Volume 6, Issue 1 April 2021

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

The CLSI **Outreach Working Group (ORWG)** is providing this News Update to highlight some recent issues related to antimicrobial susceptibility testing (AST) and reporting. We are listing links to some new educational materials and reminding you where you can find information about the CLSI AST Subcommittee proceedings.

Inside This Issue:

CLSI and the AST Subcommittee During COVID-19

Specific scheduling modifications for the AST SC include:

1. Winter 2021 meeting was held virtually during January and February 2021. Content from that meeting is available **here.**
2. Summer 2021 meeting will be held virtually from May 24 through June 17, 2021. Registration is available **here.**
3. M100, 31st Ed. was published in late March 2021 instead of January 2021.
4. The 2021 AST Annual Update Webinar will be held April 28 and 29, 2021.

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 "New Attendee Orientation" video presentation **here.**

Interested in becoming a CLSI volunteer? Learn more here.

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

CLSI AST Subcommittee Partnerships

Representatives with expertise in antimicrobials from the following organizations attend and participate in CLSI AST Subcommittee meetings and aid in dissemination of information regarding CLSI decisions and AST issues.

American College of Clinical Pharmacy Infectious Diseases Practice and Research Network (ACCP INFD PRN)
American Society for Microbiology (ASM)
Association of Public Health Laboratories (APHL)
ASTM International
College of American Pathologists (CAP)
European Committee on Antimicrobial Susceptibility Testing (EUCAST)
Infectious Diseases Society of America (IDSA)
Pediatric Infectious Diseases Society (PIDS)
Society for Healthcare Epidemiology of America (SHEA)
Society of Infectious Diseases Pharmacists (SIDP)
Susceptibility Testing Manufacturers Association (STMA)

Instructions for Accessing Topics/Articles in Previous CLSI News Updates:

1. Access the searchable CLSI AST SC Files and Resources **here.**
2. Enter keyword (eg, *Candida auris*) in the "Search" box.
3. A listing will display items in which this keyword appears. In columns 2 "Document" and 4 "Details," the notation "AST News Update" identifies the News Update edition where the keyword appears.
4. Click on the link in column 2 "Document" to access the specific News Update edition and retrieve the article.

Note that additional AST SC Files and Resources can be accessed by following these same steps.

Webinars

For information on upcoming webinars please visit the CLSI website **here.**

Upcoming Webinar

2021 AST Annual Update Webinar

Wednesday, April 28, 2021 | 1:00–2:30 PM Eastern (US) Time
Thursday, April 29, 2021 | 3:00–4:30 PM Eastern (US) Time

Moderator:

Janet A. Hindler, MCLS, MT(ASCP), F(AAM),
Microbiologist, Los Angeles County Department of Health
Los Angeles, CA

Presenters:

Romney M. Humphries, PhD, D(ABMM)
Professor of Pathology, Microbiology, and Immunology;
Medical Director of Microbiology, Vanderbilt University Medical Center
Nashville, TN

Audrey Schuetz, MD, MPH, D(ABMM)
Professor of Laboratory Medicine and Pathology, Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine
Rochester, MN

Archived and Free On-Demand Webinars:

Recently archived CLSI webinars can be accessed on demand (it is best to search by date) **here.** Archived on-demand webinars are available free of charge **six months** after the scheduled event for CLSI members. Some recent webinars are listed below:

- CLSI-CAP Annual Webinar: Ensuring Quality Beyond the Test: Reporting Antimicrobial Susceptibility Results (January 2021)
- *CLSI-SIDP ACCP Annual Webinar: Incorporating the Newest CLSI Recommendations for Antimicrobial Susceptibility Testing Into Your Stewardship Activities (January 2021)
- What's New in the 2020 Standards for Antimicrobial Susceptibility Testing (FREE February 2020)
- Understanding Breakpoint Decisions: CLSI Rationale Documents (FREE December 2019)
- CLSI-CAP Annual Webinar: Rational Approach to Antibacterial and Antifungal Breakpoints (FREE November 2019)
- Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings (FREE July 2019)
- CLSI 2019 Antimicrobial Susceptibility Testing Update (FREE, February 2019)
- Resources for Implementation of Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) in the Clinical Microbiology Laboratory (FREE, November 2018)
- Preparation, Presentation, and Promotion of Cumulative Antibiograms to Support Antimicrobial Stewardship Programs (FREE, October 2018)
- CLSI Documents for AST: What's Available for You? (FREE, May 2018)

*This webinar was not hosted by CLSI, but can be purchased on demand **here**

ASM FEMS World Microbe Forum 2021 (Virtual)

ORWG will host a session June 21, 2021 6:00–7:30 AM EST which will subsequently be available on demand for Forum registrants.

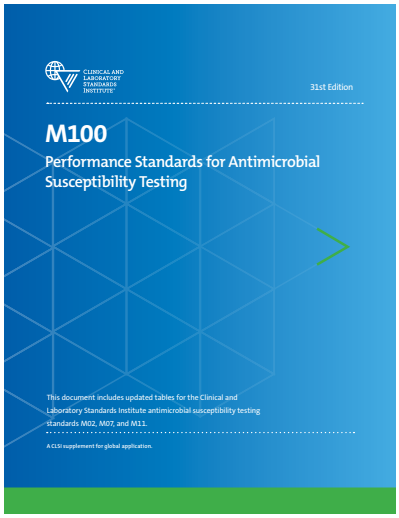
Antimicrobial Testing Meets Antimicrobial Stewardship: What Works and What's Needed?

- Modern Approaches to Antimicrobial Susceptibility Testing
  Romney Humphries, PhD, D(ABMM)
  Vanderbilt University Medical Center
  Nashville, TN

- Antimicrobial Stewardship Practice and Personalized Medicine: Where's the Connection?
  Navaneeth Narayanan, PharmD, BCPS
  Rutgers Ernest Mario School of Pharmacy
  Piscataway, NJ

New/Updated CLSI AST Documents Are Here!

M100 | Performance Standards for Antimicrobial Susceptibility Testing, 31st Edition



Major changes include:

New Breakpoints:

- Azithromycin
  - MIC and disk diffusion breakpoints for *Shigella* spp. (ECV eliminated)*
  - Disk diffusion breakpoints for *Neisseria gonorrhoeae*
- Ceftolozane-tazobactam
  - MIC breakpoints for *Haemophilus influenzae*
- Imipenem-relebactam
  - MIC and DD breakpoints for Enterobacterales and *Pseudomonas aeruginosa*
  - MIC breakpoints for anaerobes
- Lefamulin
  - MIC and DD breakpoints for *Staphylococcus aureus, H. influenzae,* and *Streptococcus pneumoniae*

Revised Breakpoints:

- Oxacillin
  - MIC breakpoints for *Staphylococcus* spp. except *S. aureus* and *Staphylococcus lugdunensis*

New Recommendations:

- Direct disk diffusion testing of isolates of Enterobacterales from positive blood culture broth
- Description of *Staphylococcus* species included in *S. aureus* complex
- Comment that linezolid susceptibility as determined by MIC testing predicts tedizolid susceptibility for *S. aureus, Enterococcus faecalis, Streptococcus pyogenes, Streptococcus agalactiae* and *Streptococcus anginosus* group

Revised Recommendations:

- Instructions for preparing zinc stock solution and iron-depleted cation-adjusted Mueller-Hinton broth for testing ceftobiprole

Expanded / Updated Recommendations:

- "Warning" for antimicrobial agents that should NOT be reported on isolates from cerebrospinal fluid
- Definition of "intermediate" (I) and addition of "I^" interpretive category for several agents that have the potential to concentrate in urine
- Oxacillin (methicillin) resistance in some *Staphylococcus* species including those not specifically addressed by species name in M100 may not be tested reliably with a cefoxitin disk diffusion test; testing for *mec*A and PBP2a are the most definitive tests for detection of methicillin (oxacillin) resistance for *Staphylococcus* spp.
- Clarified guidance for handling discrepancies when performing molecular or phenotypic testing for carbapenemases (Appendix H3)

** The CDC has described these changes **here.***

M100Ed31 Updates *(Continued)*

Quality Control:

Disk diffusion ranges revised:

- Amikacin
  - *P. aeruginosa* ATCC® 27853™
- Ceftobiprole (5 µg; deleted 30 µg disk)
  - *Escherichia coli* ATCC® 25922™
  - *S. aureus* ATCC® 25923™
- Eravacycline
  - *E. coli* ATCC® 25922™

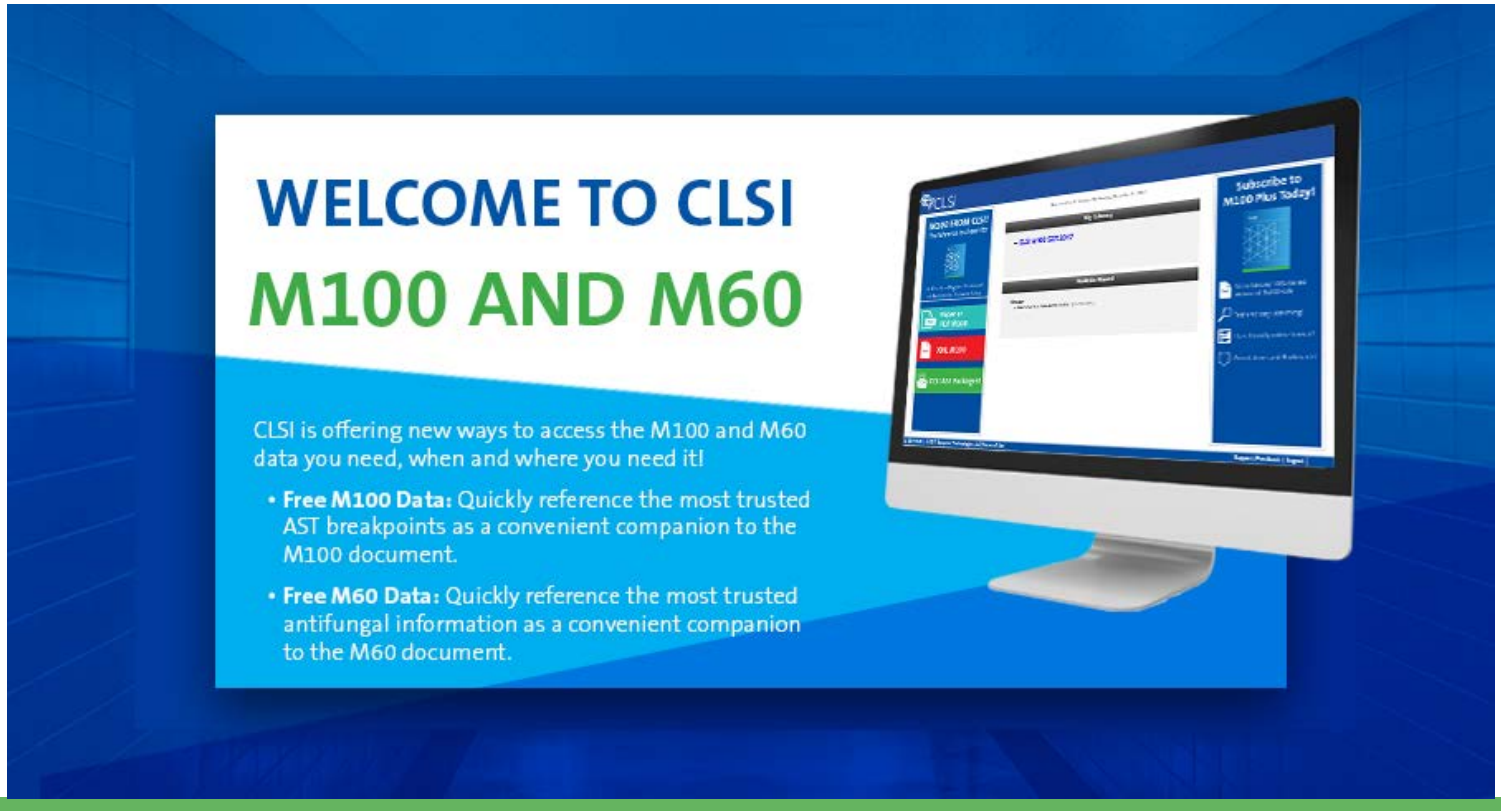
MIC ranges added:

- Aztreonam:
  - *Klebsiella pneumoniae* ATCC® BAA 2814™
- Aztreonam-nacubactam
  - *E. coli* ATCC® 25922™
  - *P. aeruginosa* ATCC® 27853™
  - *K. pneumoniae* ATCC® 700603™
  - *K. pneumoniae* ATCC® BAA-2814™
- Cefepime:
  - *K. pneumoniae* ATCC® BAA 2814™
- Cefepime-nacubactam
  - *E. coli* ATCC® 25922™
  - *P. aeruginosa* ATCC® 27853™
  - *K. pneumoniae* ATCC® 700603™
  - *K. pneumoniae* ATCC® BAA-2814™

Revised recommendation for QC when testing azithromycin against *Salmonella enterica* ser. Typhi or *Shigella* spp. by disk diffusion and MIC testing

Table Formatting Revisions:

- Table 3G separated into:
  - 3G-1 - Tests for Detecting Methicillin (Oxacillin) Resistance in *S. aureus* and *S. lugdunensis*.
  - 3G-2 - Tests for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp. Except *S. aureus* and *S. lugdunensis*
- Table footnotes numbered consecutively.



New Rationale Documents

CLSI publishes rationale documents that provide the scientific reasons behind the subcommittee's decisions, along with documentation of the standardized data and methods used to determine breakpoints. To access rationale documents, click **here.**

FDA-recognized breakpoints can be found **here.**

Archives of Retired Breakpoints and Methods

An archive of breakpoints removed from M100 since 2010 together with the rationale for their removal is available **here.**

Similarly, an archive of methods removed from M100 since 2017 is available **here.**

CLSI Educational Workshops Held at CLSI Meetings

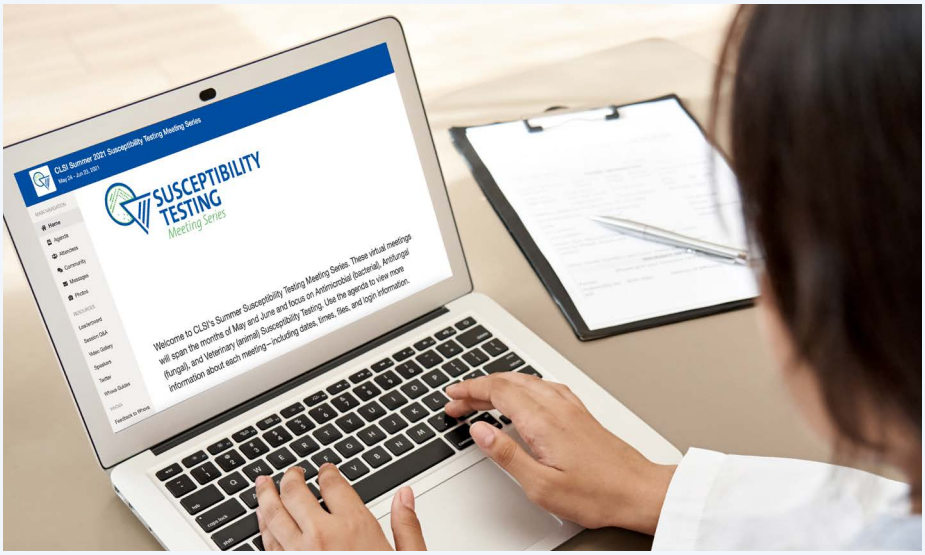
Educational Workshops, typically held on the Saturday evening prior to the AST Subcommittee Working Group meetings, are on hold until meetings can be held in person.

The slides presented for previous workshops can be found **here** listed under "Education Workshops."

Future CLSI AST Meetings

May 24–June 17, 2021
Virtual

January 2022
Ft. Lauderdale, Florida
(Virtual Options Available)



Featured Article

Re-Exploring the Intermediate Interpretive Category

Romney M. Humphries, Vanderbilt University Medical Center, Nashville, TN

Clinical breakpoints provide an interpretation of the probability of treatment success, based on the MIC value or the area of growth inhibition by disk diffusion. Isolates with results within the susceptible category are predicted to be associated with a high chance of treatment success when the patient is administered that antimicrobial, whereas those in the resistant category are associated with low chance of treatment success (see Figure 1). Factors that improve the chances of treatment success include the dosing regimen and the concentration of the antimicrobial at the site of infection. These variables make defining a single MIC or zone diameter cut-off value for a susceptible or a resistant result that applies to all infections and dosing regimens extremely difficult. This challenge is amplified by the inherent variability of susceptibility tests as MIC values are only reproducible within +/- 1 $\log_2$ dilution.

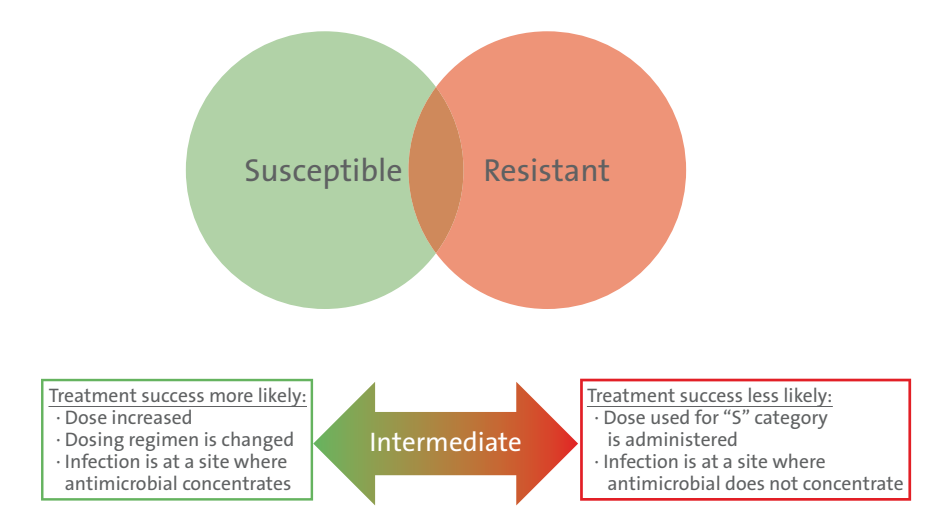


Figure 1. Implication of the Intermediate Interpretive Category

CLSI traditionally applied the intermediate category to address these challenges. Various uses for the intermediate category include:

1. Provide flexibility when variable dosing regimens are possible for an antimicrobial. In this case, "I" means increasing the dose may improve the chance of treatment success.
2. Acknowledge that at some anatomical sites, the antimicrobial is more concentrated. In this case, "I" means that if the infection is restricted to that site, success is likely (eg, urinary tract infections for many antimicrobials that are renally excreted).
3. Provide a buffer zone between susceptible and resistant categories to prevent resistant isolates from being incorrectly categorrized as susceptible, or vice versa.

Historically, CLSI did not clearly define which of the above "I" definitions applied to which breakpoints, leaving some uncertainty on how to best interpret results reported as intermediate. In practice, many clinicians interpret an intermediate result to mean resistant, when in fact in some instances it may indicate susceptible.

Over the past several years, CLSI has reevaluated the intermediate category and subsequently added two new categories: susceptible dose-dependent (SDD) and "I^" (Table 1). SDD was introduced to M100 in 2014 to provide clarity on when alternative dosing may be possible. In 2020, "I^" was added to highlight those antimicrobial agents that concentrate in urine and the likelihood of treatment success when the agent is prescribed for uncomplicated urinary tract infections. In addition, in 2020 the intermediate category was adapted for colistin and polymyxin B to highlight the low response rates associated with these antimicrobials. In this case, no susceptible category exits, just intermediate and resistant categories.

Re-Exploring the Intermediate Interpretive Category (*Continued*)

Table 1. Evolution of the Intermediate Category

Year / M100 Edition	Description
Pre-2014 (pre-M100- S24)	Single definition for intermediate category, which included: • Isolates with MIC or zone diameter values that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. • Implies clinical efficacy in body sites where drugs are physiologically concentrated or when a higher-than-normal dosage of a drug can be used. • A buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.
2014 (M100-S24)	Introduction of susceptible-dose-dependent (SDD) category for cefepime and the Enterobacterales, defined as: "Susceptibility of an isolate with an MIC in the SDD range is dependent on the dosing regimen that is used in the patient. It is necessary to use a dosing regimen (ie, higher doses, more frequent doses, or both) that results in higher drug exposure than the dose that was used to establish the susceptible breakpoint. Consideration should be given to the maximum approved dosage regimen, because higher exposure gives the highest probability of adequate coverage of an SDD isolate."
2019 (M100, 29th Ed)	Addition of SDD categories for: • Daptomycin and *Enterococcus faecium*. • Ceftaroline and *Staphylococcus aureus*.
2020 (M100, 30th Ed)	• Introduction of "I," defined as: • Agents that have the potential to concentrate at an anatomical site, ie, in the urine. • Application of "I" for colistin/polymyxin B breakpoints and highlighting the lack of a susceptible interpretive category and the low response rates for isolates with MICs ≤2 µg/mL.

The US Food and Drug Administration (FDA) does not presently recognize either SDD or "I^," which means commercial test systems cannot achieve FDA clearance using these expanded categories and must continue to apply the undifferentiated intermediate category, which includes SDD, "I," and "I^." As such, implementation of SDD and "I^" categories by clinical laboratories is complex, requiring information technology (IT) solutions and careful consideration as to which (if any) of these categories would provide a significant impact to optimal patient treatment. The laboratory should discuss how to prioritize implementation of SDD and "I^" categories with the antimicrobial stewardship team, pharmacy, infectious diseases clinicians, information technology experts, and other vested stakeholders. Implementation of "I^" specifically can be approached in a variety of ways, as described in Table 2. Of note, because "I^" merely reflects a change to the interpretive category designation and not to the breakpoint, validation of a laboratory's susceptibility test system is not needed outside of validating IT changes and results reporting. The options listed in Table 2. are not mutually exclusive, and laboratories may opt for multiple approaches, in a stepwise manner.

Re-Exploring the Intermediate Interpretive Category (*Continued*)

Table 2. Options for Implementation of "I^"

Option	Description	Considerations
1	Make M100, 31st Edition available to the antibiotic stewardship team; they may provide education on use of select agents with "I" result that are now classified as "I^" to providers in treatment guidelines, or for use in select cases (eg, treatment of multidrug resistant isolates). No changes made to patient reports.	Minimal effort required.
2	Add a footnote to the patient's report which indicates the possibility for treatment of uncomplicated urinary tract infections when "I^" is listed in M100 and an intermediate MIC or disk diffusion result is obtained.	• Prioritize which "I^" to implement. • Determine if comment is added to all reports, or only select reports (eg, for all isolates recovered from urine or those with an "I" result). • Determine appropriate report comment (examples provided in Case Study of this News Update). • Determine education required at institutional level as part of "I^" reporting roll-out.
3	Implement new interpretive category designation (denoted as "I^," or another specialized abbreviation) on patient's report.	• Prioritize which "I^" to implement. • Determine possibility of a new interpretive category with information technology department. • Determine appropriate report comment. Examples are provided in the Case Study of this News Update. • Determine education required at institutional level as part of "I^" reporting roll-out.

Case Study

The Value of Intermediate^ or "I^"

Graeme Forrest, Rush University Medical Center, Chicago, IL

Case 1: A 75-year-old male with diabetes and chronic kidney disease with an estimated glomerular filtration rate (eGFR) of 40 ml/min presented to the clinic with onset of urinary frequency and dysuria over 2 days without any systemic symptoms. A urinalysis showed 4+ glucose, 1+ blood, many white blood cells (WBCs,) and was positive for nitrites and leukocyte esterase. A urine culture was obtained, but given the lack of systemic symptoms and pending culture results, he was not prescribed an antimicrobial agent. The culture grew >10^5 CFU/mL *Escherichia coli* with susceptibility results as shown in Table 1.

Table 1. *Escherichia coli* Results

Antimicrobial Agent	MIC (µg/mL)	Interpretation
Ampicillin	16	R
Cefazolin (urine breakpoint)[a]	16	S
Cefuroxime (oral)	**16**	**I**
Ciprofloxacin	0.5	S
Nitrofurantoin	8	S
Trimethoprim-sulfamethoxazole	>4/76	R

[a] Predicts the activity of the oral agents cefaclor, cefdinir, cefpodoxime, cefprozil, cephalexin, and loracarbef..

Cefuroxime has been shown to be an effective choice for treatment of uncomplicated urinary tract infections (uUTIs) in the elderly population seen at this clinic. Although cefazolin can be tested as a surrogate for oral cephalosporins, cefazolin may overcall resistance to cefuroxime (also for cefdinir and cefpodoxime) for some isolates. Consequently, the antimicrobial stewardship team who works with this clinic requested that the laboratory report both cefazolin and cefuroxime on all urine isolates of *E. coli, K. pneumoniae* and *P. mirabilis*.

For this particular patient, the clinician called the laboratory to ask why there was a discrepancy between the cefuroxime and cefazolin results. The laboratory indicated that the cefuroxime MIC of 16 µg/mL should be considered "I^," meaning likely susceptible when cefuroxime is prescribed orally for isolates from uUTIs.

An optional comment can be added to the laboratory report, to help explain this discrepancy such as: "Cefuroxime concentrates in the urine. An MIC of ≤16 µg/mL indicates a high probability of clinical efficacy for treatment of uncomplicated urinary tract infections."

This case reflects a common clinical scenario for managing uUTIs in elderly patients. Where possible, clinicians should avoid unnecessary use of the fluoroquinolones for treatment of uUTIs as the risks of tendon rupture, neurologic abnormalities and other adverse events outweigh the value of using these agents for treating relatively mild infections. These risks are outlined in black box warnings for the fluoroquinolones in the drug label. In addition, because this patient has poor kidney function (indicated by low eGFR), some clinicians would avoid use of nitrofurantoin. The clinician prescribed cefuroxime for 5 days and the patient's symptoms resolved within 2 days. Other oral cephalosporins (eg, cephalexin) also attain very high concentrations in the urine. Cefuroxime can be given less frequently than some of the other agents, and as such is an attractive choice for this type of case.

The Value of Intermediate ^ or "I^" (*Continued*)

Case 2: After 8 days of hospitalization, a 55-year-old female with spinal cord injury and a chronic indwelling catheter developed fever, chills, and hypotension. The patient was well known to the treating team for having had prior urosepsis due to multidrug resistant *Pseudomonas aeruginosa*. Based on culture and susceptibility results from prior clinic visits, she was started empirically on meropenem 2 g every 8 hours and amikacin 20 mg/kg x one dose. Blood cultures were negative. Urine cultures grew >10^5 CFU/mL *P. aeruginosa* with susceptibility results as shown in Table 2.

Table 2. *Pseudomonas aeruginosa* Results

Antimicrobial Agent	MIC (µg/mL)	Interpretation
Cefepime	32	R
Ceftazidime	32	R
Ciprofloxacin	**1**	**I**
Gentamicin	**8**	**I**
Meropenem	>8	R
Piperacillin-tazobactam	>128/4	R

This patient represents a challenging clinical and laboratory case. The patient has a chronic source for *Pseudomonas* infection which is the indwelling catheter. Indwelling catheters are frequently colonized by bacteria, especially gram-negative rods, and antimicrobial agents should only be prescribed if there are clinical symptoms of infection. It appears this patient has an active lower urinary tract infection without bacteremia. Complicating management of this patient is the apparent lack of suitable first-line antimicrobial agents available for treatment. The source of infection and the organism's susceptibility results can aid in selection of an antimicrobial course. Catheter replacement, with antimicrobial therapy are indicated for the management of these complex infections. As both ciprofloxacin and gentamicin concentrate in the urine, "I" in this case can be considered "I^." Clinically, both should be efficacious for treatment of uUTIs due to *P. aeruginosa*. Understanding "I^" could help the clinician avoid using newer, broad-spectrum and expensive β-lactam combination agents (eg, ceftolozane-tazobactam or ceftazidime-avibactam), preserving these agents for more serious infections. The patient was prescribed intravenous ciprofloxacin 400 mg every 12 hours, with rapid resolution of fever and was discharged on oral ciprofloxacin 750 mg every 12 hours for a complete 7-day course.

In summary, the "I^" can be applied to specific antimicrobial agents administered orally or parenterally, that concentrate well in the urine. This may not be applicable for all isolates from urine and would depend on the source and extent of the infection. The intent of the "I^" is to encourage providers to use narrower-spectrum antimicrobial agents more appropriately and avoid unnecessary use (for uncomplicated cases) of newer agents. To learn more about the "I^" concept and how to communicate this concept with various stakeholders, refer to the feature article in this News Update.

Practical Tips

What's Wrong With This Picture?

Stella Antonara, OhioHealth, Columbus, OH
Lars F. Westblade, Weill Cornell Medicine, New York, NY

Case 1: One of the most frequently encountered bacterial species causing sepsis is *Staphylococcus aureus*. Clinical microbiology laboratories can provide fast results to differentiate MRSA (methicillin resistant *S. aureus*) from MSSA (methicillin susceptible *S. aureus*) by utilizing molecular methods on positive blood culture samples. If the isolate is an MRSA, the recommendations from IDSA (Infectious Diseases Society of America) for treatment for adults with uncomplicated bacteremia include vancomycin or daptomycin.[1] Table 1. shows susceptibility results obtained using a commercial automated system on a blood culture isolate of *S. aureus*. What's wrong with this picture?

Table 1. *Staphylococcus aureus* (unconfirmed results)

Antimicrobial Agent	MIC (µg/mL)	Interpretation
Clindamycin	>4	R
Doxycycline	≤0.5	S
Erythromycin	>8	R
Oxacillin	>4	R[a]
Trimethoprim-sulfamethoxazole	≤2/38	S
Vancomycin	**>8**	**R**

[a] Oxacillin-resistant staphylococci are resistant to cefazolin and all other β-lactams except ceftaroline.

Solution to Case 1: A vancomycin-resistant *S. aureus* (VRSA) is extremely unusual. The first VRSA (MIC ≥16 µg/mL) was described in the early 2000s.[2] The Centers for Disease Control and Prevention (CDC) indicated that as of 2014, 14 cases had been reported in the USA and all were MRSA but none were isolated from blood.[3] It was shown that VRSA arises when *van*A genes are transferred from vancomycin-resistant *Enterococcus* (VRE) to *S. aureus*.[4] In several patients, vancomycin-resistant *Enterococcus faecalis* was isolated from the same specimens harboring the VRSA.[5] A recent review described 54 isolates of VRSA reported worldwide from various specimen types.[6]

The following steps as described in Appendix A of CLSI M100[7] should be taken when a suspect VRSA is encountered:

1. Check the purity plate for contamination.
2. Check for a defective susceptibility panel/card.
3. Repeat organism identification and antimicrobial susceptibility test (AST) with initial method to ensure results reproduce. If possible, at the same time, repeat AST with a second method to confirm these highly unusual and significant results.
4. If results reproduce and are confirmed, contact your local public health laboratory immediately.

Due to the urgent need to contain the spread of VRSA, CDC's recommendations for handling a possible VRSA include: "Immediately, while performing confirmatory susceptibility tests, notify the patient's primary caregiver, patient-care personnel, and infection-control personnel regarding the presumptive identification of VRSA so that appropriate infection control precautions can be initiated promptly."[3] However, the decision to do this should be made in consultation with the laboratory director after careful consideration of all aspects of the case at hand and the possibility that the results may be erroneous. Previous experience with the AST system must factor into the decision.

In this case, the purity plate did not suggest contamination. The patient's record indicated that he was already in contact isolation as previous cultures grew MRSA. There were no previous reports of this patient having had VRE; if this had been a true VRSA, public health authorities would have wanted to know whether VRE was found concomitantly. The AST was repeated using the initial method with a fresh isolate; no other method was available in the laboratory for testing vancomycin and *S. aureus*. Repeat MIC results for all drugs except vancomycin were the same as the initial results; the repeat vancomycin MIC was ≤1 µg/mL, which is susceptible. If the result had repeated as vancomycin resistant, then all the actions recommended by the CDC as mentioned above should have been taken. The isolate should be sent to a public health laboratory for confirmation. However, in this case the result did not repeat and it was concluded that the initial vancomycin-resistant result was probably due to an issue with the susceptibility card and that this was a random event.

What's Wrong With This Picture? (*Continued*)

Case 2: A blood culture collected from a 37-year-old female with appendicitis turned positive with Gram stain showing gram-negative bacilli and gram-positive cocci in chains. A rapid blood culture identification panel was run on the positive blood culture broth and the organisms identified were *Klebsiella pneumoniae* and *Streptococcus agalactiae* or Group B *Streptococcus* (GBS). The susceptibility results for the *K. pneumoniae* isolate were unremarkable, considered acceptable and reported (Table 2a.). However, the GBS MIC results for ampicillin, penicillin and ceftriaxone were nonsusceptible. What's wrong with this picture?

Table 2a. *Klebsiella pneumoniae* (final results)

Antimicrobial Agent[a]	MIC (µg/mL)	Interpretation
Ampicillin-sulbactam	>32/16	R
Cefazolin	8	R
Ceftriaxone	≤1	S
Ciprofloxacin	≤0.25	S
Gentamicin	≤1	S
Piperacillin-tazobactam	≤4/4	S
Trimethoprim-sulfamethoxazole	≤2/38	S

[a] Additional agents on the panel that tested susceptible were suppressed according to a cascade reporting protocol. These included cefepime, ertapenem, meropenem, levofloxacin, amikacin and aztreonam.

Table 2b. *Streptococcus agalactiae* (GBS) (unconfirmed results)

Antimicrobial Agent[a]	MIC (µg/mL)	Interpretation
Ampicillin	**2**	**NS**
Ceftriaxone	**4**	**NS**
Penicillin G	**4**	**NS**
Vancomycin	1	S

NS, nonsusceptible

Solution to Case 2: Isolates of GBS that are not susceptible to penicillin are very rare[8] and CLSI only lists susceptible breakpoints for ampicillin, penicillin, and ceftriaxone. In addition, CLSI notes that routine susceptibility testing of β-hemolytic streptococci with ampicillin and penicillin is not needed although many laboratories routinely test isolates of GBS from sterile sites.

The susceptibility results for GBS were withheld, pending further investigation. Steps 1 to 3 as listed in Case 1 for handling unusual AST results were taken for the GBS. The purity plate and the original subculture plates were examined more closely. A second colony type was noted on both plates and subsequently identified as *Enterococcus faecalis*. The blood culture broth was subcultured again to confirm the presence of *E. faecalis* and rule out contamination of the initial GBS AST. All three organisms grew again, and AST was performed on fresh subcultures of the GBS and the *E. faecalis*. The presence of *E. faecalis* explained the elevated MICs to ampicillin, penicillin and ceftriaxone for the initial GBS AST. *E. faecalis* are intrinsically resistant to cephalosporins[8,9] and typical *E. faecalis* MICs for ampicillin are 2 µg/mL and for penicillin are 4 µg/mL.[8] These are above the ampicillin and penicillin susceptible breakpoints for GBS which are ≤0.25 and ≤0.12 µg/mL, respectively.[7] The repeated susceptibility results were as expected (Tables 2c. and 2d.) and released by the laboratory. In polymicrobial infections, organisms that are not in abundance may not reach a critical threshold needed by molecular methods to be detected. That could explain the fact that *E. faecalis* was not detected by the molecular blood culture panel.

Table 2c. *Streptococcus agalactiae* (GBS) (final results)

Antimicrobial Agent[a]	MIC (µg/mL)	Interpretation
Ampicillin	≤0.25	S
Ceftriaxone	≤0.12	S
Penicillin G	≤0.06	S
Vancomycin	0.5	S

What's Wrong With This Picture? (*Continued*)

Table 2d. *Enterococcus faecalis* (final results)

Antimicrobial Agent[a]	MIC (µg/mL)	Interpretation
Ampicillin	≤2	S
Gentamicin Synergy	Syn-S[a]	S
Vancomycin	1	S

[a] Synergy Susceptible

Case 3: A 63-year-old male underwent transurethral prostate resection. On postoperative day 1, he was febrile and had leukocytosis. Blood and urine cultures were ordered, and the following day blood cultures remained negative; however, the urine culture grew >10^5 CFU/mL *Escherichia coli*. AST was performed on an automated platform and results are shown in Table 3a. What's wrong with this picture?

Table 3a. *Escherichia coli* AST (unconfirmed results)

Antimicrobial Agent	MIC (µg/mL)	Interpretation
Amoxicillin-clavulanate	>32/16	R
Ampicillin	>32	R
Cefazolin	>16	R
Cefepime	>16	R
Ceftriaxone[a]	**2**	**I**
Ciprofloxacin	>4	R
Ertapenem[a]	**≤0.5**	**S**
Gentamicin	≤1	S
Meropenem	>4	R
Nitrofurantoin	≤16	S
Piperacillin-tazobactam	>128/4	R
Trimethoprim-sulfamethoxazole	>4/76	R

[a] Repeat testing revealed resistant results for both ceftriaxone and ertapenem.

Solution to Case 3: A wild-type isolate of *E. coli* would be susceptible to all drugs on the panel. Although this isolate appears to have acquired resistance to multiple agents, the observed susceptible results for ceftriaxone and ertapenem in the setting of resistance to cefepime and meropenem is concerning. What might be an explanation for this profile?

1. Very unusual acquired resistance. However, resistance mechanisms known to date for β-lactams and *E. coli* do not explain this profile.
2. Contamination of the AST panel.
3. Improper inoculum preparation and/or automated AST panel set up.

The results were NOT released. The steps taken to troubleshoot the problem and observations made are shown in Table 3b.

What's Wrong With This Picture? (*Continued*)

Table 3b. Troubleshooting Unusual AST Results in This Case

Step	Observation
Purity plate was closely examined.	• Culture was pure. • Colony morphology was suggestive of *E. coli.* • Colony count was within an acceptable range.
AST panel was visually inspected.	• Growth controls wells were acceptable. • Growth in drug wells aligned with the AST results reported.
Results were discussed with the technologist who prepared the inoculum and set up the panel.	• This was the first day the technologist was on her own doing set ups. • May have been some slight "sticking" of inoculator.
Loopfuls of the positive control, cefepime (16 µg/mL), and meropenem (4 µg/mL) wells were subcultured to tryptic soy agar with 5% sheep blood to assess for potential contamination.	No apparent contamination on subcultures.
Isolate was retested using the same automated AST method and by disk diffusion in parallel.	• **Isolate was resistant to ceftriaxone and ertapenem by both methods.** • All remaining results were identical to those resulted on initial AST.

After review of each step taken to address the problem, it was determined the unusual results were probably due to improper inoculation of the panel. Fortunately, the initial AST results were flagged by the bench technologist as highly unusual, as the isolate was intermediate to a third-generation cephalosporin (ceftriaxone) yet resistant to a fourth-generation cephalosporin (cefepime), and susceptible to ertapenem but resistant to meropenem which have similar antibacterial activity against isolates of Enterobacterales.[10]

The approach taken by the laboratory to investigate the root cause of the unusual AST profile was comprehensive and designed to resolve the issue quickly. An alternate approach might be taken in some cases of unusual AST results. For example, if the isolate was susceptible to all agents on the panel except ceftriaxone, the action taken might be to repeat the AST using the original method only. However, in the case of a more complicated AST profile in a post-surgical patient who may be infected with a multidrug-resistant organism such as carbapenem-resistant Enterobacterales (CRE), time is of the essence. Accurate, rapid identification of CRE is not only important for determining appropriate therapy but also for informing appropriate infection control measures.[11] By confirming the AST profile using the original and a second method in parallel, the laboratory was able to determine if there was a technical issue (human or instrument) associated with the automated AST panel, or if the *E. coli* isolate exhibited an unusual antimicrobial susceptibility profile. Although the latter scenario would be highly unlikely, if it were true, the subsequent step would be to send the isolate to a reference laboratory for testing by a reference broth microdilution method. If confirmed, the local public health laboratory should be informed.[7]

What's Wrong With This Picture? (*Continued*)

References

1 Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis*. 2011;52(3):e18–e55.

2 Centers for Disease Control and Prevention (CDC). *Staphylococcus aureus* resistant to vancomycin--United States, 2002. *MMWR Morb Mortal Wkly Rep*. 2002 Jul 5;51(26):565-7.

3 Centers for Disease Control and Prevention (CDC). CDC reminds clinical laboratories and healthcare infection preventionists of their role in the search and containment of vancomycin-resistant *Staphylococcus aureus* (VRSA). https://www.cdc.gov/hai/settings/lab/vrsa_lab_search_containment.html. Accessed April 7, 2021.

4 Zhu W, Clark N, Patel JB. pSK41-like plasmid is necessary for Inc18-like *van*A plasmid transfer from *Enterococcus faecalis* to *Staphylococcus aureus* in vitro. *Antimicrob Agents Chemother*. 2013;57(1):212-219.

5 Walters MS, Eggers P, Albrecht V, et al. Notes from the field: vancomycin-resistant *Staphylococcus aureus*—Delaware, 2015 *MMWR Morb Mortal Wkly Rep*. 2015; 64(37);1056.

6 Cong Y, Yang S and Rao C. Vancomycin resistant *Staphylococcus aureus* infections: A review of case updating and clinical features. *J Adv Res*. 2020; 21:169-176.

7 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

8 Kasahara K, Baltus AJ, Lee SH, et al. Prevalence of non-penicillin-susceptible Group B *Streptococcus* in Philadelphia and specificity of penicillin resistance screening methods. *J Clin Microbiol*. 2010;48(4):1468-1469.

9 Weinstein MP. Comparative evaluation of penicillin, ampicillin, and imipenem MICs and susceptibility breakpoints for vancomycin-susceptible and vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*. *J Clin Microbiol*. 2001;39(7):2729–2731.

10 Bush K, Bradford PA. β-Lactams and β-lactamase inhibitors: an overview. *Cold Spring Harb Perspect Med*. 2016;6(8):a025547.

11 Banerjee R, Humphries R. Clinical and laboratory considerations for the rapid detection of carbapenem-resistant Enterobacteriaceae. *Virulence*. 2017;8(4):427-439.

Hot Topic

Imipenem-Relebactam and Aztreonam-Avibactam: What Do Clinical and Public Health Microbiologists Need to Know?

Paula Snippes Vagnone, Minnesota Department of Health, St. Paul, MN
Priyanka Uprety, Robert Wood Johnson Medical School, New Brunswick, NJ
Arryn Craney, Weill Cornell Medicine, New York, NY

Novel antimicrobial agents like imipenem-relebactam (IMR), aztreonam-avibactam (AZT-AVI), and cefiderocol have been recently added to the antimicrobial armamentarium to combat multidrug resistant gram-negative infections. Guidelines for clinical microbiology laboratories regarding cefiderocol were addressed in the July 2020 **CLSI AST News Update** and included questions a laboratory should consider when deciding how to approach testing of these new agents. This current issue provides an update on IMR and AZT-AVI. AZT-AVI will be discussed in terms of *in vitro* testing and its investigational use for specific multidrug-resistant organisms (MDROs) as it is not currently US Food and Drug Administration (FDA)-approved for clinical use.

Table 1. Basic Features of Imipenem-relebactam and Aztreonam-avibactam

	Imipenem-relebactam[1,2,3]	Aztreonam-avibactam[4,5,6]
Trade Name	Rebcarbrio™	Not available
Manufacturer	Merck & Co., Inc.	Pfizer
Drug Class	β-lactam combination agent	β-lactam combination agent
Route of Administration	Intravenous	Intravenous
FDA approval date	July 2019 (cUTI/cIAI) June 2020 (HABP/VABP)	Not currently FDA approved, in Phase III clinical trials
FDA approved for treatment of infections	cUTI, including pyelonephritis and cIAI in patients 18 years of age and older with limited or no alternative treatment options. HABP/VABP in patients 18 years of age of older.	Not currently FDA approved, pending Phase III clinical trials to assess treatment of infections due to metallo-β-lactamase (MBL)-producing gram-negative bacteria in hospitalized adults with: • cIAI • Nosocomial pneumonia including HABP and VABP • cUTI • BSI

Imipenem-Relebactam and Aztreonam-Avibactam: What Do Clinical and Public Health Microbiologists Need to Know? (*Continued*)

Table 1. (*Continued*)

	Imipenem-relebactam[1,2,3]	Aztreonam-avibactam[4,5,6]
Organisms for which clinical efficacy has been demonstrated as listed in the FDA drug label	cUTI, including pyelonephritis: *Enterobacter cloacae* *Escherichia coli* *Klebsiella aerogenes* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* cIAI: *Citrobacter freundii* *Enterobacter cloacae* *Escherichia coli* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* Anaerobic gram-negative bacteria: *Bacteroides* spp.* *Fusobacterium nucleatum* *Parabacteroides distasonis* HABP/VABP: *Acinetobacter calcoaceticus-baumannii* complex *Enterobacter cloacae* *Escherichia coli* *Haemophilus influenzae* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Serratia marcescens*	Not currently FDA approved, pending Phase III clinical trials Demonstrates *in vitro* effectiveness against carbapenem-resistant Enterobacterales (CRE) including those containing class A (eg, KPC), class B MBLs (eg, NDM), class C (eg, AmpC), and class D (eg, OXA-48) β-lactamases
Additional organisms for which activity has been demonstrated *in vitro* as listed in the FDA drug label	**Aerobic gram-positive bacteria:** *Enterococcus faecalis,* methicillin-susceptible *Staphylococcus aureus, Streptococcus anginosus, Streptococcus constellatus* **Aerobic gram-negative bacteria:** *Citrobacter koseri, Enterobacter asburiae* **Anaerobic gram-positive bacteria:** *Eggerthella lenta, Parvimonas micra, Peptoniphilus harei, Peptostreptococcus anaerobius* **Anaerobic gram-negative bacteria:** *Fusobacterium necrophorum, Fusobacterium varium, Parabacteroides goldsteinii*	Not currently FDA approved, pending Phase III clinical trials
Inactive against	Methicillin-resistant *Staphylococcus aureus, Enterococcus faecium, Stenotrophomonas maltophilia,* some isolates of *Burkholderia cepacia*	Not currently FDA approved, pending Phase III clinical trials
Treatment Strategy	Multidrug resistant gram-negative bacteria including some carbapenem-resistant strains and non-fermenting gram-negative rods	Not currently FDA approved, pending Phase III clinical trials; treat infections due to MBL-producing gram-negative bacteria

**Bacteroides* spp. include *B. caccae, B. fragilis, B. ovatus, B. stercoris, B. thetaiotaomicron, B. uniformis, B. vulgatus*

Abbreviations: BSI, bloodstream infection; cIAI, complicated intra-abdominal infection; cUTI, complicated urinary tract infection; HABP, hospital acquired bacterial pneumonia; VABP, ventilator acquired bacterial pneumonia.

Imipenem-Relebactam and Aztreonam-Avibactam: What Do Clinical and Public Health Microbiologists Need to Know? (*Continued*)

Imipenem-relebactam

1. What is imipenem-relebactam? Is it like any other antimicrobial agent currently tested?

Imipenem-relebactam is a combination of imipenem, the renal dehydropeptidase-1 inhibitor cilastatin and the novel β-lactamase inhibitor relebactam.[2, 3]

Imipenem binds to penicillin-binding proteins (PBPs) thereby disrupting bacterial cell-wall synthesis. Imipenem activity is low for *Proteus*, *Providencia* and *Morganella* spp., which is related to poor permeabiltiy and not the presence of a β-lactamase. Cilastatin has no antibacterial activity and is coadministered with imipenem to prevent renal metabolism of imipenem. Relebactam is a diazabicyclooctane class β-lactamase inhibitor that inhibits Class A β-lactamases (CTX-M, TEM, SHV, KPC) and Class C β-lactamases (AmpC). It does not inhibit Class D β-lactamases (OXA-48-like) or Class B β-lactamases (MBLs, VIM, IMP, NDM).[7]

2. Should imipenem-relebactam be tested routinely? When might a laboratory be asked to test imipenem-relebactam?

According to the most recent Infectious Diseases Society of America (IDSA) Guidance on the Treatment of Antimicrobial Resistant Gram-Negative Infections, new β-lactam-combination agents like ceftazidime-avibactam (CZA), meropenem-vaborbactam and IMR are the preferred treatment options for carbapenem-resistant Enterobacterales (CRE) infections when additional information on carbapenemase phenotypic/genotypic profile is not readily available.[8] Imipenem-relebactam is not recommended for the treatment of CRE infections due to MBL (Class B)- or OXA-48-like β-lactamase (Class D)-producers or members of the Morganellaceae group (i.e. *Proteus, Providencia* and *Morganella*).

The IDSA also recommends consideration of IMR as a preferred treatment option for difficult-to-treat *P. aeruginosa* infections (defined as not susceptible to piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem, ciprofloxacin and levofloxacin). Other first-line agents for difficult to treat *P. aeruginosa* include CZA or ceftolozane-tazobactam, for infections outside the urinary tract. Cefiderocol and single-dose aminoglycoside are also first-line options for urinary tract infections.

Following discussion with the antimicrobial stewardship team, laboratories may elect to test IMR routinely, by special request or by developing a reflex algorithm for specific carbapenem-resistant gram-negative bacteria.

3. How should imipenem-relebactam be tested (Table 2)? Are there any unique testing considerations?

Routine CLSI reference disk diffusion (aerobes only) and broth microdilution MIC methods can be used for testing IMR.[9,10,11]

FDA-cleared (as of the date of this publication) commercial systems for IMR, including disks, are listed in Table 2. Check with manufacturer for specific FDA-cleared applications.

Table 2. Testing Options for Imipenem-relebactam and Aztreonam-avibactam

Antibiotic	Disk Manufacturer (disk content)	Gradient Diffusion	Broth Microdilution	Automated AST Systems
Imipenem-relebactam	Hardy Diagnostics (10/25 µg) Mast Group[a] (10/25 µg)	Liofilchem bioMerieux	Thermo Scientific™ Sensititre™	Vitek®2
Aztreonam-avibactam	Not available	Not available	AR Lab Network in-house prepared broth microdilution panels	Not available

[a] Research use only; available in Europe.

4. How should imipenem-relebactam results be interpreted?

The clinical breakpoints for IMR provided to date by FDA, CLSI and EUCAST are listed in Table 3.

Imipenem-Relebactam and Aztreonam-Avibactam: What Do Clinical and Public Health Microbiologists Need to Know? (*Continued*)

5. What are expected AST results for imipenem-relebactam?

According to the SMART, surveillance program Enterobacterales isolates tested from 2015-2018 show good overall performance for IMR with ~95% of all isolates testing susceptible. Relebactam restored susceptibility to imipenem at the following percentages when testing imipenem-not susceptible isolates of: *E. coli* (48.8%), *K. pneumoniae* (74.9%), *E. cloacae* (46.1%), *K. aerogenes* (90.8%), *K. oxytoca* (37.5%) and *C. freundii* (65.2%).[12]

IMR does not offer additional advantage for isolates that are resistant to imipenem by mechanisms other than Class A and C β-lactamases. Since *Morganella* spp., *Proteus* spp. and *Providencia* spp. demonstrate elevated MICs to imipenem due to a β-lactamase independent mechanism, IMR is not useful for these species.

The activity of IMR on carbapenem-resistant *P. aeruginosa* (n=1,445) depends on the individual carbapenemase gene present. Findings from a recent study demonstrated 97.3% of isolates tested susceptible to IMR compared to 94.6% and 94.2% for ceftolozane-tazobactam and CZA, respectively.[13]

Imipenem-relebactam offers limited benefit for *Acinetobacter baumannii* infections due to the presence of Class D β-lactamases commonly found in this species and has no activity against *S. maltophilia*, which is intrinsically resistant to imipenem.

Table 3. FDA, CLSI and EUCAST Breakpoints for Imipenem-relebactam

	FDA Breakpoints						CLSI Breakpoints[a]						EUCAST Breakpoints[b]			
	MIC (µg/mL)			DD (mm)[c]			MIC (µg/mL)			DD (mm)[c]			MIC (µg/mL)		DD (mm)[c]	
Bacteria	S	I	R	S	I	R	S	I	R	S	I	R	S	R	S	R
Enterobacterales	≤1/4	2/4	s≥4/4	≥25	21-24	≤20	≤1/4	2/4	≥4/4	≥25	21-24	≤20	≤2	>2	≥22	<22
Pseudomonas aeruginosa	≤2/4	4/4	≥8/4	≥23	20-22	≤19	≤2/4	4/4	≥8/4	≥23	20-22	≤19	≤2	>2	≥22	<22
Acinetobacter spp.	≤2/4	4/4	≥8/4	-	-	-	-	-	-	-	-	-	≤2	>2	≥24	<24
Haemophilus influenzae	≤4/4	-	-	-	-	-	-	-	-	-	-	-	IE	IE	-	-
Anaerobes	≤4/4	8/4	≥16/4	-	-	-	≤4/4	8/4	≥16/4	-	-	-	≤2	>2	-	-

Abbreviations: DD, disk diffusion; I, Intermediate; IE, insufficient evidence to set clinical breakpoints; MIC, minimal inhibitory concentration; R, Resistant; S, Susceptible.
[a] CLSI breakpoints published in CLSI M100 31st Edition. Enterobacterales breakpoints do not apply to the family Morganellaceae, which includes, but is not limited to the genera *Morganella*, *Proteus*, and *Providencia*.
[b] EUCAST clinical breakpoint applies to all Enterobacterales except *Morganella* spp.; relebactam concentration is fixed at 4 µg/mL. Breakpoints for anaerobes are for gram-negative anaerobes and gram-positive anaerobes, except *Clostridioides difficile*.
[c] Disk content for imipenem-relebactam is 10/25 µg.

Aztreonam-avibactam

1. What is aztreonam-avibactam? Is it like any other antimicrobial agent currently tested?

Aztreonam-avibactam (ATM-AVI) is not yet FDA approved, but is pending Phase III clinical trials. Aztreonam is the only clinically available member of the monobactam class of antimicrobial agents and, uniquely, is not hydrolyzed by MBLs. However, aztreonam is hydrolyzed by other β-lactamases such as those in Ambler Class C (AmpC) and Ambler Class A (eg, KPC) that often are often found in isolates of Enterobacterales. Avibactam is a diazabicyclooctane non-β-lactamase β-lactamase inhibitor that has wide-ranging activity against Ambler Class A and C, and some Class D β-lactamases such as OXA-48-like. *In vitro* studies have demonstrated that avibactam restores the activity of aztreonam (ATM) against Enterobacterales containing Class B MBLs (eg, NDM, VIM, IMP).[4,5,6,14]

2. Should aztreonam-avibactam be tested routinely? When might a laboratory be asked to test aztreonam-avibactam?

Aztreonam-avibactam is not FDA approved; however, both both ATM and ceftazidime-avibactam (CZA) are clinically available. According to the most recent guidelines from IDSA, the combination of CZA with ATM is a preferred treatment option for infections due to MBL-producing CRE (eg, NDM, IMP, VIM).[8]

Imipenem-Relebactam and Aztreonam-Avibactam: What Do Clinical and Public Health Microbiologists Need to Know? (*Continued*)

Testing for ATM and CZA individually may not provide adequate information to suggest clinical treatment with ATM-AVI. However, CDC's Antibiotic Resistance (AR) Lab Network offers testing of patient isolates for ATM-AVI by broth microdilution. The panel includes ATM, CZA, and ATM-AVI, and can be performed on confirmed isolates of MBL-producing Enterobacterales at no charge. MICs and interpretations based on current CLSI M100 breakpoints are reported for ATM and CZA. Since there are no established breakpoints for ATM-AVI, MIC results are reported without interpretation. See the CDC Expanded Antimicrobial Susceptibility Testing for Hard-to-Treat Infections (ExAST) website for details regarding isolate submission criteria, available testing locations, and additional information.

3. What are expected AST results for aztreonam-avibactam?

Although breakpoints are not yet established for ATM-AVI, several *in vitro* studies of MBL-producing Enterobacterales have demonstrated MICs typically ≤2/4 µg/mL, with a range of ≤0.015/4 to 8/4 µg/mL.[5] A recent study of NDM-producing Enterobacterales found some *E. coli* with MICs within a range of ≤0.03/4 to 32/4 µg/mL.[15] For some isolates of non-MBL-producing Enterobacterales, ATM-AVI MICs were >128/4 µg/mL. These organisms may harbor other mechanisms of resistance, such as CMY-type β-lactamases.[5]

References

1 US Food and Drug Administration. Imipenem-Relebactam (RECARBRIO). https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212819Orig1s000TOC.cfm. Accessed April 7, 2021.

2 US FOOD and Drug Administration. HABP/VABP indication for imipenem-relebactam. https://www.fda.gov/news-events/press-announcements/fda-approves-antibiotic-treat-hospital-acquired-bacterial-pneumonia-and-ventilator-associated. Accessed April 7, 2021.

3 Merck. Recarbrio – Highlights of prescribing information. https://www.merck.com/product/usa/pi_circulars/r/recarbrio/recarbrio_pi.pdf. Accessed April 7, 2021.

4 Sader HS, Mendes RE, Pfaller MA, et al. Antimicrobial activities of aztreonam-avibactam and comparator agents against contemporary (2016) clinical Enterobacteriaceae isolates. *Antimicrob Agents Chemother.* 2018;62(1):e01856-17.

5 Karlowsky JA, Kazmierczak KM, de Jonge BLM, et al. *In vitro* activity of aztreonam-avibactam against Enterobacteriaceae and *Pseudomonas aeruginosa* isolated by clinical laboratories in 40 countries from 2012 to 2015. *Antimicrob Agents Chemother.* 2017;61(9):e00472-17.

6 NIH U.S. Efficacy, safety, and tolerability of ATM-AVI in the treatment of serious infection due to MBL-producing gram-negative bacteria. https://clinicaltrials.gov/ct2/show/NCT03580044?term=ATM-AVI&draw=2&rank=1. Accessed April 7, 2021.

7 Yahav D, Giske CG, Grāmatniece A, et al. New β-Lactam-β-lactamase inhibitor combinations. *Clin Microbiol Rev.* 2020;34(1):e00115-20. Erratum in: Clin Microbiol Rev. 2021;34(2):e00021-21.

8 Tamma PD, Aitken SL, Bonomo RA, et al. 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.* 2020;27:ciaa1478. doi: 10.1093/cid/ciaa1478.

9 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

10 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2020

11 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 31st ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2021.

12 Yang Q, Zhang H, Yu Y, et al. *In vitro* activity of imipenem/relebactam against Enterobacteriaceae isolates obtained from intra-abdominal, respiratory tract, and urinary tract infections in China: study for monitoring antimicrobial resistance trends (SMART), 2015-2018. *Clin Infect Dis.* 2020;71(Supplement_4):S427-S435.

Imipenem-Relebactam and Aztreonam-Avibactam: What Do Clinical and Public Health Microbiologists Need to Know? (*Continued*)

13 Fraile-Ribot PA, Zamorano L, Orellana R, et al. GEMARA-SEIMC/REIPI *Pseudomonas* Study Group. Activity of imipenem-relebactam against a large collection of *Pseudomonas aeruginosa* clinical isolates and isogenic β-lactam-resistant mutants. *Antimicrob Agents Chemother.* 2020;64(2):e02165-19.

14 Vasoo S, Cunningham SA, Cole NC, Kohner PC, et al. *In vitro* activities of ceftazidime-avibactam, aztreonam-avibactam, and a panel of older and contemporary antimicrobial agents against carbapenemase-producing gram-negative bacilli. *Antimicrob Agents Chemother.* 2015;59(12):1835–1846.

15 Lutgring JD, Balbuena R, Reese N, et al. Antibiotic susceptibility of NDM-producing Enterobacterales collected in the United States in 2017 and 2018. *Antimicrob Agents Chemother.* 2020; 64(9): e00499-20.

More News!

CLSI M100 in China

Through an agreement with CLSI, a program to translate M100 into Chinese was developed by the China Antimicrobial Resistance Surveillance System, which includes 1,500 clinical laboratories as members. Professor Wang Hui, an advisor to the CLSI AST Subcommittee, and Professor Hu Fupin have been leading this effort together with a group of young volunteers who completed the translation in just three months. Forty-six individuals were involved and the project was sponsored by bioMerieux. The Chinese version of M100 in both hard copy and electronic formats is provided free of charge. A comprehensive training program for microbiologists and clinicians has also been developed to explain how to optimally use M100.

Outreach Working Group (ORWG) Members:

Janet Hindler (Co-Chairholder), Los Angeles County Department of Health, Los Angeles, CA, USA

Audrey Schuetz (Co-Chairholder), Mayo Clinic, Rochester, MN, USA

April Abbott, Deaconess Health System, Evansville, IN, USA

Stella Antonara, OhioHealth, Columbus, OH, USA

April Bobenchik, Lifespan Academic Medical Center, Providence, RI, USA

Andrea Farrell, Becton Dickinson, Sparks, MD, USA

Graeme Forrest, Rush University Medical Center, Chicago, IL, USA

Romney Humphries, Vanderbilt University Medical Center, Nashville, TN , USA

Shawn Lockhart, Centers for Disease Control & Prevention, Atlanta, GA, USA

Rianna Malherbe, Hardy Diagnostics, Santa Maria, CA, USA

Nicole Scangarella-Oman, GlaxoSmithKline, Collegeville, PA, USA

Paula Snippes Vagnone, Minnesota Department of Health, St. Paul, MN, USA

Priyanka Uprety, Robert Wood Johnson Medical School, New Brunswick, NJ, USA

Lars Westblade, Weill Cornell Medicine, New York, NY, USA