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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.

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CLSI and the AST Subcommittee Meetings

1. Content from the Summer 2022, Winter 2023, and Summer 2023 meetings can be found **here.**
2. Save the date for the next meetings:
   - 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.**

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

Acinetobacter – the Bad, the Awful, and the Downright Ugly

By Sara Blosser, PhD, D(ABMM)

Between 2018-2021, the National Healthcare Safety Network (NHSN) reported that 0.4% (n=1,951) of hospital-acquired infections (HAIs) in the United States were caused by *Acinetobacter* spp. Of these, 28-45% were not susceptible to carbapenem antibiotics (ie, intermediate or resistant).[1] CDC's 2019 Antibiotic Resistance Threats Report estimated that there were 8,500 carbapenem-resistant *Acinetobacter* cases in hospitalized patients in 2017.[2] Consistently, the *A. calcoaceticus-A. baumannii* complex (*A. baumannii*) is the largest cause of clinical *Acinetobacter* spp. infections and is most often recovered from respiratory specimens.

Mortality for severe *A. baumannii* infection ranges from 14-73%[3-9] It is no wonder then, that *A. baumannii*, particularly carbapenem-resistant *A. baumannii* (CRAB), is one of the top antibiotic resistance threats the US faces today.[2] Several studies have documented global carbapenem resistance rates for *A. baumannii*—and they agree, as bad as the resistance rates are in the United States (36-45%), they are even worse in Asia and Latin America (66-86%).[10-11] Additionally, many CRAB isolates harbor carbapenemases, with OXA-23 being the most common.[10-12]

In Many Cases, *Acinetobacter* is Simply a Colonizer

Colonization indicates that an organism resides on or within an individual, but that the organism is not causing an active infection. Colonization with *Acinetobacter* spp. is relatively common in healthy individuals (15-43%)[13-16], but increases substantially in hospitalized or other at-risk patients (up to 75%).[15] Risk factors for *A. baumannii* colonization include intensive care unit (ICU) stay, recent surgery, mechanical ventilation, cancer, immunosuppressive treatment, presence of a central venous catheter, dialysis, and previous treatment with β-lactams (especially carbapenems) or fluoroquinolones.[17] Differentiating between colonization and infection, however, is quite complex—especially in individuals in the ICU or who are on ventilators.[17]

When identified in culture from a non-sterile source without other signs or symptoms of infection, *A. baumannii* is generally considered to represent colonization, but this clinical information is often not available to the microbiology laboratory. From an infection prevention and control perspective, colonization presents challenges, as patient-to-patient transmission can occur whether the patient is infected or colonized.

So, Why is CRAB so Hard to Treat?

First, *A. baumannii* is intrinsically resistant to penicillins, ampicillin, amoxicillin-clavulanate, 1st and 2nd generation cephalosporins, cephamycins, aztreonam, ertapenem, macrolides, and more[18-19]; therefore, treatment options are few. Second, it has an "unrivaled adaptive nature," to cite one source[20] – due to a "plethora of mechanisms," including a predilection for developing heteroresistance, and an ability to acquire (by plasmid or transposon) resistance markers. In other words, *A. baumannii* truly is bad news. Finally, as previously stated, it is difficult to distinguish colonization from infection, confounding an already complex patient landscape and making it hard to draw solid conclusions from the outcomes of clinical trials.[17,21]

What are the Treatment Options for CRAB? Let's ask an Expert!

Note: *Tremendous thanks to Dr. Pranita Tamma for her insight on the use of sulbactam-durlobactam in treating CRAB infections. Dr. Tamma is the lead author on the Infectious Disease Society of America (IDSA) Treatment Guidelines for AMR Infections. The information contained in this interview will be released in the 2024 update.*

Q: Dr. Tamma, what are some considerations for clinicians when considering treatment options for CRAB?

A: The first step in managing CRAB is distinguishing colonization and infection. Recovery of CRAB in clinical isolates in-and-of itself does not signify infection without the proper clinical context. Once it is established that a patient indeed has a CRAB infection, use of sulbactam-based therapy is recommended. Sulbactam can be administered in the form of sulbactam-durlobactam, or as high-dose ampicillin-sulbactam. Sulbactam-durlobactam is preferred, whenever available. Of note, I have no conflicts of interest with any diagnostic or therapeutic companies.

Acinetobacter – the Bad, the Awful, and the Downright Ugly (*Continued*)

Q: Sulbactam-durlobactam or (XACDURO®), is a relatively new agent in the fight against CRAB. Can you tell us some more about why and how it is used?

A: Durlobactam, as a β-lactamase inhibitor, has the ability to protect sulbactam from hydrolysis from OXA carbapenemases so sulbactam can successfully reach its penicillin binding protein targets (PBP1 and 3). In the clinical trial from which sulbactam-durlobactam was FDA-approved,[22] all patients who received sulbactam-durlobactam also received imipenem-cilastatin.

If sulbactam-durlobactam is administered, it is generally suggested to be administered in combination with a carbapenem (either imipenem-cilastatin or meropenem), at least until clinical improvement is observed. It is possible the carbapenem is serving as a decoy as it is being hydrolyzed by any OXA-carbapenemases that may happen to escape inhibition by durlobactam, indirectly protecting sulbactam. Alternatively, since carbapenems and sulbactam have different PBP targets, it is also theoretically possible that some carbapenem molecules will reach their PBP2 target under the protection of durlobactam, enabling the targeting of multiple PBPs. Clinical outcomes data are not currently available describing the outcomes of patients with CRAB infections who received sulbactam-durlobactam in the absence of a carbapenem.

Q: And if sulbactam-durlobactam is not available? Would clinicians defer to the previous IDSA recommendations?

A: If sulbactam-durlobactam is not available, an alternate approach is the administration of high-dose ampicillin-sulbactam in combination with a second agent.[21] The secondary agents to consider include minocycline, tigecycline, cefiderocol, or polymyxin B.

A Few More Notes on Sulbactam-Durlobactam

In a 16-country clinical trial of treatment options for CRAB pneumonia or bloodstream infection, patients receiving sulbactam-durlobactam, along with imipenem, had a 13.2% lower mortality rate than patients receiving colistin-imipenem regimens.[22] Additionally, nephrotoxicity was 24.4% lower for patients receiving sulbactam-durlobactam than those who received colistin. Although ineffective against Class B metallo-β-lactamases, such as NDM or IMP, sulbactam-durlobactam has good efficacy against Class A and D carbapenemases, including OXA-23.[23]

For more information on sulbactam-durlobactam (XACDURO®), see the companion article in this issue.

What About Colistin?

Because of recent clinical trials, IDSA guidelines discourage use of colistin-containing regimens for the treatment of CRAB.[21] But the truth is, colistin has been a mainstay of CRAB-therapy regimens for two decades, and much of the world continues to use colistin. So, should you be testing and reporting colistin? The answer is, maybe. It's not preferred, but sometimes it is all we have in the fight against CRAB. Here is some guidance, directly from the Rationale Document CLSI MR01 that may assist your decision-making process: "Polymyxins are last-resort agents, and if they are available, alternative agents are preferred. A susceptible category for polymyxins cannot be established because there is no MIC for which likely clinical efficacy can be predicted. When used, polymyxins should be administered at maximally tolerated doses and in combination with a second agent."[24] Work with your antimicrobial stewardship program to determine the appropriate lab testing and reporting strategy for your facility. If you are going to use a polymyxin as part of your treatment regimen, polymyxin B is preferred over colistin given its more favorable pharmacokinetic profile.[25]

Other AST Challenges

Minocycline and tigecycline are IDSA-recommended agents for treatment of CRAB, yet tigecycline continues to be a challenge from an AST-perspective due to the absence of breakpoints. No breakpoint-setting organizations—neither CLSI, FDA, nor EUCAST—provide tigecycline breakpoints for *Acinetobacter* spp. So, tigecycline and *Acinetobacter* will continue to be another clinical microbiology conundrum—used clinically—but given the absence of breakpoints, there are substantial technical challenges in performing MIC-based testing and its interpretation.[26-28]

Acinetobacter – the Bad, the Awful, and the Downright Ugly (*Continued*)

CRAB and Public Health

Finally, what is being done to combat CRAB and other multidrug-resistant *A. baumannii* infections? CRAB presents some unique challenges to prevention and detection, especially due to its tremendous abundance in long-term care facilities, its ability to survive on surfaces for long periods of time, and its poor performance on assays for phenotypic carbapenemase detection. This last point is a good one to reiterate: unlike Enterobacterales or *Pseudomonas aeruginosa*, the modified carbapenemase inactivation method (mCIM) and CarbaNP perform poorly with *A. baumannii*, and thus are not approved for use for CRAB isolates.[19] Many commercially available molecular methods also do not include OXA-23 and OXA-24, the two most common acquired carbapenemases identified in CRAB in the US.

CRAB is one of the priority targets for testing in the Centers for Disease Control and Prevention's (CDC) Antimicrobial Resistance Laboratory Network (AR Lab Network).[29] State and local public health laboratories (PHLs) actively recruit clinical labs to submit clinical isolates of *A. baumannii* resistant to imipenem, meropenem, or doripenem (MIC ≥ 8 µg/mL). At PHLs, these isolates are tested for carbapenemase activity and mechanisms (including KPC, NDM, VIM, IMP, OXA-48, OXA-23, and OXA-24) and evaluated against a broad panel of anti-gram-negative antimicrobial agents. Whole genome sequencing is utilized in the identification of additional carbapenemases, tracing intra- and inter- facility transmission, and understanding the molecular epidemiology of submitted isolates. Seven AR Lab Network Regional labs also perform colonization screening for specific carbapenemase-producing CRAB. This screening is a pillar of the prevention, infection control, and response arms of the AR Lab Network.

Conclusion

We can all agree, infections caused by *A. baumannii* are tricky to treat, test, and detect. The drugs used to treat these infections are complicated to test and determining whether a culture result represents colonization or infection only compounds the challenge. Whether a cause of colonization or infection, *A. baumannii*, particularly CRAB, can spread rapidly within health care facilities, highlighting the incredible importance of the microbiology laboratory to detect this organism and perform AST.

We have a few glimmers of hope though: public health is ramping up their support for infection control and response efforts to mitigate CRAB transmission, and sulbactam-durlobactam is giving clinicians new therapeutic options to combat infections. Our work is far from over, though, as we still need additional therapeutic options and improved test methods for existing therapies like tigecycline and polymyxin b.

Use What You Read:

- Follow the recommendations from the 33rd edition of CLSI M100-S33, including:
  - Tiered antimicrobial testing and reporting for *Acinetobacter* spp. (Table 1D).
  - Current *Acinetobacter* spp. breakpoints (Table 2B-2).
  - If testing cefiderocol, colistin, or polymyxin b, make a special note of the reporting comments (Table 2B-2).
  - Follow the recommendations in Appendix A: confirming AST, following up with your public health laboratory, and saving isolates, as appropriate.
- Work with your antimicrobial stewardship program to harmonize testing challenges and clinical practice for treating CRAB infections.
- Be familiar with the current IDSA treatment guidelines. If you are not routinely testing the drugs recommended for CRAB treatment, develop a strategy for when/how these drugs can be ordered and tested in your facility.
- Submit CRAB isolates to your local or state public health lab.

Acinetobacter – the Bad, the Awful, and the Downright Ugly (*Continued*)

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Case Study

Antifungal Body Site Reporting for *Candida* spp.

Amir Seyedmousavi, National Institutes of Health, Bethesda, MD
Audrey N. Schuetz, Mayo Clinic, Rochester, MN

A 59-year-old woman with metastatic breast cancer was admitted to a hospital for fever and suspected sepsis 12 days after chemotherapy-induced neutropenia (<1,000 neutrophils/µL in peripheral blood; normal range 2500-7000 neutrophils/µL). The patient was started empirically on cefepime, vancomycin, and anidulafungin. Two sets of blood cultures grew *Candida tropicalis* on day 3 post admission. Since this isolate was recovered from the blood, both species-level identification and susceptibility testing were performed. At the time of blood culture positivity, the patient did not report any visual symptoms, and dilated fundoscopy of the eyes on day 4 did not reveal any signs of ocular involvement.

Table 1. summarizes the antifungal susceptibility test results on the *C. tropicalis* blood culture isolate using a commercial broth microdilution method. Minimal inhibitory concentrations (MICs) were interpreted according to breakpoints and epidemiological cutoff values (ECVs) available in CLSI M27M44S-ED3:2022 and CLSI M57S-ED4:2022, respectively,[1, 2] which showed susceptibility to all echinocandin antifungal agents (anidulafungin, caspofungin, and micafungin) with an MIC of 0.06 µg/mL to each.

Table 1. Antifungal Susceptibility Test Results of *Candida tropicalis* Isolated From Blood

Antifungal Agents		MIC (µg/mL)	Interpretive Category
Polyenes	Amphotericin B	1	WT*
Azoles	Fluconazole	2	S
	Itraconazole	0.5	WT*
	Posaconazole	0.12	WT*
	Voriconazole	0.12	S
Echinocandins	Anidulafungin	0.06	S
	Caspofungin	0.06	S
	Micafungin	0.06	S
Flucytosine	Flucytosine (or, 5-FC)	<=0.06	-- **

Abbreviations: MIC, minimal inhibitory concentration; S, susceptible; WT, wild-type.
Report comments:
* There are currently no standardized susceptibility testing breakpoints or interpretive criteria for this organism and this antifungal agent. The MIC obtained is within the MIC distribution for wild-type isolates, suggesting that this isolate is not likely to have an acquired mechanism of resistance. Clinical outcomes cannot be predicted based on this information.
** No breakpoints or epidemiological cutoff values for this organism and this antifungal agent exist.

Repeat blood cultures drawn 4 days after the initial positive blood cultures were negative. The patient was improving clinically on cefepime, vancomycin, and anidulafungin. However, ten days into treatment, the patient developed progressive blurring of vision in the right eye. Ophthalmic fundoscopy showed a low visual acuity of 20/200, marked vitreous exudates, and prominent areas of chorioretinitis, all of which confirm endophthalmitis (ie, inflammation of the inner parts of the eye). Cultures of blood and vitreous fluid were collected. Blood cultures were negative, but vitreous fluid cultures grew *C. tropicalis*. Antifungal susceptibility testing was performed on the *C. tropicalis* isolated from vitreous fluid, and the results showed identical MIC values to the initial blood culture isolate. However, the MIC values for all three echinocandins (anidulafungin, caspofungin, and micafungin) were suppressed from the final report (Table 2), according to CLSI M27M44S-ED3:2022 guidelines, since the isolate was ocular in origin.[1] Based on the susceptibility results and site of infection, anidulafungin was stopped and switched to intravenous liposomal amphotericin B and voriconazole for 6 days, followed by oral fluconazole. She also received amphotericin B by intravitreal administration (ie, injected directly into the eye) and underwent vitrectomy.

Antifungal Body Site Reporting for *Candida* spp. (*Continued*)

Table 2. Antifungal Susceptibility Test Results of *Candida tropicalis* Isolated From Vitreous Fluid

Antifungal Agents		MIC (µg/mL)	Interpretive Category
Polyenes	Amphotericin B	1	WT*
Azoles	Fluconazole	2	S
	Itraconazole	0.5	WT*
	Posaconazole	0.12	WT*
	Voriconazole	0.12	S
Echinocandins	Anidulafungin	- #	-
	Caspofungin	- #	-
	Micafungin	- #	-
Flucytosine	Flucytosine (or, 5-FC)	<=0.06	-- **

#Systemic echinocandins are known to have limited ability to penetrate ocular tissues. Consider infectious disease service consultation for guidance.
*There are currently no standardized susceptibility testing breakpoints or interpretive criteria for this organism and this antifungal agent. The MIC obtained is within the MIC distribution for wild-type isolates, suggesting that this isolate is not likely to have an acquired mechanism of resistance. Clinical outcomes cannot be predicted based on this information.
**No breakpoints or epidemiological cutoff values (ECVs) for this organism and this antifungal agent exist.

Should echinocandins be reported on Candida isolates from ocular specimens? If so, how?

Case study answer and discussion:

Candida species are common causes of fungal endophthalmitis. This infection arises from either endogenous or exogenous sources. The endogenous form most often occurs following candidemia via hematogenous spread to the eye, with infection typically progressing through the retina at the back of the eyeball into the vitreous fluid. Exogenous endophthalmitis is usually a consequence of trauma, eye surgery, or progression of corneal infection (ie, fungal keratitis). Successful therapy of *Candida* endophthalmitis requires penetration of antifungal agent into the relevant compartments of the eye (ie, the choroid, retina, vitreous fluid, and aqueous humor).

In the current case, the patient developed endogenous *C. tropicalis* endophthalmitis while receiving anidulafungin therapy. Echinocandin antifungal agents are highly active and fungicidal against *Candida* spp., including isolates that are resistant to triazoles and species that form biofilms. They are first line agents for candidemia. The Infectious Diseases Society of America (IDSA) also recommends performance of a dilated retinal exam during the first week of treatment in cases of candidemia due to risk of seeding the eye, since evidence of hematogenous spread of *Candida* to the eye may impact choice of antifungal therapy.[3]

Ocular candidiasis can be treated with systemic antifungal therapy with intravitreal injection of an antifungal agent, sometimes combined with vitrectomy.[3, 4] Understanding how antifungal agents penetrate in ocular tissue (cornea, aqueous, vitreous) is a critical factor to achieving optimal outcome for endogenous *Candida* endophthalmitis.[5]

Amphotericin B has been used successfully for the treatment of invasive orbital and intraocular infections. Penetration of amphotericin B into the eyes is also enhanced by inflammation. Therefore, intravenous administration combined with direct injection of amphotericin B into the eye is the recommended route of administration in patients with severe endophthalmitis.

Among azoles, voriconazole is the ideal choice in the treatment of ocular fungal infections, as it has a high intraocular penetration profile and a broad spectrum of activity against all *Candida* species.[5] Fluconazole also achieves high levels of penetration (25-100% of the plasma concentration) into ocular tissues within hours after a single dose.[6] Voriconazole and fluconazole administered systemically are detectable in aqueous and vitreous fluids of uninflamed and inflamed eyes. Most published clinical experience of *Candida* endophthalmitis describes the use of voriconazole and fluconazole.[7] Ocular penetration of itraconazole and posaconazole appears to be low, and little or no data exist for isavuconazole.

All three echinocandin antifungal agents currently approved by the United States Food and Drug Administration (FDA) to treat candidemia show limited penetration into the eye due to their large molecular weight. After systemic administration, the echinocandins distribute well into major tissues, including lung, liver, and spleen. However, they achieve undetectable or very low vitreous concentrations relative to plasma.[4] The subtherapeutic penetration into ocular tissues has been associated with treatment failure in *Candida* endophthalmitis.

Antifungal Body Site Reporting for *Candida* spp. (*Continued*)

Candida species isolated from sterile sites (eg, blood, cerebral spinal fluid, joint fluid, pleural fluid, pericardial fluid, and ocular tissue) are recommended to routinely undergo species-level identification and susceptibility testing. For a proper appreciation of antifungal efficacy in different tissues, the CLSI M27M44S-ED3:2022 and CLSI M57S-ED4:2022 guidelines,[1,2] provide recommendations on how to report susceptibility results into the patient record for situations during which *Candida* is isolated from different anatomical sites (Table 3). Body site-specific guidelines for *Candida* reporting are provided for azoles and the echinocandins for ocular sources including cornea, aqueous humor, and vitreous fluid. Other body site sources which are covered in the CLSI documents include urine for amphotericin B, azoles, and echinocandins; cerebrospinal fluid, central nervous system tissue, and abscess material for azoles and echinocandins. No reporting restrictions are proposed for flucytosine.

Table 3. CLSI Recommendations on Reporting Antifungal Susceptibility Test Results for *Candida* spp. Isolated From Ocular Specimens[1,2]

Antifungal Agent	Specimen	Reporting	Report Comment
Amphotericin B	Ocular (cornea, aqueous, and vitreous sources)	No reporting restrictions	
Azoles		Routinely report only fluconazole and voriconazole. Report itraconazole, posaconazole, and isavuconazole only by request.	
Echinocandins		Should not be routinely reported.	Systemic administration of echinocandins is not recommended for ocular infections because echinocandins have minimal tissue penetration in the eye. Consult ophthalmology, pharmacy, or infectious diseases service for guidance.
Flucytosine (or, 5-FC)		No reporting restrictions	

Case follow-up:

The choice of antifungal agent(s) for treatment depends on the susceptibility profile of the *Candida* spp. isolate as well as penetration to anatomical site of infection. Based on the MIC breakpoints, *Candida* spp. may demonstrate susceptibility or resistance to multiple classes of antifungal drugs. In addition, the possibility of a difference in antifungal susceptibility pattern when the same isolate is isolated from different body sites (ie, blood and vitreous fluid) also supports performance of antifungal susceptibility testing of *Candida* when isolated from multiple sterile sites.

In the current case, the patient was initially begun on an echinocandin for candidemia and underwent an eye exam during the first week of candidemia treatment, as suggested by IDSA, at which time there was no evidence of ocular involvement. However, the patient experienced visual symptoms on day 10 and was confirmed to have developed *Candida* endophthalmitis while on treatment with an echinocandin. Given the positive eye cultures, treatment was changed to systemic liposomal amphotericin B and voriconazole as well as intravitreal amphotericin B. Vitrectomy combined with systemic and intravitreal antifungal treatment yielded a favorable outcome in the management of endogenous endophthalmitis due to *C. tropicalis* in the current case.

In summary, evidence from IDSA suggests that echinocandins are appropriate initial therapy for candidemia, and eye examinations should be performed during the initial week of candidemia therapy to assess whether endophthalmitis is present. If endophthalmitis is present, echinocandins are not the appropriate choice, given that they do not penetrate ocular tissues due to their large molecular weight. For the management of sight-threatening lesions in the eye, achieving adequate concentrations of the appropriate antifungal agent in the area of the eye that is infected is crucial to success. The intravitreal injection of amphotericin B or voriconazole is helpful to achieve high local antifungal activity as quickly as possible because these agents achieve adequate concentrations in the posterior part of the eye and within the vitreous fluid. The antifungal susceptibility pattern of the infecting *Candida* species is also important to assess, since multidrug resistance has been noted for many *Candida* species and raises additional treatment challenges.

Antifungal Body Site Reporting for *Candida* spp. (*Continued*)

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Practical Tips for Using Newly Formatted Tables 1 in CLSI M100 33rd Edition[1]

Priyanka Uprety, BD Life Sciences – Integrated Diagnostic Solutions, Sparks, Maryland, USA
Rebekah Dumm, Washington University School of Medicine, St. Louis, Missouri, USA
Janet Hindler, Los Angeles County Department of Public Health, Los Angeles, California, USA

Why were CLSI M100 Tables 1 revised?

In 1972, CLSI, formerly known as the National Committee for Clinical Laboratory Standards (NCCLS), published "Table 1" in one of the earliest NCCLS AST documents. Table 1 was intended to help clinical laboratories decide which antimicrobial agents to test and report on specific bacteria. New drugs and new comments were added to the Tables 1 over the ensuing decades, but the format for these tables did not change. In the early 2020s CLSI decided to systematically reconsider the value of Tables 1 in light of the: **1)** availability of new antimicrobial agents, **2)** rise of antimicrobial resistance, including multidrug-resistant organisms (MDRO), and **3)** introduction of antimicrobial stewardship programs to help manage appropriate antimicrobial use. The result was a major reformatting of Tables 1 and an expansion from 3 tables to 16 tables in the CLSI M100 33rd edition.[1] Previously, each Table 1 contained several organisms/organism groups and many footnotes.[2] Now, each Table 1 focuses on a single organism/organism group and contains only footnotes pertinent to that organism/organism group.

What should your clinical laboratory do with the new Tables 1?

It is suggested that each laboratory performing AST review the new Tables 1 in the context of antimicrobial agents currently tested and reported in their laboratory. Briefly, the steps might include:

1. Review the new Tables 1 together with the information that was added to CLSI M100 33rd edition that explains how to use these tables. These instructions are located at the beginning of Tables 1 as an "Introduction" and expanded in Section I (pages 2-7) entitled "Selection of Antimicrobial Agents for Testing and Reporting" of the "Instructions for Use" section of CLSI M100.
2. Review the antimicrobial agents currently tested and reported for each organism/organism group in your laboratory to see where gaps framed in the following questions may exist:
   - Are there agents on your panels that are not included in the respective Table 1?

     Example: Your panel for *Pseudomonas aeruginosa* includes ceftriaxone, but ceftriaxone is not listed in Table 1C and there are no breakpoints for ceftriaxone in Table 2B1. Ceftriaxone breakpoints for *P. aeruginosa* were eliminated in 2010.
   - Are there agents listed on any Table 1 that are not included on your panels?

     Example 1: Your panel for *Stenotrophomonas maltophilia* does not include minocycline, but minocycline is in Tier 1 in Table 1F. Tier 1 lists "antimicrobial agents that are appropriate for routine, primary testing and reporting."

     Example 2: You have been reporting ciprofloxacin routinely on all MRSA. In previous editions of CLSI M100, ciprofloxacin was included in the category (Category C) of agents that may require testing when resistant to primary agents. However, now ciprofloxacin is in Tier 4 in Table 1F where the suggestion is to consider testing ciprofloxacin only by physician request for *Staphylococcus* spp.
   - Are your protocols up to date with suggested "cascade" reporting rules? Tables 1 suggest that results for broader-spectrum agents are suppressed for isolates that are susceptible to similar narrow-spectrum agents.
   - Do your protocols address "selective" reporting rules, such as suppressing daptomycin on isolates from the respiratory tract?
3. Prepare a summary of the gaps that need attention.
4. Schedule a meeting with your antimicrobial stewardship program team (ASP) to determine if changes in your AST and reporting protocols are warranted.

Practical Tips for Using Newly Formatted Tables 1 in CLSI M100 33rd Edition[1] (*Continued*)

Once it is determined that changes in AST and reporting protocols are warranted in your laboratory, what factors might be considered when addressing these changes?

1. Factors to consider if an agent **will be eliminated from routine testing and reporting:**
   - Will removal of the agent from routine reports impact stakeholders beyond those discussed with the ASP?
   - For which organism/organism groups will the agent be removed from routinely reporting?
   - Was the agent tested and reported as part of a commercial MIC panel or as an individual test (eg, disk diffusion, gradient strip)?
   - Is there a need to retain the ability to test the agent by physician request?
   - Which ancillary protocols need to be modified (eg, QC, Infection Control, etc.)?
   - Are any special changes needed in the data management system for reporting the agent (eg, any expert reporting rules for which the drug has been included, or any infection control guidance linked to the particular antimicrobial agent report)?
   - Will the agent be removed from the next publication of the facility's annual antibiogram?
   - How will the change be communicated to laboratory staff and recipients of AST results?
2. Factors to consider if an agent **will be added to routine testing and reporting:**
   - For which organism/organism groups will the agent be routinely tested and reported?
   - Is the agent currently on the routine MIC panel(s) but suppressed for the organism/organism groups where the change is needed?
   - If the drug is not on the routine MIC panel(s), will it be most practical to select a new MIC panel or test the agent offline?
   - Has performance of the agent been previously verified/validated, or will a verification/validation be required?
   - Will any selective or cascade reporting rules for the new drug or other drugs be needed as a result of this change?
   - What changes will be needed within the data management system? How long will it take to make these changes?
   - Which ancillary protocols need to be modified (eg, QC, Infection Control, etc.)?
   - Will the agent be added to the next publication of the facility's annual antibiogram?
   - How will the change be communicated to laboratory staff and recipients of AST results?

What would be an example of applying the guidance in the new Tables 1?

The following case may be considered. Two sets of blood cultures from a patient who was recently transferred to the hospital from a long-term care facility grew carbapenem-resistant *Klebsiella pneumoniae* (refer to Table 1.). Results from the laboratory's routine automated MIC panel are shown below. This was the third case of carbapenem-resistant *K. pneumoniae* within a month for this laboratory, and the sixth case in the past 12 months for which an isolate of Enterobacterales tested resistant to all antimicrobials on the automated panel. As with the other cases, the provider asked that the isolate be tested for ceftazidime-avibactam, which was performed using an agar gradient diffusion test since ceftazidime-avibactam was not on the automated MIC panel.

Practical Tips for Using Newly Formatted Tables 1 in CLSI M100 33rd Edition[1] (*Continued*)

Table 1. Antimicrobial Susceptiblity Report for *Klebsiella pneumoniae* in Case Example

Antimicrobial Agent	MIC (µg/mL)	Interpretation
Ampicillin	>32	R
Cefazolin	>32	R
Cefepime	>32	R
Ceftriaxone	>32	R
Ciprofloxacin	>2	R
Gentamicin	>8	R
Meropenem	>8	R
Piperacillin-tazobactam	>128/4	R
Tobramycin	>8	R
Trimethoprim-sulfamethoxazole	>4/78	R

Abbreviation: R, resistant

A follow up to this case led the ASP to request a review of the laboratory's testing and reporting protocols, at which time the new CLSI M100 Table 1A (see below) for Enterobacterales was discussed. The ASP team shared the Infectious Diseases Society of America's guidelines for managing patients with MDROs.[3] It was noted that CLSI suggests that routine, primary testing of one or more of the β-lactam combination agents should be considered for reporting (ie, Tier 3) for institutions that serve patients at high risk for MDROs. Although the laboratory cascades to testing ceftazidime-avibactam on carbapenem-resistant Enterobacterales (CRE) the decision was made to find a new AST panel that contained ceftazidime-avibactam for testing and reporting because:

- There is a one-day delay in reporting ceftazidime-avibactam results when cascading to agar gradient diffusion testing. Most patients harboring CRE are very ill, and AST results are critical in guiding the most appropriate agent(s) for treatment as soon as possible.
- There were reports within nearby hospitals of metallo-β-lactamase (MBL)-producing isolates of Enterobacterales; this is important because ceftazidime-avibactam is not active against MBL-producing isolates.
- This institution serves patients at high risk for MDROs, and this reporting approach corresponds to suggestions in CLSI M100 Table 1A.

Practical Tips for Using Newly Formatted Tables 1 in CLSI M100 33rd Edition[1] (*Continued*)

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

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin			
Cefazolin	Cefuroxime		
Cefotaxime or ceftriaxone[b]	Cefepime[c]		
	Ertapenem Imipenem Meropenem	Cefiderocol	
		Ceftazidime-avibactam	
		Imipenem-relebactam	
		Meropenem-vaborbactam	
Amoxicillin-clavulanate Ampicillin-sulbactam			
Piperacillin-tazobactam			
Gentamicin	Tobramycin	Plazomicin	
	Amikacin		
Ciprofloxacin Levofloxacin			
Trimethoprim-sulfamethoxazole			
	Cefotetan Cefoxitin		
	Tetracycline[d]		
			Aztreonam
			Ceftaroline[b]
			Ceftazidime[b]
			Ceftolozane-tazobactam
Urine Only			
Cefazolin (surrogate for uncomplicated UTI)[e]			
Nitrofurantoin			
		Fosfomycin[f] (*Escherichia coli*)	

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

In conclusion, laboratories should review the new Tables 1 in the updated CLSI M100 document and discuss testing and reporting changes with appropriate stakeholders such as ASP.

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Hot topic

Sulbactam-Durlobactam: The "double beta-lactamase inhibitor" drug

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

Many laboratorians are familiar with sulbactam, a β-lactamase inhibitor that is commercially combined with ampicillin, which was introduced in the US in 1986 to make ampicillin-sulbactam ("Unasyn"). Like many older β-lactamase inhibitors, sulbactam is itself a β-lactam, but has poor antimicrobial activity against most bacteria. Notably, sulbactam demonstrates bactericidal activity against *Acinetobacter* spp., due to sulbactam's high affinity to penicillin-binding proteins expressed by *Acinetobacter* (PBP1 and PBP3), resulting in inhibition of bacterial cell wall synthesis and cell death.[1] For decades, ampicillin-sulbactam has been used for its sulbactam component in the treatment of *Acinetobacter* spp. However, resistance to sulbactam has become common (45-80%) among contemporary *Acinetobacter* spp. isolates worldwide, in part due to the presence of Ambler class A, C, and D β-lactamases.[2] Entasis Therapeutics, Inc. developed durlobactam, a non-β-lactam, diazabicyclooctane inhibitor of Ambler class A, C, and D β-lactamases expressed by *Acinetobacter* spp. The addition of durlobactam to sulbactam restores sulbactam's activity against most *Acinetobacter* isolates. Sulbactam-durlobactam was approved by the United States Food and Drug Administration (FDA) in May 2023 for the treatment of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia caused by susceptible strains of *Acinetobacter baumannii-calcoaceticus* complex, in adults.[3] The same breakpoints assigned by FDA were approved by CLSI in June 2023 (to be published in the 34th edition of CLSI M100 edition in February 2024), and are shown in Table 1. Resistance to sulbactam-durlobactam is rare as >97% of global isolates of *A. baumannii-calcoaceticus* complex show susceptibility to sulbactam-durlobactam. However, resistance has been shown with mutation to PBP3 or the presence of a metallo-β-lactamase such as NDM.[4-6]

The US FDA has cleared several testing options, including HardyDisk (Hardy Diagnostics), Oxoid Disc (Thermo Scientific), ETEST® (bioMérieux), and Sensititre 18-24 hour MIC (ThermoFisher Scientific). Not all tests are currently commercially available, now but are anticipated in early 2024.

Table 1. Sulbactam-Durlobactam Breakpoints for *Acinetobacter baumannii-calcoaceticus* Complex

MIC Breakpoints (µg/mL)			Disk Breakpoints (mm)		
S	I	R	S	I	R
≤4/4	8/4	≥16/4	≥17	14-16	≤13

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In Memoriam: Clyde Thornsberry, PhD

Clyde Thornsberry, who was a major figure in the field of antimicrobial susceptibility testing for many years, passed away peacefully at his home in Georgia on June 15, 2023. Early in the 1970s, Clyde joined the pioneering group of experts who had formed the Subcommittee on Antimicrobial Susceptibility Testing which had been established under the auspices of the National Committee for Clinical Laboratory Standards (NCCLS), (now CLSI). Over the course of five decades, he served the subcommittee and its working groups in various roles.

Clyde Thornsberry, PhD

After serving in the US Army in Korea and returning home, Clyde enrolled at the University of Kentucky, where he earned his undergraduate degree and a PhD in Bacteriology. He had an illustrious career at the US Centers for Disease Control and Prevention (CDC), where he established and became the first Director of the Antimicrobics Investigation Branch. He was one of the founders of the ICAAC meetings (now part of ASM MICROBE); authored more than 1400 peer-reviewed scientific articles, books, and monographs; was widely recognized as a leading authority in the field of AST; and lectured nationally and internationally on the science (and art) of antimicrobial susceptibility testing.

Into the second decade of the 2000s, Clyde continued to attend AST Subcommittee meetings and was always happy to share his wit and wisdom. Although soft spoken, he was not shy about dispensing his opinions, and his comments often were accompanied by his dry and understated humor. Lastly, and perhaps most importantly in terms of his legacy, Clyde was devoted to his community and family, in particular his wife, Glenda, and his children and grandchildren.