The CLSI Outreach Working Group (ORWG) is providing this Newsletter to highlight some recent issues related to antimicrobial susceptibility testing 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.

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)

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.

- Access that newsletter here.
- To learn more about upcoming or past meetings, click here.
- CLSI posts meeting minutes and summaries for public access here.
- If you are planning on attending a CLSI AST Subcommittee meeting, check out the Orientation presentation here.

Interested in becoming a CLSI volunteer? Learn more here.

Please remember that CLSI’s AST Subcommittee welcomes suggestions from you about any aspect of CLSI documents, educational materials, or this Newsletter.
Webinars

For information on upcoming webinars please go here.

Archived and Free On-Demand Webinars:

Recently archived CLSI webinars can be accessed on demand 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:

- **Resources for Implementation of Matrix-Assisted Laser Desorption/ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) in the Clinical Microbiology Laboratory** (November 2018)
- **Preparation, Presentation, and Promotion of Cumulative Antibiograms To Support Antimicrobial Stewardship Programs** (October 2018)
- **CLSI Documents for AST: What’s Available for You?** (FREE May 2018)
- **Current Recommendations for Antimicrobial Susceptibility Testing of Enterococcus spp.** (FREE March 2018)
- **CLSI 2018 AST Webinar: M100, M02, and M07 Updates** (FREE February 2018)

Upcoming Webinar:

**CLSI 2019 Antimicrobial Susceptibility Testing Update**

Wednesday, February 20, 2019 | 1:00–2:30 PM Eastern (US) Time
Thursday, February 21, 2019 | 3:00–4:30 PM Eastern (US) Time

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

Presenters:
Romney M. Humphries, PhD, D(ABMM)
Chief Scientific Officer, Accelerate Diagnostics
Tucson, AZ

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

Archive of Retired Breakpoints

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.

Check It Out! Educational Workshops Held at CLSI Meetings

*Nicole Scangarella-Oman, GlaxoSmithKline*

To coincide with the January and June CLSI Committees Weeks, the ORWG coordinates a biannual “live” Educational Workshop, typically held on the Saturday evening prior to the start of the AST Subcommittee Working Group meetings.

The June 2018 workshop, held in San Diego, CA, was entitled “Implementation of the 21st Century Cures Act for Breakpoints and Interpretive Categories.” This workshop provided an opportunity for various groups to provide their unique perspectives, highlight recent achievements and discuss remaining challenges regarding the implementation of the 21st Century Cures Act, and how it impacts the microbiology laboratory, drug development sponsors, device companies, and clinicians. Presenters included representatives from the FDA Center for Drug Evaluation and Research (CDER), the FDA Center for Devices and Radiological Health (CDRH), Susceptibility Testing Manufacturers Association (STMA) and CLSI Antimicrobial Susceptibility Testing Subcommittee. A question and answer session followed the presentation and was moderated by Romney Humphries.

The next workshop, “Recent Advances in PK/PD and Its Use In Setting Breakpoints,” will be held on Saturday, January 26, 2019, in St. Augustine, Florida.

PowerPoint presentations from past workshops, can be found here, by simply filtering to anything that starts with “Education.”

Future CLSI AST Meetings

**January 27–29, 2019**
St. Augustine, Florida

**June 16–18, 2019**
Dallas, Texas

**January 26–28, 2020**
Tempe, Arizona

**June 14–16, 2020**
Baltimore, Maryland
Updated CLSI AST Documents Are Here!

Check out a summary of major changes below.

**M100 | Performance Standards for Antimicrobial Susceptibility Testing, 29th Edition**

**Major changes include:**

**New/Revised Breakpoints**

**New:**
- Meropenem-vaborbactam: disk diffusion and MIC breakpoints for *Enterobacteriaceae*
- Cefiderocol: investigational MIC breakpoints for *Enterobacteriaceae, P. aeruginosa, Actinobacter spp.,* and *Stenotrophomonas maltophilia*
- Azithromycin: MIC breakpoints for *N. gonorrhoeae* [previously an epidemiological cutoff value (ECV)]

**Revised:**
- Ceftaroline: disk diffusion and MIC breakpoints for *Staphylococcus aureus* [added susceptible-dose dependent (SDD) interpretive category]
- Ciprofloxacin: disk diffusion and MIC breakpoints for *Enterobacteriaceae* and *P. aeruginosa*
- Daptomycin: MIC breakpoints for *Enterococcus* spp. (added SDD interpretive category)
- Levofloxacin: disk diffusion and MIC breakpoints for *Enterobacteriaceae* and *P. aeruginosa*

**New Recommendations**
- Colistin added as a surrogate for polymyxin B when testing *P. aeruginosa, Acinetobacter baumannii,* and select members of the *Enterobacteriaceae*

**Updated Recommendations**
- Ceftazidime-avibactam: instructions for performing MIC testing when specific disk diffusion zone diameters are observed for *Enterobacteriaceae*
- Fosfomycin: reinforced the comments that fosfomycin disk diffusion and MIC breakpoints are for *E. coli* urinary tract isolates only

**Intrinsic Resistance guidance updated for:**
- *Citrobacter koseri, Citrobacter amalonaticus* group
- *K. pneumoniae, Klebsiella oxytoca,* and *Klebsiella variicola*
- *Raoultella* spp.
- *A. baumannii/A. calcoaceticus* complex
- *Burkholderia cepacia* complex

**Reformatting / Relocating**

Tables “Using Molecular Assays for Resistance Detection now in M100” (previously listed on CLSI website only):
- **H1.** Strategies for Reporting Methicillin (Oxacillin) Results When Using Molecular and Phenotypic AST Methods for *S. aureus*
- **H2.** Strategies for Reporting Vancomycin Results When Using Molecular and Phenotypic AST Methods for *Enterococcus* spp.
- **H3.** Table H3. Reporting Results from Extended-Spectrum β-Lactamase Resistance and Carbapenemase Molecular Tests for *Enterobacteriaceae*
Applying Fluoroquinolone Pharmacokinetics, Pharmacodynamics, and Updated Clinical Breakpoints for Gram-Negative Pathogens to Determine Optimal Dosing

Eric Wenzler, University of Illinois at Chicago, Chicago, IL

Antimicrobial-resistant bacteria are responsible for significant morbidity, mortality, and excess healthcare costs, and their prevalence is increasing at a rate faster than the antimicrobial development pipeline. FDA-approved antimicrobial doses represent estimates across a patient population in order to maximize generalizability and probability of clinical effect as it would apply to “most” patients. Unfortunately, the increase in bacterial resistance and medical complexity of our patients means that these population estimates no longer apply to “most” patients, or at least not to the patients in whom optimizing antimicrobial doses matters the most. Fortunately, the principles of antimicrobial pharmacokinetics (PK) and pharmacodynamics (PD) can help optimize dosing regimens and tailor therapy for each patient individually.

In the January 2018 CLSI AST News Update, the basic principles behind PK and PD were presented. This article expands on that knowledge, demonstrating how PK/PD principles are applied in practice by clinicians when managing patients with challenging infections.
Applying Fluoroquinolone Pharmacokinetics, Pharmacodynamics, and Updated Clinical Breakpoints for Gram-Negative Pathogens to Determine Optimal Dosing (Continued)

Pharmacokinetic Principles of Fluoroquinolones

In the previous CLSI AST News Update, PK was summarized as the evolution of a drug’s concentration, throughout the body and over time, after a dose of drug is administered.

PK is governed by 4 principles: absorption, distribution, metabolism, and elimination, which are evaluated when deliberating the optimum treatment regimen for a patient. **Absorption** applies primarily to oral antimicrobials, as there is no absorption phase for antimicrobials administered intravenously (modern antimicrobials are rarely administered by topical or intramuscular routes). For oral antimicrobials, absorption is most commonly discussed in the context of bioavailability (F or fraction absorbed, expressed as %).

**Absolute bioavailability** is the total amount of drug in circulation after oral administration compared to that after IV administration, whereas **relative bioavailability** is one oral formulation compared to another. Bioavailability incorporates both the rate and extent of absorption of the drug and can be affected by many factors including: gastrointestinal transit time, drug disintegration and dissolution, the activity of physiological transporters (ie, molecules in the human body that transport molecules to the cytoplasm of cells, for example in the kidney), interactions with other drugs, and food. Among the commonly used fluoroquinolones, levofloxacin and moxifloxacin have virtually 100% bioavailability meaning that the route of administration can be converted between IV and PO in a 1:1 ratio with no adjustment.2,3 The bioavailability of ciprofloxacin and delafloxacin are 70% and 59% respectively, meaning that a slightly higher dose must be given orally in order to match the exposure achieved after an IV dose.4,5

**Distribution** is most often referred to in the context of the volume of distribution of a drug (Vd). This is most correctly termed the apparent Vd, as Vd is not an actual biological or physiologic parameter. Vd is the volume that would be necessary to account for the total amount of drug in the body, if that drug were present in the entire body at the same concentration as in the plasma relative to the dose administered. Simply put, apparent Vd is merely a conversion factor required to convert a dose (expressed as mg or g) into a concentration (expressed as µg/mL or mg/L), as it is measured in the body. Vd is commonly normalized to body weight and expressed as L/kg. For example, if a 1000 mg dose of vancomycin is administered intravenously and then the concentration measured in the patient’s blood 1 hour later is 17 mg/L, the apparent volume that that dose must have been dissolved in is 58.9 L (1000 mg / 17 mg/L = 58.9 L, or 0.7 L/kg for 80 kg patient). A volume of distribution ≥ 0.7 L/kg is typically considered large and is associated with tissue concentrations exceeding those in plasma and adequate penetration into extravascular body sites. The fluoroquinolones have Vd values ranging from 0.6 to ≥ 2 L/kg and are widely distributed throughout the body into lung, skin, fat, muscle, cartilage, bone, and many bodily secretions. The distribution of a drug is affected by its intrinsic properties such as its molecular size, charge, hydrophilicity or lipophilicity, and protein binding.

**Metabolism** occurs primarily in the liver and intestines. The vast majority of antimicrobials (including the fluoroquinolones) are not extensively metabolized, and for those that are, formed metabolites are rarely microbiologically active and thus do not contribute to overall drug efficacy.

**Elimination** is the most important PK parameter for antimicrobials and is discussed in terms of total body clearance (CLt) as the volume of blood removed of drug per unit time (eg; L/h). Almost all antimicrobials are primarily cleared through the kidneys and this is affected by the size of the drug, its protein binding, and the health of the patient’s kidneys. Clearance is used in part to determine the elimination half-life of the drug, which is the time it takes for the drug concentration in the body to be halved. Half-life is a more clinically useful parameter as steady-state, or the state in which drug concentration is in equilibrium in the body, occurs mathematically after 5 half-lives (eg, 50%, 75%, 87.5%, 93.8%, 96.9%, see Figure 1). The frequency at which a drug is dosed is therefore based in part on the half-life, and a typical rule of thumb is to dose a drug after at least 3 half-lives, or once ~87.5% of the dose has been eliminated from the body. For example, the half-life of levofloxacin in plasma is approximately 7.5 hours, therefore the drug is dosed clinically every 24 hours.6 Estimates of the patient’s clearance and subsequent drug half-life are used to make dosing adjustments for patients with increased or decreased renal function, including patients on dialysis.
Applying Fluoroquinolone Pharmacokinetics, Pharmacodynamics, and Updated Clinical Breakpoints for Gram-Negative Pathogens to Determine Optimal Dosing (Continued)

Application of Fluoroquinolone PK/PD Principles: Case Examples

A 54-year-old Caucasian female patient is admitted to your institution from her skilled nursing facility and diagnosed with bacteremia due to *Pseudomonas aeruginosa*. The microbiology laboratory reports a MIC of 2 µg/mL to levofloxacin for this isolate, which was within the susceptible range, according to the M100 2018 edition.6 The patient’s estimated creatinine clearance (CrCl, a measure of kidney function) is normal at 80 mL/min. The team would like to de-escalate from intravenous piperacillin-tazobactam and send her back to her facility on oral levofloxacin. Can oral levofloxacin be used to treat this infection? If so, what dose should be used?

To answer this question, we first need to know the PK/PD index of efficacy for levofloxacin, which is the AUC/MIC ratio. Next, we need to know the AUC/MIC target for Gram-negative pathogens. AUC/MIC targets are initially established by *in vitro* or *in vivo* dose fractionation studies and (ideally) validated in human clinical studies. We can refer to a clinical study in patients with hospital-acquired bacterial pneumonia, which demonstrated higher rates of pathogen eradication when a levofloxacin AUC/MIC of at least 87 was achieved.7 Therefore, we know we need an AUC of at least 174 mg x h/L for this patient, as the MIC is 2 µg/mL.

We can next approach this question in two ways. First, the levofloxacin package insert is referenced to see what AUC is achievable with oral dosing. The maximum oral dose (750 mg every 24 h) provides an AUC of 101 ± 20 mg x h/L in healthy volunteers.2 Based on this initial check, it does not seem as if oral levofloxacin would be an appropriate recommendation for this patient. However, more accurate calculations can be used to answer this question definitively. To do this, the patient’s specific total body clearance (CLT) of levofloxacin is evaluated. This can be achieved by using an equation relating patient-specific CrCl to CLT of levofloxacin from a population PK study from the published literature, and then determining the AUC via the equation AUC = Dose/CL. Ideally, the population PK analysis would have been performed in a population that matches or closely resembles the patient in question. In this example we will utilize the equation from a group of patients with serious community-acquired infections.8 The CL equation is:

- 5.945 + race + (age x -0.032) + (CrCl x 0.07)

To solve for our patient: CL = 5.945 – 1.486 + (54 x -0.032) + (80 x 0.07) = 8.33 L/h

- So an AUC of 174 = dose/8.33. Solving for dose we get 1449.4 mg, which is almost twofold the maximum daily dose of 750 mg.

Therefore, despite the fact that the MIC in this case is considered susceptible, levofloxacin given PO would not be an appropriate recommendation for this patient. Because fluoroquinolone oral bioavailability is the same as IV, IV levofloxacin is also not an option. This discordance between fluoroquinolone breakpoints and achievable systemic PK has been recognized and reported for years.9 This discordance is one reason the fluoroquinolone susceptibility breakpoints are now lower in CLSI M100-Ed29.10 The impetus for this change stems from several factors including the increase in MICs to fluoroquinolones among Gram-negative pathogens, advances in PK/PD knowledge and technology, and the paucity of clinical data supporting the original breakpoints.11

The impact of this change can be examined in part through evaluating the PK of ciprofloxacin and revised clinical breakpoints against *Enterobacteriaceae*. From the ciprofloxacin package insert, the AUCs after a 500 mg and 750 mg every 12-hour oral dose, respectively, are 27.4 and 31.6 mg x h/L. To achieve the aforementioned AUC/MIC target of 87, these two doses could be used effectively for MIC values up to 0.25 µg/mL (the updated susceptibility breakpoint for ciprofloxacin against *Enterobacteriaceae* approved for CLSI M100-Ed29). Under the old breakpoints, both 0.5 and 1 µg/mL would have been considered susceptible but would not be achievable from a PK/PD perspective in a patient with normal renal function. In addition to correlating with PK/PD targets, the revised breakpoints are closer to those published by EUCAST.12 CLSI has submitted a rationale document outlining the data supporting the change to the US FDA for their review and potential recognition. In anticipation of the delay until FDA recognition of the revised breakpoints, the performance of fluoroquinolone disk diffusion and Etest has been compared to reference BMD and demonstrated adequate performance using the updated 2019 CLSI breakpoints.11
Applying Fluoroquinolone Pharmacokinetics, Pharmacodynamics, and Updated Clinical Breakpoints for Gram-Negative Pathogens to Determine Optimal Dosing (Continued)

To illustrate further, we can examine a case of a patient with pneumonia due to *Klebsiella pneumoniae*. The patient is a 72-year-old female with a CrCl of 45 mL/min and an MIC to ciprofloxacin of 0.25 µg/mL. Given her age and slightly impaired renal function, we can assume her patient-specific AUC after either a 500 mg or 750 mg every 12 hour dose would be even higher than those listed in the package insert for patients with normal renal function, and therefore either dose would be appropriate to achieve the needed AUC of 21.75 mg x h/L. To be sure we can apply a population PK equation relating CrCl to $CL_T$ derived from 44 elderly patients with lower respiratory tract infections:\(^13\)

\[
\begin{align*}
&CL_T = 8 + 0.21 \times CrCl \\
&\text{Therefore, } CL_T = 8 \text{ L/h} + 0.21 \times 45 = 17.5 \text{ L/h}. \\
&AUC = \text{Dose/}CL_T; 21.75 = \text{Dose/17.5}; 380.63 \text{ mg}.
\end{align*}
\]

These estimations suggest that the 500 mg every 12-hour dose would be adequate for this patient and that the 750 mg dose can be avoided, which may reduce potential dose-related toxicities in this elderly patient.

**Summary**

While these practical examples provide patient-specific dosing recommendations and allow us to assess the appropriateness of an antimicrobial dosing regimen beyond simply referring to the pathogen’s MIC, more advanced applications of these principles that incorporate infection site and variability in both MIC and PK measurements are available.\(^14\) The probability of optimal clinical outcomes is maximized when antimicrobial PK/PD targets are achieved. Due to the rise in bacterial resistance and medical complexity of patients, standard population-based doses of these agents rarely meet PK/PD targets in the patients who are most dependent on it for survival, such as those who are critically ill and have pathophysiological derangements affecting their PK. As such, it is necessary for clinicians to understand and employ precision medicine via PK/PD principles when determining antimicrobial dosing regimens.

**Figure 1:** Half-lives required to achieve steady-state and complete drug elimination

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Applying Fluoroquinolone Pharmacokinetics, Pharmacodynamics, and Updated Clinical Breakpoints for Gram-Negative Pathogens to Determine Optimal Dosing (Continued)

References


Digging Deeper Into Understanding Cefazolin Reporting for Enterobacteriaceae

Audrey N. Schuetz, Mayo Clinic, Rochester, MN
Stella Antonara, Nationwide Children’s Hospital, Columbus, OH

In the Spring 2018 CLSI AST News Update, we provided a case study illustrating various ways by which to report cefazolin as a surrogate for oral cephalosporins for applicable Gram-negative organisms isolated from the urine. The cases concentrated solely on testing and reporting of cefazolin as a surrogate. However, cefazolin, which is administered intramuscularly (IM) or intravenously (IV) may also be used itself for the treatment of complicated urinary tract infections. Additionally, cefazolin may be used in treatment of systemic infections due to susceptible isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*.

Here, we present two cases that relate to using parenteral cefazolin as the therapeutic agent and one additional case of use of oral cephalosporins for uncomplicated urinary tract infections, as follows:

Case 1 - Reporting of cefazolin for complicated urinary tract infections (cUTIs) when a parenteral agent is indicated

Case 2 - Reporting of cefazolin for treatment of systemic infections due to a susceptible isolate of *Escherichia coli*

Case 3 - Testing and reporting of the oral agent cefdinir when requested on urine isolates of *Citrobacter koseri*

We also provide additional Q&As. The cefazolin breakpoints recommended for use in various settings as listed in CLSI M100-Ed29 are listed in Table 1.

### Table 1. CLSI Cefazolin Breakpoints

<table>
<thead>
<tr>
<th>Reason for Testing</th>
<th>Susc (µg/ml)</th>
<th>Int (µg/ml)</th>
<th>Res (µg/ml)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predict cefazolin use for systemic infections</td>
<td>≤ 2</td>
<td>4</td>
<td>≥ 8</td>
<td></td>
</tr>
<tr>
<td>Predict cefazolin use for uUTI</td>
<td>≤ 16</td>
<td>–</td>
<td>≥ 32</td>
<td>2 g every 8 h (IM or IV)</td>
</tr>
<tr>
<td>Surrogate for oral cephalosporins to use for uUTI</td>
<td>≤ 16</td>
<td>–</td>
<td>≥ 32</td>
<td>1 g every 12 h (IM or IV)</td>
</tr>
</tbody>
</table>

Abbreviations: IM = intramuscular; Int = intermediate; IV = intravenous; Res = resistant; Susc = susceptible; uUTI = uncomplicated UTI.

**Case Study 1:** A 35-year-old female presents to the Emergency Department (ED) with dysuria and hematuria and lower back pain. The patient is suspected of suffering from acute pyelonephritis which is supported by her clinical symptoms and urinalysis results. Due to severity of symptoms, she is admitted to the hospital. A urine culture is collected and demonstrates > 100,000 CFU/mL *E. coli*. The cefazolin MIC is 8 µg/mL. Which breakpoints should be applied to cefazolin in this scenario?

**Answer to Case Study 1:** Due to the status of this patient (acute pyelonephritis), this is considered a case of complicated UTI. According to M100-Ed29, cefazolin MIC breakpoints are S ≤2 µg/mL; I 4 µg/mL; and R ≥ 8 µg/mL when cefazolin is considered for therapy of infections other than uncomplicated UTIs due to *E. coli*, *K. pneumoniae*, and *P. mirabilis*.

Rarely do laboratories receive information from clinicians about whether a UTI is considered uncomplicated or not. Sometimes, it can be unclear to the clinicians themselves whether a UTI qualifies as complicated or not. Therefore, laboratories should offer reporting options that can guide clinicians as they narrow or broaden their differentials. The laboratory report may appear as below in Table 2.
Digging Deeper Into Understanding Cefazolin Reporting for Enterobacteriaceae (Continued)

Table 2: Case Study 1 Laboratory Report  
Specimen: Urine  
Organism: > 100,000 CFU/mL E. coli

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC (µg/mL)</th>
<th>Interpretive Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;32</td>
<td>R</td>
</tr>
<tr>
<td>Cefazolin, complicated UTI or systemic infection</td>
<td>8</td>
<td>R</td>
</tr>
<tr>
<td>Cefazolin, uncomplicated UTI</td>
<td>8</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤1</td>
<td>S*</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>≤32</td>
<td>S</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>≤16/4</td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>&gt;4/76</td>
<td>R</td>
</tr>
</tbody>
</table>

Abbreviations: R, resistant; S, susceptible; UTI: urinary tract infection.  
*M100 28th edition breakpoint

In conclusion, the clinician will likely select another antimicrobial to which the organism tests susceptible since a cefazolin MIC of 8 µg/ml is interpreted as resistant for a complicated UTI.

Case Study 2: A 50-year-old male presents to the ED with an abscess in his thigh, fever, and urinary symptoms. He has been living on the streets. Methicillin-susceptible Staphylococcus aureus grows from the abscess, and E. coli grows from urine and blood cultures. He was admitted as an inpatient. The clinician had empirically treated with vancomycin and piperacillin-tazobactam. When the culture results were provided, the clinician expressed interest in de-escalating therapy by covering for both S. aureus and E. coli with cefazolin. Ceftriaxone cannot be given due to hyperbilirubinemia in this patient. Which breakpoints should be applied to cefazolin in this scenario?

Table 3: Case Study 2 Laboratory Report  
Specimen: Blood  
Organism: E. coli

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC (µg/mL)</th>
<th>Interpretive Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;32</td>
<td>R</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>1</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤1</td>
<td>S</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>≤32</td>
<td>S</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>≤16/4</td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>&gt;4/76</td>
<td>R</td>
</tr>
</tbody>
</table>

Abbreviations: R, resistant; S, susceptible.

Answer to Case Study 2: The cefazolin systemic MIC breakpoints for isolates of Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis are S ≤ 2 µg/mL; I 4 µg/mL; and R ≥ 8 µg/mL. These breakpoints are to be applied for these isolates causing infections other than uUTIs. The clinician would be able to use cefazolin for treatment of this patient’s systemic infection due to E. coli, while covering for confirmed methicillin-susceptible S. aureus. In some laboratories using commercial systems, the lowest cefazolin concentration included in panels is 4 µg/mL. If the commercial system does not extend below 4 µg/mL and the MIC reported by the instrument is ≤4 µg/mL, the isolate could either be intermediate (if the MIC is truly 4 µg/mL) or susceptible (if the MIC is truly 2 µg/mL or less). The laboratory would need to test the isolate with cefazolin by an alternate method, in order to cover the dilutions below 4 µg/mL and report the interpretation of the result based on the systemic breakpoints.
Digging Deeper Into Understanding Cefazolin Reporting for Enterobacteriaceae (Continued)

Case Study 3: A 32-year-old female presents to the ED with dysuria and hematuria. The patient is diagnosed with urinary tract infection which is supported by urinalysis results. A urine culture is collected. The patient is sent home from the ED on cefdinir. The urine culture demonstrates > 100,000 CFU/mL Citrobacter koseri. Antimicrobial susceptibility testing (AST) results are shown in Table 4. The provider asks the laboratory if cefazolin predicts cefdinir results. How can the laboratory address this request?

Answer to Case Study 3: This is a case of uncomplicated UTI in a female patient. According to CLSI, use of cefazolin as a surrogate for oral cephalosporins only applies to E. coli, K. pneumoniae, and P. mirabilis. When the cefazolin surrogate studies were performed, there were only enough data from isolates of these three species to establish surrogacy with confidence.

Thus, the laboratory technologist should tell the clinician that cefazolin results cannot predict results for oral cephalosporin agents such as cefdinir. The provider could communicate with the patient and ask if her condition is improving. If she is not improving, the provider can switch the patient to another antibiotic such as nitrofurantoin since it tested susceptible. If the provider insists on testing the isolate for cefdinir susceptibilities, the laboratory could test the C. koseri isolate using an MIC method. Breakpoints for cefdinir exist for the Enterobacteriaceae family. However, the laboratory should be aware that disk diffusion testing for cefdinir and loracarbef should not be performed for Citrobacter, Providencia, or Enterobacter spp., as false-susceptible results have been reported.

Supplemental Q&A:

Question #1
A laboratory is in the process of performing a validation study for the current CLSI cefazolin breakpoints. The lowest concentration of cefazolin on the commercial antimicrobial susceptibility test panel is 4 µg/ml. How can the laboratory validate the cefazolin systemic breakpoints?

Answer #1
If the lowest concentration is 4 µg/ml, the laboratory cannot validate or apply the systemic breakpoints. The laboratory should consult with the Antibiotic Stewardship Team as to how often providers may need cefazolin results for systemic infections and explain the current testing situation. Although there are breakpoints for cefazolin against E. coli, K. pneumoniae, and P. mirabilis, wide use of cefazolin for treatment of non-urinary tract infections due to these species is unlikely. If cefazolin were specifically requested on a non-urine isolate, the laboratory could perform another method of AST (eg, disk diffusion). Nevertheless, the laboratory can still validate the urine breakpoints.

Question #2
Is it likely that manufacturers of commercial antimicrobial susceptibility test systems will obtain FDA clearance for the current CLSI systemic and urine cefazolin breakpoints on their systems in the near future?

Answer #2
No. The FDA cefazolin MIC breakpoints are currently S ≤1 µg/ml, I 2 µg/ml, R ≥ 4 µg/ml and there is no distinction for systemic versus urine breakpoints. Manufacturers of commercial antimicrobial susceptibility test systems must use FDA breakpoints. Consequently, it is unlikely that such systems will be updated with the current CLSI cefazolin breakpoints any time soon.

Reference:
Practical Tips

#1 When Should Antifungal Susceptibility Testing Be Performed For Candida Species Isolated From Clinical Specimens?

Cecilia Carvalhaes, JMI Laboratories, North Liberty, Iowa

Background

There has been a greater demand for antifungal susceptibility testing (AFST) in recent years as a result of the increased use of antifungals, the recognition of innate resistance in some fungal species, and the emergence of resistance during therapy. AFST results can be useful to:

- Predict the likely outcome and guide antifungal therapy in specific clinical circumstances, including candidemia and mucosal candidiasis.
- Detect resistance that develops during therapy
- Monitor local susceptibility patterns that will guide empiric therapy

Although Candida spp. are the most prevalent organisms associated with invasive fungal infections, each species has its unique virulence potential, antifungal susceptibility patterns, and epidemiology. Of note, Candida auris, a pathogen first described in 2009 that has reduced susceptibility to the major antifungal classes, has emerged as a cause of rapidly spreading nosocomial outbreaks associated with high mortality rates.

In 2017, CLSI combined broth and disk susceptibility testing recommendations for Candida and species-specific MIC interpretive breakpoints into CLSI document M60. This document includes breakpoints for the most frequent Candida species for selected agents (echinocandins, fluconazole, and voriconazole, see Table 1). Note that not all Candida species are represented in CLSI document M60 and breakpoints have not been set for all antifungal agents that may warrant testing. In the absence of breakpoints, the epidemiological cutoff value (ECV) published in CLSI document M59 is useful to alert for the possibility of intrinsic or acquired resistance based on the MIC value (see Table 1). ECV is the MIC value that presumptively separates populations into those with and without intrinsic or acquired resistance traits. ECVs are not meant to predict the clinical outcome to therapy, and a susceptibility category (eg, Susceptible/ Intermediate/ Susceptible-Dose Dependent/ Resistant) should not be reported with an ECV since the value does not take into consideration clinical or pharmacodynamic and pharmacokinetic data. However, ECVs should help to detect those strains that are likely to harbor antifungal resistance mechanisms.

The Infectious Diseases Society of America (IDSA) and the European Society of Clinical Microbiology and Infectious Disease recommend routine antifungal susceptibility testing for all clinically relevant Candida isolates. Clinically relevant isolates are those recovered from blood and other sterile body sites, including tissue biopsies, bone, and fluids, such as cerebrospinal, peritoneal, pericardial, pleural, and synovial fluid. Candida spp. isolates recovered from body sites where colonization is common, such as respiratory tract, gastrointestinal tract, vagina or skin should not be considered clinically relevant and susceptibility testing is generally not performed for isolates from these sites.

This article addresses the susceptibility testing for Candida species for which CLSI has established breakpoints or ECV recommendations. CLSI also offers ECVs for Cryptococcus, but such testing is not covered in this article. CLSI is also currently assessing the possibility of setting breakpoints for some antifungals for Candida auris, but those have not yet been set. Antifungal susceptibility testing of C. auris will thus be discussed in a future article.

Practical Microbiology Situations

Based on published CLSI breakpoints and ECVs and literature review, Table 2 contains expert opinion guidelines for when AFST should be performed and Table 3 suggests the antifungal agents that may be considered for primary and secondary panel testing according to Candida species, also according to expert opinions published in a variety of journals. In many situations, decisions for antifungal testing must be made on a case-by-case basis with input from the microbiologist, infectious diseases practitioners and other healthcare providers.
When Should Antifungal Susceptibility Testing Be Performed For Candida Species Isolated From Clinical Specimens? (Continued)

Several examples related to antifungal testing in situations that might be encountered by clinical microbiologists are reviewed below.

1. **Candida glabrata** from blood culture
   - Discussion: Routine AFST should be performed for all Candida species from blood, at least for fluconazole. In this case, *C. glabrata* is a pathogen of special interest due to common acquired resistance to azoles. An MIC and interpretation should be reported for fluconazole and testing with echinocandins is also recommended. Since no clinical breakpoints are currently available for *C. glabrata* and voriconazole or posaconazole, the laboratory may consider reporting the ECVs if these agents are requested. Amphotericin B susceptibility may also be tested, and an ECV reported for this agent.

2. **Candida krusei** from urine specimen
   - Discussion: If a Candida spp. is recovered from urine and is confirmed to be linked to a urinary tract infection, further workup beyond species identification is not needed. If treatment is indicated, fluconazole is usually prescribed. Echinocandins are not used to treat urinary tract infections, due to minimal excretion of active drug in the urine. If fluconazole susceptibility cannot be inferred by species identification, such as with *C. glabrata* for which acquired resistance to fluconazole is not uncommon, AFST may be warranted. And if the isolate is *C. krusei* which is intrinsically resistant to fluconazole, testing of posaconazole or amphotericin B may be performed (see Table 3). ECVs are available for posaconazole and amphotericin B for *C. krusei* (Table 1).

3. **Candida albicans** from oropharyngeal specimen
   - Discussion: Mucosal candidiasis is commonly observed in immunocompromised patients who have received antifungal prophylaxis. In these immunocompromised patients, AFST may be indicated. In addition, AFST would be recommended in case of treatment failure to rule out fluconazole-resistant isolates and to determine susceptibility of other agents.

4. **Candida albicans** from vaginal specimen
   - Discussion: Candida albicans colonizes healthy human skin, mucosal surfaces, and the reproductive tract. Since approximately 10%–20% of women harbor Candida spp. and other yeasts in the vagina, identifying Candida by culture in the absence of symptoms or signs of infection is not an indication for treatment. In general, azole susceptibility testing is not warranted for individual treatment guidance, but fluconazole susceptibility testing may be requested for treating complicated vulvovaginal candidiasis.

5. **C. tropicalis** from tracheal aspirate specimen
   - Discussion: The isolation of Candida species from the respiratory tract is commonly encountered among patients who are in the ICU and are intubated or have a chronic tracheostomy. This almost always reflects colonization of the airways and not infection. Candida tropicalis possesses a remarkable capacity to form biofilms in medical devices. AFST for *C. tropicalis* from tracheal aspirate is not needed.

In summary, it is helpful for laboratories to have a clear approach outlining when performance of AFST is necessary and with which antifungals. Selective antifungal susceptibility testing coupled with more frequent identification of Candida to the species level has proven useful, especially in difficult-to-manage cases of invasive candidiasis. In addition, periodic epidemiological studies should also be done to determine the susceptibility profiles and resistance rates for individual centers. Finally, the use of antifungal susceptibility testing to detect emerging resistance and predict the therapeutic potential of newly discovered investigational agents is of primary importance for continuing medical progress.
When Should Antifungal Susceptibility Testing Be Performed For Candida Species Isolated From Clinical Specimens? (Continued)

Table 1. Antifungal Agents for which Clinical Breakpoints or ECVs Are Published for Candida Species in CLSI Documents

<table>
<thead>
<tr>
<th>Species</th>
<th>Fluconazole</th>
<th>Voriconazole</th>
<th>Posaconazole</th>
<th>Itraconazole</th>
<th>Caspofungin</th>
<th>Micafungin</th>
<th>Anidulafungin</th>
<th>Amphotericin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>BP / ECV</td>
<td>BP / ECV</td>
<td>ECV</td>
<td>–</td>
<td>BP</td>
<td>BP / ECV</td>
<td>BP / ECV</td>
<td>ECV</td>
</tr>
<tr>
<td>Candida dubliniensis</td>
<td>ECV</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ECV</td>
<td>ECV</td>
<td>–</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>BP / ECV</td>
<td>ECV</td>
<td>ECV</td>
<td>ECV</td>
<td>BP</td>
<td>BP / ECV</td>
<td>BP / ECV</td>
<td>ECV</td>
</tr>
<tr>
<td>Candida guilliermondii</td>
<td>ECV</td>
<td>–</td>
<td>ECV</td>
<td>–</td>
<td>BP</td>
<td>BP / ECV</td>
<td>BP / ECV</td>
<td>–</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>–</td>
<td>BP / ECV</td>
<td>ECV</td>
<td>–</td>
<td>BP</td>
<td>BP / ECV</td>
<td>BP / ECV</td>
<td>ECV</td>
</tr>
<tr>
<td>Candida lusitaniae</td>
<td>ECV</td>
<td>–</td>
<td>ECV</td>
<td>ECV</td>
<td>–</td>
<td>ECV</td>
<td>ECV</td>
<td>–</td>
</tr>
<tr>
<td>Candida parapsilosis complexa</td>
<td>BP / ECV</td>
<td>BP / ECV</td>
<td>ECV</td>
<td>–</td>
<td>BP</td>
<td>BP / ECV</td>
<td>BP / ECV</td>
<td>ECV</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>BP / ECV</td>
<td>BP / ECV</td>
<td>ECV</td>
<td>ECV</td>
<td>BP</td>
<td>BP / ECV</td>
<td>BP / ECV</td>
<td>ECV</td>
</tr>
</tbody>
</table>

Abbreviations: BP, clinical breakpoints; ECV, epidemiological cut-off values.
a CLSI M60
b CLSI M59
c ECV established for C. parapsilosis species complex, which may include isolates of C. orthopsilosis and C. metapsilosis.

Table 2. Recommendations for Antifungal Susceptibility Testing of Candida Species According to Specimen Source (Adapted from Pfaller and Diekema10 and Pfaller8)

<table>
<thead>
<tr>
<th>Specimen source</th>
<th>Antifungal Recommendations Species/agents to test/report</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Test fluconazole and echinocandins on all Candida species.</td>
<td>Test additional antifungal agents according to species listed in Table 3. If invasive disease and clinical failure with initial therapy is suspected, test amphotericin B, fluconazole, voriconazole, and an echinocandin for any Candida species.</td>
</tr>
<tr>
<td>CSF</td>
<td>Test fluconazole on all Candida species.</td>
<td>Test additional antifungal agents according to species listed in Table 3.</td>
</tr>
<tr>
<td>Other sterile source (body fluids, tissue, bone, etc.)</td>
<td>Test fluconazole and echinocandins on all Candida species.</td>
<td></td>
</tr>
<tr>
<td>Oropharyngeal</td>
<td>Test fluconazole on Candida species isolated from patients unresponsive to fluconazole therapy.</td>
<td>Test additional antifungal agents according to species listed in Table 3. If clinical significance is established, and AFST is requested by health care providers, test suggested antifungal agents according to Candida species listed in Table 3. Note: do not test echinocandins against isolates from the urinary tract.</td>
</tr>
<tr>
<td>Upper respiratory tract (sputum)</td>
<td>Do not perform AFST routinely.</td>
<td></td>
</tr>
<tr>
<td>Lower respiratory tract (BAL, tracheal aspirate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin / wounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Monitoring local susceptibility profiles with an antifungal antibiogram is recommended.
When Should Antifungal Susceptibility Testing Be Performed For Candida Species Isolated From Clinical Specimens? (Continued)

Table 3. Suggested Antifungal Agents for Primary and Secondary Panel Testing Consideration According to Candida Species Based on CLSI Documents, Pfaller and Diekema, and Pfaller.

<table>
<thead>
<tr>
<th>Species</th>
<th>Azolesa</th>
<th>Echinocandinsb,c</th>
<th>Polyenes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluconazole</td>
<td>Voriconazole</td>
<td>Posaconazole</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Primary</td>
<td>Secondary</td>
<td>Secondary</td>
</tr>
<tr>
<td>Candida dubliniensis</td>
<td>Primary</td>
<td>Secondary</td>
<td>Secondary</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>Primary</td>
<td>Secondary</td>
<td>Secondary</td>
</tr>
<tr>
<td>Candida guilliermondii</td>
<td>Secondary</td>
<td>Secondary</td>
<td>Secondary</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>Intrinsic R, should not be tested</td>
<td>Primary</td>
<td>Primary</td>
</tr>
<tr>
<td>Candida lusitaniae</td>
<td>Primary</td>
<td>Secondary</td>
<td>Secondary</td>
</tr>
</tbody>
</table>

a Itraconazole is not generally clinically useful for the treatment of infections due to Candida species and is not included in this table.

b Test at least one of the echinocandins drugs.

c Do not test echinocandins against isolates from the urinary tract.

d No BP or ECV published by CLSI

Note: Other antifungal drugs may be tested upon request.
When Should Antifungal Susceptibility Testing Be Performed For Candida Species Isolated From Clinical Specimens? (Continued)

References:


Practical Tips

#2 Differences in Disk Content Recommended by CLSI and EUCAST for Disk Diffusion Testing

Romney M. Humphries, Accelerate Diagnostics, Tucson, AZ

Disk diffusion testing is used by a variety of laboratories throughout the world. Disk diffusion breakpoints are dependent on the concentration of antimicrobial impregnated into the disk, otherwise known as the disk content. This disk content in microgram concentrations is printed on each disk and is listed in both CLSI’s M100 and M45 and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables.

Drug manufacturers and standards organizations attempt to identify a disk content that clearly differentiates a susceptible from a resistant isolate. Additionally, attempts are made to ensure the disk content selected does not produce exceedingly large zones for susceptible isolates. For some antimicrobials, no disk content produces acceptable results and consequently disk diffusion testing is not recommended for the drug or drug/organism combination (e.g., vancomycin for staphylococci).

There are some differences between CLSI and EUCAST recommended disk contents and not all of these types of disks are commercially available in all countries (Table 1). If a laboratory chooses to apply EUCAST breakpoints to tests performed in the US, it is imperative that the FDA-cleared disk used contains the disk content assigned by EUCAST, and vice versa. Similarly, the disk quality control ranges are based on the disk content, and laboratories must ensure they are using the correct quality control range for the disk content employed.

Table 1. Agents for Which There Are Differences Between CLSI and EUCAST in Disk Content Recommendations

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>CLSI Disk Content (µg)</th>
<th>EUCAST Disk Content (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>Ceftaroline</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Ceftazidime-avibactam</td>
<td>30-20</td>
<td>10-4</td>
</tr>
<tr>
<td>Gentamicin (Enterococcus HLAR screen)</td>
<td>120</td>
<td>30</td>
</tr>
<tr>
<td>Linezolid</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Penicillin</td>
<td>10 Units*</td>
<td>1 Unit*</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>100-10</td>
<td>30-6</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>30</td>
<td>5</td>
</tr>
</tbody>
</table>

* disk content in Units, not micrograms

In 2017, CLSI and EUCAST formed a joint disk-diffusion working group endeavored to harmonize disk contents for new antimicrobial agents going forward.
Infections due to methicillin-sensitive *Staphylococcus aureus* (MSSA) continue to be a significant source of morbidity and mortality. While antistaphylococcal penicillins, such as nafcillin, have been the mainstay of therapy for MSSA, the use of cefazolin has grown increasingly popular. Indeed, recent data from observational clinical studies suggest that cefazolin is as efficacious as nafcillin, with less toxicity, has a more favorable dosing schedule, and possibly improves survival. Thus, cefazolin has become first line therapy for the treatment of MSSA infections.

Failures of cefazolin for deep-seated MSSA infections (ie, endocarditis) are well documented and have been associated with the activity of certain types of the staphylococcal $\beta$-lactamase. This enzyme, encoded by the *blaZ* gene, was originally divided into four isotypes (A, B, C, and D) based on serological assays, and now defined by amino acid sequence. Clinical failures of cefazolin occur most frequently with enzyme types A and C and have been linked to the cefazolin inoculum effect (CIE). This phenomenon is the loss of therapeutic efficacy of cefazolin and other early generation cephalosporin antibiotics when large numbers of organisms are present. In *in vitro*, strains exhibiting the CIE may show minimum inhibitory concentrations (MICs) in the susceptible range at a standard inoculum of ~5 x 10^5 CFU/mL. At a high inoculum of 5 x 10^7 or greater, such strains have a pronounced increase in the MIC of cefazolin to > 16 µg/mL. This effect is abolished if BlaZ is absent or functionally inactivated by a $\beta$-lactamase inhibitor.

The clinical impact of the CIE is an area of active study. In a 2017 narrative review of the published literature regarding the safety and efficacy of cefazolin versus anti-staphylococcal penicillins in MSSA bacteremia, Loubet and colleagues concluded that no significant differences in mortality or relapse rates between the two therapies were evident, although they advised caution due to the limitations of the available studies. Two studies published subsequently suggest that the presence of the CIE may have an impact on clinical outcomes for MSSA bacteremia with the use of cephalosporins as first-line therapy. A recent prospective observational study of 77 patients treated for MSSA bacteremia in Argentina (where antistaphylococcal penicillins are not available) showed a high prevalence of the CIE in MSSA (54.5%). This effect was associated with an increase in 30-day all-cause mortality (Risk ratio [RR] 2.65; 95% confidence interval [CI] 1.1-6.42) in patients with CIE-positive MSSA as compared to patients with MSSA lacking the CIE. Similarly, a study from Korea comparing nafcillin to cefazolin showed that ca. 22% of patients had isolates positive for the CIE. Most importantly, those patients infected with isolates that exhibited the CIE and treated with cefazolin were more likely to experience treatment failure (61.5% vs 28.9%; $p = 0.049$) and death at one month (15.4% vs. 0%; $p = 0.047$), as compared to patients with CIE-negative isolates. No difference in either outcome was noted with the nafcillin treatment group, regardless of the presence of the inoculum effect, suggesting that there were no differences in virulence between isolates from the two groups.

Currently, the only way to detect the CIE is by standard MIC determination at high bacterial inoculum. This test is both time consuming and cumbersome to perform, limiting its utility in the clinical microbiology lab. In addition, high inoculum MICs have not been evaluated and standardized by the CLSI, and we would discourage use of this technique outside of a research laboratory setting. It is also important to note that $\beta$-lactamase testing as described in the M100 document is for identification of staphylococci which are penicillin-resistant due to production of the BlaZ $\beta$-lactamase. This test cannot determine the presence or absence of the CIE and must not be used as a surrogate marker. The rapid identification of MSSA isolates exhibiting the CIE effect would permit clinicians to stratify patients at risk of cefazolin treatment failure and adjust therapy accordingly. CIE testing has the potential to improve patient outcomes in serious MSSA infections, in addition to cost savings and prevention of relapses.

In summary, MSSA producing staphylococcal $\beta$-lactamase that exhibit the CIE may contribute to treatment failure with cefazolin. Emerging data are conflicting, but some suggest that the CIE may have a direct clinical impact on patient mortality. The CIE is not currently detected by routine laboratory testing, and requests for testing of clinical isolates should be deferred until a standardized testing methodology becomes available. Ongoing efforts to develop rapid diagnostics are needed.
The Cefazolin Inoculum Effect for MSSA (Continued)

References:


Quality Corner

Antimicrobial Susceptibility Testing Quality Control of β-lactam/β-lactamase Inhibitor Combination Agents in Clinical Development

Ayuen Lual and Heena Shah, Culture Collections, NIS Laboratories, National Infection Service, Public Health England, UK
Jane E. Ambler, Wockhardt, USA, and Suyog Vaidya, Wockhardt, India
Brian Beck, Microbiologics, USA

The Spring issue featured an article that highlighted important considerations for clinical laboratories conducting antimicrobial susceptibility testing (AST) of new β-lactam combination agents1-2. The authors acknowledged that as more β-lactam combination agents are made available to clinicians, CLSI will need to recommend additional quality control (QC) strains to ensure that both components of these novel β-lactam combinations are active.

For the susceptibility testing of non-fastidious organisms and β-lactam combination agents, Tables 4A-2 and 5A-2 were added to the disk diffusion and MIC QC Tables of M100-S28, respectively.3 For antimicrobial drugs in clinical development, QC ranges are established through data generated by multi-laboratory CLSI M23 Tier 2 QC studies.4 In brief, these studies involve the determination of MICs of the investigational drug for 10 replicates of each QC strain, using 3 different media lots by at least seven independent laboratories. In addition, the same laboratories also test 10 replicates of the same QC strains by disk diffusion using 2 lots of disks and 3 different media lots. These data are critically reviewed by both the CLSI AST QC working group and the CLSI AST Subcommittee prior to publication in M100.

For the high dose β-lactam combination cefepime-tazobactam5-8 currently in Phase III development, CLSI recommends use of Escherichia coli NCTC 13353, a CTX-M-15 ESBL producer for QC testing to assess the β-lactamase inhibition effect by tazobactam, as cefepime is labile to CTX-M-15 and tazobactam inhibits CTX-M-15.7 More recently, when establishing QC expected ranges for cefepime-zidebactam (a novel diazabicyclooctane β-lactamase inhibitor/β-lactam enhancer with high-affinity for PBP2 binding in Gram-negatives combined with cefepime),9 Acinetobacter baumannii NCTC 13304 was introduced as a new QC strain, as current CLSI QC strains did not differentiate cefepime, zidebactam and cefepime-zidebactam activity. A. baumannii NCTC 13304 produces OXA-27 which allows discrimination between the activity of cefepime-zidebactam versus standalone cefepime or zidebactam by MIC3 and disk diffusion tests (approved by CLSI AST Subcommittee, January 2018).

These new QC strains have been introduced to monitor the quality of the test materials used and reproducibility of the test results. Subsequently, during late stage clinical development additional QC data are collected prospectively by drug sponsors from a variety of sources including AST results from clinical trials, an antimicrobial surveillance program, in-house and external in vitro susceptibility studies, and commercial AST device development. These data are continually monitored to ensure accurate and reproducible AST results and to reassess existing QC expected ranges, which may as a result of this review remain unchanged, change (shifted) or expand (eg, to a four dilution MIC range), as appropriate.4

Laboratories following CLSI standards are generally familiar with routine QC strains recommended for AST from the American Type Culture Collection (ATCC®), but they may be less familiar with the alternative options for accessing QC strains. Sourcing and maintaining AST QC strains needed to follow CLSI standards has become burdensome. To streamline QC programs, many laboratories around the world are seeking sources from which to acquire QC strains. Culture collections such as National Collection of Type Cultures (NCTC) and commercial biological control manufacturers such as Microbiologics are common resources for all the AST QC reference strains recommended by CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). NCTC is one of four Culture Collections operated by Public Health England (PHE) in the UK. The NCTC collection holds nearly 6000 type and reference bacterial strains, including many unique reference strains with established and novel resistance mechanisms.

How to access QC strains
While QC strains are available directly from individual culture collections such as NCTC and ATCC, necessary import regulations and permit requirements complicate the laboratory procurement process of receiving strains from overseas culture collections. NCTC strains can be ordered through contacting Microbiologics and by searching for “Microbiologics NCTC” on the Internet.
Antimicrobial Susceptibility Testing Quality Control of β-lactam/β-lactamase inhibitor Combination Agents in Clinical Development (Continued)

Serially subculturing (passaging) bacteria can cause genetic variation; therefore, stock control strains should not be more than four subcultures from the ATCC or NCTC authenticated strain. Laboratories may wish to record the number of subcultures. Checking the purity of stock cultures is paramount, regardless of whether they are stored on beads or slants. Storage (-60°C or below) is important for QC strains, especially QC strains recommended for β-lactam combination agents. These strains have plasmids encoding the β-lactamase genes and spontaneous loss of the plasmids has been documented. If stored at temperatures above -60°C or if repeatedly subcultured, these strains may lose their resistance characteristics and QC may be reported outside acceptable ranges.

References:

Related links:
www.phe-culturecollections.org.uk/NCTC
www.microbiologics.com

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