time in CLSI M100-Ed33.

Aminoglycoside use is associated with nephrotoxicity and ototoxicity. Nephrotoxicity is mitigated through use of off-label, high-dose extended interval (once-daily) dosing, which is now the standard of care, as opposed to multiple daily dosing.[1]

The aminoglycoside (gentamicin, tobramycin, and amikacin) breakpoints had not been reexamined since their introduction in the 1980s. However, review of modern pharmacokinetic and pharmacodynamic (PK/PD) data against members of the Enterobacterales and *P. aeruginosa* demonstrated:

- No safe aminoglycoside dosing regimen was predicted to achieve bacterial 1- or 2-log killing, regardless of the breakpoint applied (2022 or 2023).
- Bacterial stasis (ie, growth inhibition) was achievable with the aminoglycosides using extended interval dosing, but only for isolates with minimal inhibitory concentrations (MIC) below the susceptible breakpoints listed in CLSI M100-Ed32 (see Table 2).

CLSI M100-Ed33: Updated Aminoglycoside Breakpoints for Enterobacterales and *Pseudomonas aeruginosa* (*Continued*)

An important change with the updated breakpoints is the elimination of gentamicin as a suggested treatment option for *P. aeruginosa*. A maximum gentamicin MIC of 0.5 µg/mL for *P. aeruginosa* was predicted to achieve the exposure required for bacterial stasis, which is far below the *P. aeruginosa* epidemiological cutoff value (ECV) of 8 µg/mL. In other words, the data demonstrated wild-type isolates (with MICs below the ECV) were not treatable with gentamicin, which raises the possibility of intrinsic resistance, although intrinsic resistance has not been formally addressed by the Intrinsic Resistance Working Group of the CLSI AST Subcommittee. Increasing the dose of gentamicin is not possible due to the risk of toxicity.

Importantly, the breakpoints in CLSI M100-Ed33 were established using a stasis (rather than a 1- or 2-log kill) endpoint. In other words, isolates that are susceptible by these breakpoints are anticipated to have their growth inhibited, but not be killed, by the aminoglycosides when given as monotherapy. Bacteriostasis endpoints like these are suitable for infections with lower bacterial burden, good source control, and for patients with fewer comorbidities and for whom the consequences of inadequate therapy are low, such as urinary tract infections (UTIs). For this reason, CLSI cautions that monotherapy with the aminoglycosides should be only used for UTIs. Combination therapy for indications other than UTIs should be considered, along with consultation with an infectious diseases specialist.

Important comments related to the updated aminoglycoside breakpoints that appear in CLSI M100-Ed33 are listed in Table 3.

Next Steps

Laboratories should discuss the aminoglycoside breakpoint changes listed in CLSI M100-Ed33 with their institution's antimicrobial stewardship programs, as well as with infectious diseases clinicians and pharmacy. The US Food and Drug Administration (FDA) has not yet recognized these revised breakpoints, meaning no commercial manufacturer can obtain FDA-clearance for the updated Enterobacterales and *P. aeruginosa* aminoglycoside breakpoints. Laboratories may consider adoption of the updated breakpoints, off-label, following a validation study, if their test system includes MIC dilutions low enough to accommodate the breakpoints (see Table 4).

Interim steps may include:

1. Enterobacterales and *P. aeruginosa*:
   - Add comment when aminoglycosides are reported.

     Example:

     "Aminoglycosides should not be used as monotherapy for systemic infections. Consultation with an infectious diseases specialist is recommended."
   - Suppress aminoglycosides and report on request only, using disk diffusion validated using the 2023 breakpoints.
   - Implement new aminoglycoside breakpoints on commercial AST system, if possible .
2. *P. aeruginosa*:
   - Suppress or report gentamicin as "R".
   - Report amikacin only on urine isolates.

CLSI M100-Ed33: Updated Aminoglycoside Breakpoints for Enterobacterales and *Pseudomonas aeruginosa* (*Continued*)

Table 2. Enterobacterales and *P. aeruginosa* MIC Breakpoints (µg/mL) for Gentamicin, Tobramycin, Amikacin, and Plazomicin [1]

Organism / Agent	Obsolete CLSI M100-Ed32			Updated CLSI M100-Ed33		
	S	I	R	S	I	R
Enterobacterales						
Gentamicin	≤4	8	≥16	≤2	4	≥8
Tobramycin	≤4	8	≥16	≤2	4	≥8
Amikacin	≤16	32	≥64	≤4	8	≥16
Plazomicin	–	–	–	≤2	4	≥8
P. aeruginosa						
Gentamicin[2]	≤4	8	≥16	–	–	–
Tobramycin	≤4	8	≥16	≤1	2	≥4
Amikacin[3]	≤16	32	≥64	≤16	32	≥64
Plazomicin		–	–	–	–	–

[1]Disk diffusion breakpoints have also been updated. Refer to CLSI M100-Ed33.

[2]Intrinsic resistance of *P. aeruginosa* to gentamicin has not been formally addressed by the Intrinsic Resistance Working Group.

[3]Amikacin for use only for infections that originate in the urinary tract when CLSI M100-Ed33 breakpoints are applied.

Table 3. New Comments Published in CLSI M100-Ed33 to Accompany the Updated Aminoglycoside Breakpoints

Location	New Comment
Table 2A Enterobacterales	(55) Breakpoints for gentamicin, tobramycin, and amikacin are based on population distributions of various species, PK/PD target attainment analyses with an endpoint of net bacterial stasis and limited clinical data. Clinical outcomes data for aminoglycosides as monotherapy for systemic infections are limited and have resulted in worse treatment outcomes for infections outside of the urinary tract compared with other therapies. Combination therapy for most indications other than urinary tract infections should be considered. Consultation with an infectious diseases specialist is recommended.
Table 2B-1 *P. aeruginosa*	(28) Breakpoints for tobramycin and amikacin are based on population distributions of various species, PK/PD target attainment analyses with an endpoint of net bacterial stasis, and limited clinical data. Clinical outcomes data for aminoglycosides as monotherapy for systemic infections are limited and have resulted in worse treatment outcomes for infections outside of the urinary tract compared with other therapies. Combination therapy for most indications other than urinary tract infections should be considered. Consultation with an infectious diseases specialist is recommended.

CLSI M100-Ed33: Updated Aminoglycoside Breakpoints for Enterobacterales and *Pseudomonas aeruginosa* (*Continued*)

Table 4. Availability of Low Aminoglycoside Dilutions on Automated AST Systems to Accommodate CLSI M100-Ed33 Breakpoints

Organism / Agent	BD Phoenix	Beckman Coulter MicroScan	bioMérieux Vitek2	ThermoFisher Sensititre	Accelerate PhenoTest BC
Enterobacterales					
Amikacin	No, lowest dilution is 8 µg/mL	Yes, on select panels	Yes	Yes, on select panels	Yes
Gentamicin	Yes	Yes	Yes	Yes	Yes
Tobramycin	Yes	Yes	Yes	Yes, on select panels	Yes
P. aeruginosa					
Amikacin	Yes	Yes	Yes	Yes	Yes
Tobramycin	No, lowest dilution is 2 µg/mL	Yes, on select panels	Yes	Yes, on select panels	Yes

References

1 Nicolau DP, Freeman CD, Belliveau PP, Nightingale CH, Ross JW, Quintiliani R. Experience with a once-daily aminoglycoside program administered to 2,184 adult patients. *Antimicrob Agents Chemother.* 1995;39(3):650-655. doi: 10.1128/AAC.39.3.650. PMID: 7793867; PMCID: PMC162599.

2 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 32nd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2022.

3 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 33rd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2023. Access here.

Case Study

Aminoglycoside Case Study

Graeme N Forrest, MBBS, Professor of Medicine, Division of Infectious Diseases, Rush University, Chicago, Illinois, USA

A 45-year-old patient with acute myeloid leukemia (AML) received induction chemotherapy 2 weeks ago and currently has an absolute neutrophil count of <100 cells/µL. The patient had been on levofloxacin prophylaxis for his neutropenia, but developed a fever a week ago, at which point meropenem was started. All cultures and imaging were negative.

The patient developed hypotension and was transferred to the intensive care unit, where he was intubated and placed on vasopressors. A chest x-ray showed dense right lower lobe pneumonia. Vancomycin and amikacin were added to the meropenem therapy a day later.

Blood and sputum cultures grew gram-negative rods, identified as *Pseudomonas aeruginosa* by rapid diagnostic testing. The patient's condition continued to deteriorate and repeat blood cultures one day later grew *P. aeruginosa*. Unfortunately, the patient expired on day 3 of hospitalization. Final cultures grew carbapenem-resistant *P. aeruginosa*, susceptible to amikacin and tobramycin, but resistant to gentamicin by applying CLSI M100-Ed32 breakpoints (see Table 1).[1]

Important Takeaways

This case reflects 2 important points:

1. By applying the updated breakpoints published in CLSI M100-Ed33, tobramycin would have been the only aminoglycoside predicted to achieve bacterial stasis for systemic infections due to *P. aeruginosa*.[2] It was inappropriate to use amikacin, since amikacin is effective against *P. aeruginosa* only in treatment of urinary tract infections.

2. When gram-negative bacteremia occurs as a breakthrough infection (ie, in a patient who is already receiving gram-negative-directed therapy such as this patient on levofloxacin), addition of an aminoglycoside to meropenem is not an appropriate therapeutic approach. Addition of an aminoglycoside would not add a significant amount of efficacy to the meropenem. Rather, the clinician should have treated with a different beta-lactam drug such as piperacillin-tazobactam or cefepime, or perhaps one of the newer beta-lactam inhibitor combinations (eg, ceftolozane-tazobactam or ceftazidime-avibactam).[3]

Changes to the aminoglycoside breakpoints require careful conversation with antimicrobial stewardship programs and possible revisiting of current clinical guidelines (such as those for the intensive care unit [ICU] where aminoglycosides are cornerstone antimicrobials for the management of gram-negative infections).

Table 1. AST Profile of the *Pseudomonas aeruginosa* Isolate

Antimicrobial Agent	MIC (µg/mL)	Interpretive Category	
		CLSI M100-Ed32[1]	CLSI M100-Ed33[3]
Amikacin	16	S	N/A
Aztreonam	>32	R	R
Cefepime	>32	R	R
Ceftolozane-tazobactam	2/4	S	S
Ciprofloxacin	>2	R	R
Gentamicin	16	R	N/A
Piperacillin-tazobactam	>128/4	R	R
Tobramycin	2	S	I
Meropenem	>16	R	R

Abbreviations: I, intermediate; N/A, not applicable; R, resistant; S, susceptible.

Aminoglycoside Case Study (*Continued*)

References

1 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 32nd Edition. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2022.

2 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 33rd Edition. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2023.

3 Tamma P, Aitken S, Bonomo R, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America guidance on the treatment of extended-spectrum β-lactamase producing Enterobacterales (ESBL-E), carbapenem-resistant Enterobacterales (CRE), and *Pseudomonas aeruginosa* with difficult-to-treat resistance (DTR-*P. aeruginosa*). *Clin Infect Dis.* 2021:72(7):e169-e183. Doi:10.1093/cid/ciaa1478.

The Latest on Testing Cefiderocol

Andrea Ferrell, BD Life Sciences, Sparks, Maryland, USA
Janet Hindler, Los Angeles County Department of Public Health, Los Angeles, California, USA

In the July 2020 issue of this News Update, cefiderocol (trade name Fetroja®, manufactured by Shionogi) was discussed to include its projected use, requirements for testing, and more. Since that time, several new developments have occurred for cefiderocol testing. This article will bring clinical laboratorians up to date with key facts about testing cefiderocol and a recent warning statement that was added to CLSI M100-Ed33.

In 2020, CLSI cefiderocol breakpoints were considered investigational (INV) because cefiderocol was not yet approved by the US Food and Drug Administration (FDA) for clinical use. FDA has since approved cefiderocol for treatment of adults with complicated urinary tract infections (cUTIs) and hospital-acquired and ventilator-associated bacterial pneumonia due to *Pseudomonas aeruginosa*, *Acinetobacter baumannii* complex (pneumonia only), and several members of the Enterobacterales as described here.

Key facts about *in vitro* testing of cefiderocol:

1. Cefiderocol is considered a last resort drug for treatment of infections due to multidrug resistant gram-negative aerobic bacteria[1,2] and is typically tested and reported on these bacteria only.
2. Cefiderocol has no clinically relevant *in vitro* activity against most gram-positive bacteria or anaerobic bacteria, and there are no recommendations for testing these organism groups.
3. Broth dilution testing of cefiderocol requires the use of iron-depleted, cation-adjusted Mueller-Hinton broth.
4. Disk diffusion testing of cefiderocol is performed on routine Mueller-Hinton agar. There is no need to obtain special iron-depleted Mueller-Hinton agar for disk diffusion testing.
5. FDA-cleared cefiderocol disks are available from three manufacturers (see Table 1).
6. For manual broth microdilution, two FDA-cleared assays are available (ComASP and Sensititre), in addition to a Sensititre research use only (RUO) test (see Table 1). An RUO gradient strip from Liofilchem is available for testing *P. aeruginosa* only (see Table 1).
7. Currently, cefiderocol is not available on any automated AST system.
8. Isolates for verification or validation of cefiderocol tests are available from:
   - CDC & FDA Antibiotic Resistance (AR) Isolate Bank
   - Laboratory Specialists, Inc. (LSI)
9. LSI provides reference broth microdilution minimal inhibitory concentration (MIC) testing of cefiderocol and is a CLIA-certified laboratory.
10. Breakpoints for cefiderocol provided by CLSI and FDA differ for *P. aeruginosa* and *A. baumannii* complex (see Table 2). FDA-cleared tests are based on FDA breakpoints, and use of alternative breakpoints for FDA-cleared tests require validation. EUCAST has cefiderocol breakpoints only for Enterobacterales and *P. aeruginosa*; the breakpoints can be found here.

Shionogi maintains an up-to-date listing of available testing methods here.

Table 1. Testing Options for Cefiderocol in Addition to Reference Broth Microdilution

Disk Manufacturer (30 µg disk)	Gradient Diffusion	Broth Microdilution
BD[a] Hardy Diagnostics[a] Oxoid[a] Liofilchem[b]	Liofilchem[b]	Liofilchem ComASP® Cefiderocol[a] Sensititre panels (MDRGN2F[a] and MDRGNX2F[b]) from Thermo Fisher

[a]FDA cleared
[b]Research use only in the USA

The Latest on Testing Cefiderocol (*Continued*)

Table 2. FDA and CLSI Breakpoints for Cefiderocol[3,4]

Bacteria	FDA Breakpoints						CLSI Breakpoints					
	MIC (µg/mL)			DD (mm)[a]			MIC (µg/mL)			DD (mm)[a]		
	S	I	R	S	I	R	S	I	R	S	I	R
Enterobacterales[b,c]	≤4	8	≥16	≥16	9–15	≤8	≤4	8	≥16	≥16	9–15	≤8
P. aeruginosa	≤1	2	≥4	≥22	13–21	≤12	≤4	8	≥16	≥18	13–17	≤12
Acinetobacter baumannii complex	≤1	2	≥4	≥19	12–18	≤11	≤4	8	≥16	≥15[d]	–	–
S. maltophilia	–	–	–	–	–	–	≤1	–	–	≥15	–	–

Abbreviations: DD, disk diffusion; I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible.
[a] Disk content = 30 µg.
[b] Clinical efficacy was shown for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae* complex in patients with complicated urinary tract infections (cUTI).
[c] Clinical efficacy was shown for *E. coli*, *K. pneumoniae*, *E. cloacae* complex, and *S. marcescens* in patients with hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia (HABP/VABP).
[d] Disk diffusion zone diameters ≤ 14 mm should not be interpreted or reported because zone diameters ≤ 14 mm occur with resistant, intermediate, and susceptible isolates. For isolates with zone diameters ≤ 14 mm, do not report cefiderocol without performing an MIC test.
[e] CLSI breakpoints are based on PK/PD properties, MIC distributions, and limited clinical data.

There are challenges with reading results from both disk diffusion and reference broth microdilution MIC testing of cefiderocol. These were addressed early after the drug achieved FDA approval, in a minireview by Simner and Patel,[5] and broth microdilution endpoints are also shown in CLSI M100 Appendix I.[3] Since that time, additional testing issues have come to light, leading CLSI to add the following statement to the 2023 edition of CLSI M100, to emphasize these challenges: "The accuracy and reproducibility of cefiderocol testing results by disk diffusion and broth microdilution are markedly impacted by iron concentration, inoculum preparation, and may vary by disk and media manufacturer. Depending on the type of variance observed, false resistant or false susceptible results may occur. Testing subsequent isolates is encouraged. Discussion with prescribers and antimicrobial stewardship members about the potential for inaccuracies is recommended." Members of the CLSI AST Subcommittee have provided additional guidance to support this statement.[6]

References

1 Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America antimicrobial-resistant treatment guidance: Gram-negative bacterial infections. Infectious Diseases Society of America 2022; Version 1.1. Available at www.idsociety.org/practice-guideline/amr-guidance/. Accessed June 1, 2023.

2 Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America Guidance on the treatment of AmpC β-lactamase-producing Enterobacterales, carbapenem-resistant *Acinetobacter baumannii,* and *Stenotrophomonas maltophilia* infections. Infectious Diseases Society of America 2022; Version 2.0. Available at www.idsociety.org/practice-guideline/amr-guidance-2.0/. Accessed June 1, 2023.

3 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 33rd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2023. Access here.

4 US Food and Drug Administration. Antibacterial Susceptibility Test Interpretive Criteria (STIC). https://www.fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteria, 2023. Accessed June 1, 2023

5 Simner PJ, Patel R. Cefiderocol antimicrobial susceptibility testing considerations: the Achilles' heel of the Trojan horse? *J Clin Microbiol.* 2020;59(1). DOI: https://doi.org/10.1128/JCM.00951-20.

6 Simner PJ, Palavecino EL, Satlin MJ, et al. Potential of inaccurate cefiderocol susceptibility results: a CLSI AST subcommittee advisory. *J Clin Microbiol*. 2023. 61:e0160022. doi: 10.1128/jcm.01600-22.

Case Study

New CLSI Intrinsic Resistance Guidance for Fungi

Audrey N. Schuetz, Mayo Clinic, Rochester, MN

Case:

A 60-year-old female with a history of colon cancer presented to the Emergency Department with acute onset of abdominal pain. She had a recent proctocolectomy (removal of rectum and part of colon). Her temperature was normal, blood pressure was 136/70, and heart rate was 149 beats per minute.

Her abdomen was tender throughout. A pelvic abscess was seen on abdominal imaging. Draining of the abscess produced dark debris that looked like stool, suggestive of a bowel leak. The patient's peripheral white blood cell count was elevated at 10.7 x 10^9 cells/L (normal range 3.4 – 9.6 x 10^9 cells/L).

Gram stain of the pelvic fluid showed:

- Few white blood cells.
- Few gram-positive cocci.
- Few gram-positive bacilli.
- Few yeast.

The patient was started empirically on cefepime, metronidazole, vancomycin, and voriconazole to cover for aerobes, anaerobes, and yeasts. Aerobic bacterial and fungal cultures of the abscess were positive for multiple organisms: *Enterococcus hirae, Lacticaseibacillus* (*Lactobacillus*) *rhamnosus*, and *Candida krusei* (*Pichia kudriavzevii*). Anaerobic bacterial culture was not ordered. Blood cultures were negative. The *E. hirae* was susceptible to daptomycin and vancomycin, but susceptibility testing was not performed on *L. rhamnosus*. Antifungal susceptibility testing (AFST) was performed on *C. krusei* since yeasts were present on the direct Gram stain and due to the patient's complicated medical course. Refer to Table 1 for preliminary AFST results for *C. krusei* performed using a commercial broth microdilution method. Minimal inhibitory concentrations (MICs) were interpreted according to breakpoints and epidemiological cutoff values (ECVs) available in CLSI documents M27M44S and M57S.[1,2]

Table 1. Preliminary Antifungal Susceptibility Test Results and Report Comment for *Candida krusei*

Antifungal Agent	MIC (µg/mL)	Interpretive Category
Amphotericin B	1	WT*
Caspofungin	0.12	S
Fluconazole	**4**	**???**
Itraconazole	0.25	WT*
Posaconazole	0.25	WT*
Voriconazole	0.25	S

Abbreviations: MIC, minimal inhibitory concentration; S, susceptible; WT, wild type.
* Report comment: There are currently no breakpoints or interpretive criteria for this organism and this antifungal agent. The MIC is below the wild type MIC, which suggests that this isolate is not likely to have an acquired mechanism of resistance. Clinical outcomes cannot be predicted based on this information.

Breakpoints for caspofungin and voriconazole were applied to the organism. ECVs were applied to amphotericin B, itraconazole and posaconazole according to CLSI M57S because breakpoints do not exist.[2] There is no breakpoint or ECV for *C. krusei* and fluconazole.

New CLSI Intrinsic Resistance Guidance for Fungi (*Continued*)

Should fluconazole be reported? If so, how?

Case Study Answer:

Over the past several years, the Intrinsic Resistance Working Group of the CLSI Subcommittee on Antifungal Susceptibility Tests (AFST SC) has been developing fungal intrinsic resistance (IR) guidance for laboratories. This guidance was developed, in part, due to the microbiology checklist item (MIC.42740) introduced by the College of American Pathologists in 2015 which indicates that unusual or inconsistent antifungal test results should be further investigated by the laboratory. The AFST SC realized at the time that there was limited guidance concerning antifungal test results that are inconsistent with the species identification. Thus, the AFST Intrinsic Resistance Working Group was formed to study IR in both yeasts and molds. IR is defined as inherent or innate (not acquired) antimicrobial resistance which is reflected in wild-type antimicrobial patterns of all or almost all representatives of a species. IR is so common that susceptibility testing is unnecessary. Members of the IR Working Group review population MIC distributions for organisms, gather clinical data on outcomes, and review expert opinion by professional societies on various organism-antifungal combinations. This group's approach was modeled after that of the IR Working Group of CLSI's Antimicrobial Susceptibility Testing Subcommittee (AST SC) which investigates bacterial IR.

After formal assessment, CLSI concluded that *C. krusei* is intrinsically resistant to fluconazole. Many studies with thousands of isolates tested by reference CLSI methodology demonstrate high modal MICs of 16 µg/mL or greater for *C. krusei* against fluconazole. Professional organizations such as the Infectious Diseases Society of America also recommend against use of fluconazole to treat infections due to *C. krusei* based on poor clinical response.

In this Case Study example, fluconazole should be reported as resistant, despite the relatively low MIC obtained. This can be achieved by removing the MIC value and reporting a "resistant" categorical result. Table 2 demonstrates the final AFST report for the *C. krusei* isolate.

Table 2. Final Antifungal Susceptibility Test Results and Report Comment for *Candida krusei*

Antifungal Agent	MIC (µg/mL)	Interpretive Category
Amphotericin B	1	WT*
Caspofungin	0.12	S
Fluconazole	–	R
Itraconazole	0.25	WT*
Posaconazole	0.25	WT*
Voriconazole	0.25	S

Abbreviations: MIC, minimal inhibitory concentration; R, resistant; S, susceptible; WT, wild type.
* Report comment: There are currently no breakpoints or interpretive criteria for this organism and this antifungal agent. The MIC is below the wild type MIC, which suggests that this isolate is not likely to have an acquired mechanism of resistance. Clinical outcomes cannot be predicted based on this information.

Because antifungal treatment is often empiric, IR comments may be helpful to report before AFST results are available so certain drugs might be avoided. IR comments may be linked with an isolate (rather than with AFST results) so that they are released with the organism name alongside other comments such as "AFST results pending." A simple report comment, such as "*C. krusei* is intrinsically resistant to fluconazole," can be helpful in guiding empiric therapy. Such IR comments are particularly helpful when the fungus must be sent out to a reference laboratory for testing and the turnaround time to results will be prolonged. They can also be helpful even if AFST is not performed on an isolate, in order to lead the clinician away from using a certain antifungal agent.

Reporting decisions for IR should be undertaken by the microbiology laboratory in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders.

New CLSI Intrinsic Resistance Guidance for Fungi (Continued)

Over 20 fungal-antifungal combinations have been assessed for IR by CLSI, and IR has been determined for several fungi, including yeasts and molds. Intrinsic resistance tables are available in appendixes of the M27M44S (yeast) and M38M51S (mold) documents.[1,3] M27M44S is now freely available online. Refer to Table 3 below which was extracted from M27M44S for a list of yeasts which are intrinsically resistant to certain antifungal agents. The M57S document on ECVs for fungi includes a comprehensive summary table outlining available breakpoints, ECVs, and IR for all fungi (yeasts and molds) in Table 6.[2]

Table 3. Intrinsic Resistance of Yeasts[1]

	Candida krusei	*Cryptococcus* spp.	*Rhodotorula* spp.	*Trichosporon* spp.
Anidulafungin		IR	IR	IR
Caspofungin		IR	IR	IR
Fluconazole	IR		IR	
Micafungin		IR	IR	IR

Abbreviation: IR, intrinsic resistance.

Case Follow-up:

The patient's symptoms resolved with antibiotics and drainage of the abscess. She was discharged home on daptomycin, ertapenem, and voriconazole. The ertapenem was maintained for gram-negative and anaerobic coverage. It is common practice to include such antimicrobials to provide coverage for the variety of bowel microbiota which may be present but may not be recovered in culture.

References

1. CLSI. *Performance Standards for Antifungal Susceptibility Testing of Yeasts*. 3rd ed. CLSI supplement M27M44S. Clinical and Laboratory Standards Institute; 2022.
2. CLSI. *Epidemiological Cutoff Values for Antifungal Susceptibility Testing*. 4th ed. CLSI supplement M57S. Clinical and Laboratory Standards Institute; 2022.
3. CLSI. *Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi*. 3rd ed. CLSI supplement M38M51S. Clinical and Laboratory Standards Institute; 2022.