



CLSI Subcommittee on Antimicrobial Susceptibility Testing

# CLSI AST News Update

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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.

## Upcoming Webinar:

### Preparation, Presentation, and Promotion of Cumulative Antibiograms To Support Antimicrobial Stewardship Programs

October 16, 2018 | 1:00–2:00 PM Eastern (US) Time

#### Presenters:

Sharon Erdman, PharmD  
*Clinical Professor, Purdue University College of Pharmacy  
 Infectious Diseases Clinical Pharmacist/Co-Director OPAT Program, Eskenazi Health*

Patricia J. Simner, PhD, D(ABMM)  
*Associate Professor of Pathology, Johns Hopkins University  
 Director of Medical Bacteriology and Parasitology Laboratories, Johns Hopkins Hospital*

## 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.

## Inside This Issue:

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## Webinars

For information on upcoming webinars please go [here](#).

Recently archived CLSI webinars can be accessed on demand (it is best to search “by date”). Learn more about program availability [here](#).

### Recent On-Demand Webinars:

- *CLSI Documents for AST; What’s Available for you? (FREE, May 2018)*
- *Current Recommendations for Antimicrobial Susceptibility Testing of Enterococcus spp. (March 2018)*
- *CLSI 2018 AST Webinar: M100, M02, and M07 Updates (February 2018)*
- *CLSI/CAP Webinar: Digging Deeper Into Antimicrobial Susceptibility Challenges (October 2017)*

### Free Webinars

Archived on-demand webinars are available free of charge six months after the scheduled event for CLSI members. Visit [here](#) or contact CLSI for more information about accessing these on-demand webinars.

### Upcoming Webinars:

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Johns Hopkins Hospital*

#### Resources for Implementation of Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) in the Clinical Microbiology Laboratory

Tuesday, November 6, 2018 | 1:00–2:00 PM Eastern (US) Time

##### Presenters:

Carey-Ann Burnham, PhD, D(ABMM), FIDSA, F(AAM)  
*Professor of Pathology & Immunology  
Washington University in St. Louis School of Medicine*

Kaede Ota Sullivan, MD, MSc, FRCPC, FAAP, FCCM, D(ABMM)  
*Associate Professor of Pathology and Laboratory Medicine  
Lewis Katz School of Medicine at Temple University*

### 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 Disease Pharmacists  
(SIDP)

Susceptibility Testing Manufacturers  
Association (STMA)

### 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](#).

## What's new for CLSI AST Documents?

Lots of changes with antifungal documents! Check out a summary of major changes below.



### M27 | Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, 4th edition.

**Replaces former document M27-A3**

Describes broth dilution method for yeasts.

#### New Recommendations:

- Compared to prior M27-A3, the current M27 document does not include guidance concerning results interpretation for ketoconazole
- New antifungal susceptibility testing process flow chart

#### Updated Recommendations:

- Updated recommended reading time for broth microdilution to 24 hours only (prior M27-A3 stated 24 and/or 48 hours was acceptable for some antifungal agents)
- Explanation provided for deletion of former *Candida* itraconazole and flucytosine breakpoints



### M59 | Epidemiological Cutoff Values for Antifungal Susceptibility Testing, 2nd edition.

**Replaces former document M59, 1st edition**

Provides epidemiological cutoff values (ECVs) for select species of *Candida*, *Cryptococcus*, and *Aspergillus*.

#### New Recommendations:

- Expanded list of *Candida* spp. for which fluconazole ECVs are available
- Addition of posaconazole ECVs for select species of *Candida*
- Addition of voriconazole ECV for *C. glabrata*
- Addition of ECVs for various *Cryptococcus* spp.
- Explanation provided regarding the need to validate other susceptibility testing methods against the broth microdilution method before reporting ECVs



### M38 | Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi, 3rd edition.

**Replaces former document M38-A2**

Describes broth dilution method for filamentous fungi.

#### New Recommendation:

- New indications for testing of filamentous fungi

#### Updated Recommendations:

- Updated information on reading results for echinocandins and isavuconazole
- Guidance for reading and interpreting results for filamentous fungi, including dermatophytes



### M60 | Performance Standards for Antifungal Susceptibility Testing of Yeasts, 1st edition.

**Replaces former documents M27-S4 and M44-S3**

Provides MIC and zone diameter breakpoints for select *Candida* spp..

#### New Recommendations:

- Changed voriconazole “susceptible dose-dependent” category to “intermediate”
- Addition of footnote regarding variability in results for susceptibility testing with caspofungin
- Addition of footnote regarding intrinsic resistance of *C. krusei* to fluconazole

#### Updated Recommendation:

- Explanation for deletion of former *Candida* itraconazole and flucytosine breakpoints



### M61 | Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi, 1st edition.

**Replaces former documents M38-A2 and M51-S1**

Provides MIC and zone diameter for QC and reference strains for select filamentous fungi.

#### New Recommendation:

- Addition of QC ranges for isavuconazole

#### Updated Recommendation:

- Revised zone diameter limits and modes for QC and reference strains

## Check It Out! Educational Workshops Held at CLSI Meetings

Nicole Scangarella-Oman, GlaxoSmithKline

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

The January 2018 workshop, held in Dallas, TX, was

**“Epidemiological cutoff values (ECVs): their development and use.”** ECVs (also known as ECOFFs) have been a hot topic of debate recently. This workshop provided an opportunity for various standards setting groups to provide their unique perspectives, highlight recent achievements, and discuss remaining challenges regarding the development and use of ECVs/ECOFFs, both in the microbiology laboratory and clinical setting. Presentations included representatives from EUCAST, FDA, and CLSI (anti-fungal and AST committees).

PowerPoint presentations from past workshops can be found [here](#).

The next workshop – “Implementation of the 21st Century Cures Act for Susceptibility Test Interpretive Criteria” will be held on Saturday June 2nd, 2018 in San Diego, CA.

In addition to review of workshop materials, you can learn more about the 21st Century Cures Act by accessing two recently published papers:

Humphries RM, Ferraro MJ, and Hindler JA. Impact of 21st Century Cures Act on Breakpoints and Commercial Antimicrobial Susceptibility Test Systems: Progress and Pitfalls. *J Clin Microbiol.* 2018;56 no. 5 e00139-18.

Humphries RM, Hindler JA, Ferraro MJ, Mathers A. 21st Century Cures Act and Antimicrobial Susceptibility Testing: Clinical Implications in the Era of Multidrug Resistance. *Clin Infect Dis.* 2018; doi.org/10.1093/cid/ciy432.

### Future CLSI AST Meetings

**May 31–June 6, 2018**

San Diego, California

**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

## Feature Article | Part 1

# New $\beta$ -lactam Combination Agents for the Treatment of Gram-Negative Bacterial Infections: What the Clinical Microbiologist Needs to Know!

Stephanie L. Mitchell, University of Pittsburgh and Children’s Hospital of Pittsburgh

Romney M. Humphries, Accelerate Diagnostics

Antimicrobial resistant Gram-negative bacteria have become a major public health threat in the last decade. Clinicians are severely limited in the number of effective treatment options for infections caused by multi-drug resistant (MDR) or extensively-drug resistant (XDR) Gram-negative bacteria. Emphasis has been placed on the development of novel antimicrobials that are active against these organisms. Several new  $\beta$ -lactam combination agents (BLCs), which consist of a  $\beta$ -lactam and a  $\beta$ -lactamase inhibitor, have recently come to market. These include ceftolozane-tazobactam (C/T), ceftazidime-avibactam (CZA) and meropenem-vaborbactam (MEV). While not yet on market, imipenem-relebactam is completing Phase III clinical trials and expected to come to market in the near future. However, susceptibility of Gram-negative organisms to these BLC agents is never guaranteed. Susceptibility testing by the clinical laboratory of the BLCs is critical to informing their use for the clinician. We introduce the newer Gram-negative BLCs, including testing and reporting considerations. While physicians may occasionally consider these newer BLCs to treat highly resistant nonfermenters other than *Pseudomonas aeruginosa*, testing should be performed with caution, and only after consultation with infectious disease physicians and/or pharmacists as test methods have not been validated against these organisms for the newer BLCs and breakpoints are not available from FDA or CLSI.

### Ceftolozane-tazobactam (C/T; Zerbaxa™)

C/T is composed of an antipseudomonal cephalosporin (ceftolozane) and the  $\beta$ -lactamase inhibitor tazobactam. Ceftolozane binds to penicillin-binding proteins (PBPs) and interferes with cell wall synthesis, and tazobactam competitively binds to  $\beta$ -lactamases and

## New $\beta$ -lactam Combination Agents for the Treatment of Gram-Negative Bacterial Infections: What the Clinical Microbiologist Needs to Know! (Continued)

prevents  $\beta$ -lactamases from inactivating ceftolozane. C/T is active against some *Enterobacteriaceae* isolates that produce extended-spectrum  $\beta$ -lactamases (ESBLs), including those that express CTX-M, which is the predominant ESBL in the US. However, C/T does not have activity against isolates that produce carbapenemases as tazobactam does not inhibit carbapenemase activity and most carbapenemases hydrolyze ceftolozane (see Table 1). Because carbapenem resistance in *P. aeruginosa* is not typically due to the presence of a carbapenemase, C/T may be active against some carbapenem-resistant *P. aeruginosa* isolates.

**Table 1. Activity of  $\beta$ -lactam Combination Agents Against Various Organism Groups and  $\beta$ -lactamases Commonly Produced by Gram-negative Organisms.**

Agent	Spectrum of Activity								
	ESBL	Carbapenemase ( $\beta$ -lactamase) Class				<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter baumannii</i>	<i>Stenotrophomonas maltophilia</i>	<i>Burkholderia cepacia complex</i>
		A	B	C	D				
C/T	+	-	-	+	+*	+	+/-	+/-	+/-
CZA	+	+	-	+	+	+	-	-	ND
MEV	+	+	-	+	+*	-	-	-	ND
I/R	+	+	-	+	-	+/-	-	-	ND

C/T, ceftolozane-tazobactam; CZA, ceftazidime-avibactam; I/R, imipenem-relebactam; MEV, meropenem-vaborbactam

'+' drug is active; '-' drug is inactive; '+/-' drug may or may not have activity

ND, not determined. \* May not have activity against some oxacillinases and carbapenemases of this class.

In most instances, C/T testing will be requested for isolates of *P. aeruginosa* that are resistant to more than one antipseudomonal  $\beta$ -lactam or BLC, such as ceftazidime, cefepime, aztreonam, meropenem, imipenem, piperacillin-tazobactam, or ceftazidime-avibactam.<sup>1</sup> Requests to test isolates of *Enterobacteriaceae* are less frequent, but C/T might be considered by the clinician for cases of a mixed infection with *P. aeruginosa*, or as a carbapenem-sparing option for an ESBL-producing *Enterobacteriaceae*. Testing should be performed in these instances.<sup>2</sup>

FDA-cleared tests available for C/T are listed in Table 2. Disk and Etest have been shown to perform well compared to reference broth microdilution (BMD), and the MIC Test Strip (MTS) gradient diffusion test consistently measures 1-2 two-fold dilutions higher than BMD.<sup>1</sup>

### Ceftazidime-avibactam (CZA; Avycaz<sup>®</sup>)

CZA is composed of the antipseudomonal cephalosporin ceftazidime and the non- $\beta$ -lactam,  $\beta$ -lactamase inhibitor, avibactam. Avibactam is a  $\beta$ -lactam ring mimic that reversibly binds to many  $\beta$ -lactamases, preventing their activity.<sup>3</sup> CZA activity is similar to that of C/T for ESBL- and AmpC-producing *Enterobacteriaceae*. Unlike some other  $\beta$ -lactamase inhibitors (like clavulanate), avibactam does not appear to induce chromosomal AmpC and may be used against organisms that carry resistance via AmpC. The real advantage of CZA is its ability to inhibit the activity of serine carbapenemases (eg, KPC), but not metallo- $\beta$ -lactamases (see Table 1). Resistance to CZA by *Klebsiella pneumoniae* isolates that harbor a mutated KPC gene or that co-express a metallo- $\beta$ -lactamase have been described, but are rare. CZA is active against *P. aeruginosa*, including some isolates that are resistant to other antipseudomonal  $\beta$ -lactams.<sup>1,4-5</sup>

Carbapenem-resistant *Enterobacteriaceae* and *P. aeruginosa* that are resistant to antipseudomonal  $\beta$ -lactams may be considered for testing. FDA-cleared testing methods are listed in Table 2. Of note, there is no "intermediate" breakpoint for CZA, which appears to be problematic for disk diffusion tests, and recent studies suggest this method may overcall resistance.<sup>6</sup> If used, it is recommended that isolates that test resistant by disk should be confirmed by an alternate method. CZA Etest performs well.<sup>6</sup>

## New $\beta$ -lactam Combination Agents for the Treatment of Gram-Negative Bacterial Infections: What the Clinical Microbiologist Needs to Know! (*Continued*)

Table 2. Testing Options for Newer  $\beta$ -lactam Combination Agents.

BLC	FDA-Approved Testing Methods				Isolates Available from CDC AR Bank
	Disk Diffusion (Vendor)	Gradient Diffusion (Vendor)	BMD (Vendor)	Automated US AST Systems (Vendor*)	
C/T	Y (Hardy)	Y (bioMerieux, Liofilchem)	Y (Thermo-Fisher)	Y (BD, Beckman Coulter)	Y
CZA	Y (BD, Hardy)	Y (bioMerieux)	Y (Thermo-Fisher)	Y (BD, Beckman Coulter)	Y
MEV	Y (Hardy)	Y (Liofilchem)	Y (Thermo-Fisher)	Y (BD)	N

C/T, ceftolozane-tazobactam; CDC AR Bank, Centers for Disease Control and Prevention Antimicrobial Resistance Bank; CZA, ceftazidime-avibactam; FDA, Food and Drug Administration; MEV, meropenem-vaborbactam; N, no; Y, yes.

Imipenem-relebactam is not listed as this drug is currently not available for clinical use in the US.

\* Vendors have FDA-clearance but panels not currently available commercially in the US.

### Meropenem-vaborbactam (MEV; Vabomere™)

MEV is the newest BLC, composed of a carbapenem backbone, which makes MEV stable against most  $\beta$ -lactamases. Vaborbactam is a novel boronic acid  $\beta$ -lactamase inhibitor that binds and inhibits KPC serine carbapenemases.

MEV activity has been best demonstrated for members of the *Enterobacteriaceae* (see Table 1), with significant activity against ESBL, AmpC, and KPC expressing isolates.<sup>7</sup> MEV does not have activity against MBLs or oxacillinases that have carbapenemase activity (eg, OXA-48).<sup>7</sup> MEV is not anticipated to provide additional coverage against the non-*Enterobacteriaceae*, including *P. aeruginosa*, unless these express KPC.<sup>7</sup>

Requests for MEV testing will predominantly be for isolates of carbapenem resistant *Enterobacteriaceae*. Given the lack of data, testing non-*Enterobacteriaceae* against MEV should generally not be performed. CLSI is evaluating breakpoints for the *Enterobacteriaceae* in June 2018 for inclusion in M100-S29. Other than the FDA submission data, there are no published studies that have evaluated performance of tests listed in Table 2 for MEV.

### Imipenem-relebactam (I/R; under evaluation)

I/R is the second BLC to include a carbapenem. Unique to I/R is the novel class A and C  $\beta$ -lactamase inhibitor, relebactam. Relebactam has no activity against class B or D carbapenemases (see Table 1). To date, excellent activity of I/R against KPC-producing *Enterobacteriaceae*, including KPC mutants that are resistant to avibactam, has been shown.<sup>8-10</sup> I/R has variable activity against *P. aeruginosa* isolates, including some with loss of OprD, a common imipenem resistance mechanism. This activity is thought to be due to inhibition of low-level imipenem hydrolysis by *P. aeruginosa*'s endogenous AmpC enzyme.<sup>9</sup> I/R is not currently FDA-cleared for clinical use. At this time, the clinical laboratory should not perform clinical testing for I/R.

### Implementing BLC ASTs in the clinical laboratory

Many laboratories may struggle with how to best approach testing of the newer BLCs. However, these agents are potentially life-saving for patients with critical infections due to highly resistant pathogens and cannot be effectively used without AST data. Laboratories should identify a strategy to perform testing, either in house, or by contacting a reference laboratory. The approach taken may depend on patient populations, local antibiogram data, and hospital formulary. Collaboration with infectious diseases and critical care practitioners, the local antibiotic stewardship team, infection control, and pharmacy is critical for determining testing needs.

Verification study design has been reviewed in a prior issue of the CLSI AST News Update (**Vol. 1, Issue 2, Winter 2016**), and detailed guidance is available in the CLSI M52 guideline.<sup>11</sup> A key resource for verification of BLC tests is the CDC AR Bank (see Table 2), which can provide isolates with known MICs to CZA and C/T. A collection of isolates with MEV data is under development. Additional sources of isolates may include the AST manufacturer or the pharmaceutical company. Verification studies may include as few as 5 clinical isolates (for disk diffusion) and up to 30 isolates (for gradient strips and other MIC tests).

## New $\beta$ -lactam Combination Agents for the Treatment of Gram-Negative Bacterial Infections: What the Clinical Microbiologist Needs to Know! (*Continued*)

### References

- 1 Humphries RM, Hindler JA, Magnano P, Wong-Beringer A, Tibbetts R, Miller SA. Performance of ceftolozane-tazobactam Etest, MIC Test Strips, and disk diffusion compared to reference broth microdilution for beta-lactam resistant *Pseudomonas aeruginosa* isolates. *J Clin Microbiol*. 2018;56(3):e01633-17.
- 2 Arizpe A, Reveles KR, Patel SD, Aitken SL. Updates in the Management of Cephalosporin-Resistant Gram-Negative Bacteria. *Curr Infect Dis Rep*. 2016;18(12):39.
- 3 Zhanel GG, Lawson CD, Adam H, et al. Ceftazidime-avibactam: a novel cephalosporin/beta-lactamase inhibitor combination. *Drugs*. 2013;73(2):159-77.
- 4 Sader HS, Castanheira M, Flamm RK, Mendes RE, Farrell DJ, Jones RN. Ceftazidime/avibactam tested against Gram-negative bacteria from intensive care unit (ICU) and non-ICU patients, including those with ventilator-associated pneumonia. *Int J Antimicrob Agents*. 2015;46(1):53-9.
- 5 Mushtaq S, Warner M, Livermore DM. *In vitro* activity of ceftazidime+NXL104 against *Pseudomonas aeruginosa* and other non-fermenters. *J Antimicrob Chemother*. 2010;65(11):2376-81.
- 6 Shields RK, Clancy CJ, Pasculle AW, Press EG, Haidar G, Hao B, Chen L, Kreiswirth BN, Nguyen MH. Verification of Ceftazidime-Avibactam and Ceftolozane-Tazobactam Susceptibility Testing Methods against Carbapenem-Resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa*. *J Clin Microbiol*. 2018;56(2):e01093-17.
- 7 Castanheira M, Huband MD, Mendes RE, Flamm RK. Meropenem-Vaborbactam Tested against Contemporary Gram-Negative Isolates Collected Worldwide during 2014, Including Carbapenem-Resistant, KPC-Producing, Multidrug-Resistant, and Extensively Drug-Resistant *Enterobacteriaceae*. *Antimicrob Agents Chemother* 2017;61(9):e00567-17.
- 8 Haidar G, Clancy CJ, Chen L, et al. Identifying Spectra of Activity and Therapeutic Niches for Ceftazidime-Avibactam and Imipenem-Relebactam against Carbapenem-Resistant *Enterobacteriaceae*. *Antimicrob Agents Chemother*. 2017;61(9):e00642-17.
- 9 Livermore DM, Warner M, Mushtaq S. Activity of MK-7655 combined with imipenem against *Enterobacteriaceae* and *Pseudomonas aeruginosa*. *J Antimicrob Chemother*. 2013;68(10):2286-90.
- 10 Lob SH, Hackel MA, Kazmierczak KM, Young K, Motyl MR, Karlowsky JA, Sahm DF. *In Vitro* Activity of Imipenem-Relebactam against Gram-Negative ESKAPE Pathogens Isolated by Clinical Laboratories in the United States in 2015 (Results from the SMART Global Surveillance Program). *Antimicrob Agents Chemother*. 2017;61(6):e02209-16.
- 11 CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. 1st ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2015

## Feature Article | Part 2

# Why All the Fuss Over Quality Control of $\beta$ -lactam Combination Agents?

Janet A Hindler, UCLA Health System; Los Angeles County Department of Health

### Background

Quality control (QC) of  $\beta$ -lactam combination agents requires use of strains that will “control” both components of the combination. A  $\beta$ -lactam combination agent includes a  $\beta$ -lactam (eg, drug A) and a second drug that “protects” or “inhibits” the  $\beta$ -lactam from destruction by  $\beta$ -lactamase produced by a microorganism (eg, drug B). A  $\beta$ -lactamase-producing QC strain is required and this strain must be resistant to drug A alone but susceptible to the combination of drug A and drug B. This strain is needed to ensure the  $\beta$ -lactamase inhibitor is present and functional.

Figures 1A-2B show reactions for two of several strains recommended by CLSI for QC of  $\beta$ -lactam combination agents. *E. coli* ATCC® 35218 produces a TEM-1  $\beta$ -lactamase and *Klebsiella pneumoniae* ATCC® 700603 produces an ESBL (SHV-18). The  $\beta$ -lactam alone is included to illustrate the differences in activities of the single versus combination agents.

- Figure 1A (*E. coli* ATCC® 35218)
  - There is no zone around ampicillin, as TEM-1 hydrolyzes ampicillin.
  - There is a zone around amoxicillin-clavulanate, as clavulanate inhibits the TEM-1  $\beta$ -lactamase allowing unhydrolyzed amoxicillin to retain antibacterial activity.
  - Therefore, this strain is satisfactory for routine QC testing of amoxicillin-clavulanate.  
(Note: ampicillin and amoxicillin have comparable activity against *Enterobacteriaceae*.)
- Figure 1B (*E. coli* ATCC® 35218)
  - There is a zone around ceftazidime as ceftazidime is NOT hydrolyzed by TEM-1.
  - There is a zone around ceftazidime-avibactam as a result of the ceftazidime component. The avibactam is not needed here to “protect” ceftazidime from the TEM-1  $\beta$ -lactamase produced by the *E. coli*.
  - Therefore, this strain is NOT satisfactory for routine QC testing of ceftazidime-avibactam.
- Figure 2A (*K. pneumoniae* ATCC® 700603)
  - There is no zone around ampicillin, as SHV-18 hydrolyzes ampicillin.
  - There is a zone around amoxicillin-clavulanate, as clavulanate inhibits the SHV-18  $\beta$ -lactamase allowing unhydrolyzed amoxicillin to retain antibacterial activity.
  - Therefore, this strain is satisfactory for routine QC testing of amoxicillin-clavulanate (currently only MIC QC ranges are available).
- Figure 2B (*K. pneumoniae* ATCC® 700603)
  - There is a small zone around ceftazidime, as SHV-18 hydrolyzes ceftazidime.
  - There is a much larger zone around ceftazidime-avibactam, as avibactam inhibits the SHV-18  $\beta$ -lactamase allowing unhydrolyzed ceftazidime to retain antibacterial activity.
  - Therefore, this strain is satisfactory for routine QC testing of ceftazidime-avibactam.

### Selecting strains for QC of $\beta$ -lactam combination agents

There are new tables (see Table 4A-2 for disk diffusion and Table 5A-2 for MIC methods) in CLSI M100, 28th edition,<sup>1</sup> that are to be used exclusively for QC of  $\beta$ -lactam combination agents and contain acceptable ranges for strains suggested for QC. In some cases, there is more than one strain that would be acceptable, as described in the example for amoxicillin-clavulanate above. Tables 4A-2 and 5A-2 contain some  $\beta$ -lactam combination agents that are not yet FDA cleared for human use but may be used in the future.

### Maintaining and checking the integrity of $\beta$ -lactamase producing QC strains

CLSI QC strains used for  $\beta$ -lactam combination agents contain  $\beta$ -lactamase genes that are located on plasmids. In order to avoid loss of these plasmids, the QC strains must be handled properly. Specifically, they must be stored at  $-60^{\circ}\text{C}$  or below and must not be repeatedly subcultured (see CLSI M02 or CLSI M07, Appendix C, which describes maintenance of QC strains).<sup>2,3</sup> If using lyophilized controls, it is important to store and maintain them following the manufacturer’s recommendations.

## Why All the Fuss Over Quality Control of $\beta$ -lactam Combination Agents? (Continued)

Following initial subculture from frozen or lyophilized stocks, the  $\beta$ -lactamase producing QC strain must be checked one time to ensure it has not lost the plasmid encoding the  $\beta$ -lactamase gene. This “integrity check” is done by testing the QC strain with a  $\beta$ -lactam agent to which the strain is known to be resistant (ie, the  $\beta$ -lactamase produced by the strain hydrolyzes or inactivates the agent). The disk and MIC ranges for antimicrobials which may be used for integrity checks are highlighted in orange boxes in Tables 4A-2 and 5A-2, respectively, in M100.<sup>1</sup> An “integrity check” can be done using the standard CLSI disk diffusion method or any MIC method; the check is only to demonstrate that the plasmid containing  $\beta$ -lactamase genes is present in the strain, the genes are expressed, and the strain can be reliably used to QC  $\beta$ -lactam combination agents. For example, if using a commercial MIC system, this system or a disk diffusion or other MIC test could be used to perform the integrity check. Steps for handling QC strains and checking their integrity are listed below. Table 1 contains a list of available  $\beta$ -lactamase producing QC strains.

Steps for handling  $\beta$ -lactamase producing QC Strains and performance of QC; piperacillin-tazobactam MIC testing as an example:<sup>1-3</sup>

1. Retrieve the  $\beta$ -lactamase producing QC strain from a frozen or lyophilized stock culture. For piperacillin-tazobactam, either *E. coli* ATCC® 35218 or *K. pneumoniae* ATCC® 700603 can be used (see Table 2 below).
2. Perform an initial integrity check of the QC strain (after two subcultures) to ensure it has not lost plasmids encoding for  $\beta$ -lactamase genes. This one-time check can be done prior to or concurrent with performance of the QC test.
  - a. Using a disk diffusion or any MIC method, test one  $\beta$ -lactam agent to which the *E. coli* ATCC® 35218 or *K. pneumoniae* ATCC® 700603 is known to be resistant.
  - b. Zone measurements or MIC values must fall within the acceptable range (refer to orange highlighted boxes) as listed in Tables 4A-2 and 5A-2 in M100.
3. Continue subculturing the strain according to the CLSI recommended QC strain maintenance protocol (See CLSI M02 or CLSI M07 Appendix C).
4. Perform daily or weekly QC for piperacillin-tazobactam MIC testing with the selected  $\beta$ -lactamase producing QC strain. Refer to green highlighted boxes as listed in Table 5A-2 in M100.
5. When it is time to obtain a new subculture of *E. coli* ATCC® 35218 or *K. pneumoniae* ATCC® 700603 from a frozen or lyophilized stock, perform an integrity check on the new subculture (as in step 1).

### Evolving QC recommendations

CLSI reviews proposed QC ranges submitted by pharmaceutical manufacturers at each CLSI AST Subcommittee meeting. As new  $\beta$ -lactam combination agents are developed, Tables 4A-2 and 5A-2 will be expanded.

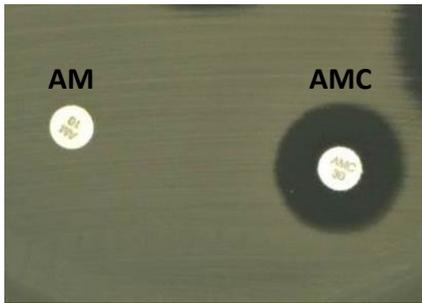
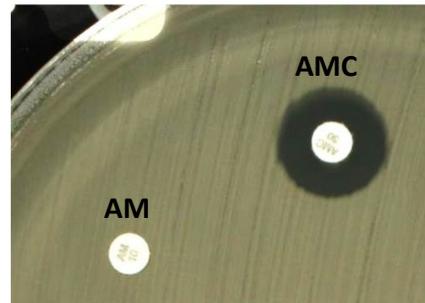
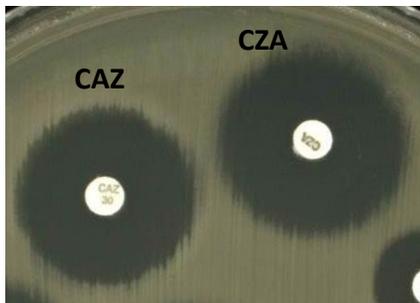
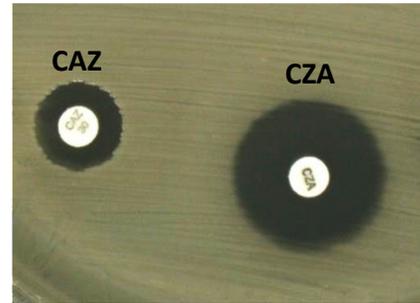
Why All the Fuss Over Quality Control of  $\beta$ -lactam Combination Agents? (Continued)Table 1. Examples of  $\beta$ -Lactamase QC Strains to be Used for  $\beta$ -Lactam Combination Agents QC<sup>a</sup>

$\beta$ -lactamase Producing QC Strain	$\beta$ -lactamase(s) Produced	Appropriate to use for routine QC of: <i>Refer to green highlighted boxes in Tables 4A-2 and 5A-2 of M100, 28th ed.</i>	Check integrity of the QC strain using one of the following agents: <sup>b</sup> <i>Refer to orange highlighted boxes in Tables 4A-2 and 5A-2 of M100, 28th ed.</i>
<i>E. coli</i> ATCC® 35218	TEM-1	Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam Ticarcillin-clavulanate	Ampicillin Piperacillin Ticarcillin
<i>K. pneumoniae</i> ATCC® 700603	SHV-18 (ESBL) OXA-2	Amoxicillin-clavulanate (MIC only) Ampicillin-sulbactam (MIC only) Ceftazidime-avibactam Ceftolozane-tazobactam Piperacillin-tazobactam (MIC only) Ticarcillin-clavulanate (MIC only)	Amoxicillin (MIC only) Ampicillin (MIC only) Aztreonam Cefotaxime (disk only) Cefpodoxime (disk only) Ceftaroline (MIC only) Ceftazidime Ticarcillin (MIC only)
<i>K. pneumoniae</i> ATCC® BAA-1705	KPC-2 TEM SHV	Imipenem-relebactam (MIC only) Meropenem-vaborbactam	Imipenem (MIC only)
<i>K. pneumoniae</i> ATCC® BAA-2814 <sup>c</sup>	KPC-3 TEM-1 SHV-11	Imipenem-relebactam (MIC only) Meropenem-vaborbactam	Imipenem (MIC only) Meropenem

<sup>a</sup> Extracted from CLSI M100 Tables 4A-2 and 5A-2.<sup>1</sup> For QC of additional  $\beta$ -lactam combination agents not yet cleared by FDA, refer to the tables in M100.

<sup>b</sup> The  $\beta$ -lactamase produced by the respective QC strain confers resistance to agents listed in this column.

<sup>c</sup> *K. pneumoniae* ATCC® BAA-2814 has been shown to be stable for  $\beta$ -lactamase production and QC integrity check is not required for this strain.

Why All the Fuss Over Quality Control of  $\beta$ -lactam Combination Agents? (Continued)Figure 1A. *E. coli* ATCC® 35218Figure 2A. *K. pneumoniae* ATCC® 700603Figure 1B. *E. coli* ATCC® 35218Figure 2B. *K. pneumoniae* ATCC® 700603

AM, ampicillin; AMC, amoxicillin-clavulanic acid; CAZ, ceftazidime; CZA, ceftazidime-avibactam

## References:

- 1 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
- 2 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
- 3 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

## Case Study

## Cefazolin, Urine, and *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*: Entertaining Solutions for Antimicrobial Susceptibility Testing and Reporting

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Stella Antonara, Nationwide Children's Hospital

**Case Study:** A urine culture is ordered on a 72-year-old female who was seen at an outpatient clinic and diagnosed with acute cystitis. The culture demonstrated >100,000 CFU/mL *Escherichia coli*. Upon receipt of these results, the clinician asks that the isolate be tested for cefdinir since the patient was sent home with a prescription for this narrow-spectrum cephalosporin that is administered orally. How can the laboratory approach this request?

**Answer:** According to CLSI, there are three different approaches for the interpretation of cefazolin results for *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* and one of these addresses cefdinir:<sup>1</sup>

1. When cefazolin might be used for systemic infections, MIC breakpoints are ( $\mu\text{g/mL}$ ): S  $\leq 2$ ; I 4; R  $\geq 8$ .
2. When cefazolin might be used for uncomplicated urinary tract infections (uUTIs), or infections confined to the bladder, MIC breakpoints are ( $\mu\text{g/mL}$ ): S  $\leq 16$ ; R  $\geq 32$ .
3. When an oral cephalosporin might be used for uUTIs or infections confined to the bladder, cefazolin is tested as a surrogate and MIC breakpoints are ( $\mu\text{g/mL}$ ): S  $\leq 16$ ; R  $\geq 32$ .

Note: disk diffusion breakpoints are also available in M100.<sup>1</sup>

When cefazolin is tested as a surrogate as noted in option #3 above, cefazolin results predict results for the oral agents cefaclor, **cefdinir**, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef. A CLSI publication regarding the adoption of this surrogate test provides a brief description of the rationale.<sup>2</sup>

The laboratory should indicate to the clinician that cefazolin is being tested as a surrogate, either by appending results comments as shown in Table 1, or by another method.

**Table 1: Case Study Laboratory Report**

Specimen: Urine

Organism: >100,000 CFU/mL *Escherichia coli*

Antimicrobial	MIC ( $\mu\text{g/mL}$ )	Interpretive Category
Ampicillin	>32	R
Cefazolin Oral Cephalosporin Surrogate*	8	S
*For uncomplicated urinary tract infections, cefazolin results predict results for oral cephalexin, cefaclor, cefdinir, cefpodoxime, cefprozil, and cefuroxime.		
Ciprofloxacin	>4	R
Nitrofurantoin	$\leq 16$	S
Trimethoprim-sulfamethoxazole	>4/76	R

S, susceptible; R, resistant

## Cefazolin, Urine, and *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*: Entertaining Solutions for Antimicrobial Susceptibility Testing and Reporting (Continued)

Since there are several oral cephalosporins predicted by the “cefazolin as a surrogate” rule, the laboratory may wish to list within the result comment the specific oral cephalosporins commonly used by their clinicians and/or on formulary at the institution, rather than listing all cephalosporins as shown in Table 1.

Alternatively, the laboratory could report the “cefazolin as a surrogate” results by listing “Oral Cephalosporins” on the laboratory report instead of “Cefazolin.” A report comment specifying which oral cephalosporins are predicted by the result should accompany this report as shown below in Table 2:

**Table 2. Option for reporting results when “cefazolin as a surrogate” tests susceptible for urine isolates of *E. coli*, *K. pneumoniae*, and *P. mirabilis***

Antimicrobial	MIC (µg/mL)	Interpretive Category
Oral Cephalosporins*	8	S
*Oral cephalosporins include cephalexin, cefaclor, cefdinir, cefpodoxime, cefprozil, and cefuroxime		

S, susceptible

In this case study, the laboratory need not test cefdinir separately. The isolate is predictably susceptible to cefdinir, since the surrogate cefazolin tested within the susceptible range.

However, if the isolate were resistant to the surrogate cefazolin, there are some oral cephalosporins which may be more active than cefazolin and would be susceptible. The surrogate cefazolin may overcall resistance to cefdinir, cefpodoxime, and cefuroxime axetil. CLSI states that “if a [surrogate] cefazolin tests resistant, test these drugs individually if needed for therapy.” Each of these antimicrobial agents has both MIC and disk diffusion CLSI breakpoints. There are a variety of ways by which laboratories can handle this through the use of report comments. Refer to Table 3 example below.

**Table 3. Option for reporting results when “cefazolin as a surrogate” tests resistant for urine isolates of *E. coli*, *K. pneumoniae*, and *P. mirabilis***

Antimicrobial	MIC (µg/mL)	Interpretive Category
Oral Cephalosporin*	32	R
*Oral cephalosporins include cephalexin, cefaclor, cefdinir, cefpodoxime, cefprozil, and cefuroxime. If treatment with cefdinir, cefpodoxime or cefuroxime is being considered, please contact the laboratory for further testing. Some isolates may test susceptible to these agents while testing resistant to cefazolin.		

R, resistant

More case studies regarding **Cefazolin, Urine, and *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*** will be provided in upcoming issues of CLSI Outreach Working Group Newsletters. Stay tuned!

### Supplemental Q&A:

**Question #1:** My laboratory only utilizes a commercial system for cefazolin testing of *E. coli*, *K. pneumoniae*, or *P. mirabilis*, but the lowest concentration of cefazolin included on the test panel is 4 µg/mL which does not cover the “S” and “I” non-urine breakpoints which are S ≤2 µg/mL; I 4 µg/mL; R ≥8 µg/mL. If a clinician calls for a cefazolin result on a blood isolate of *E. coli*, *K. pneumoniae*, or *P. mirabilis*, what options do I have?

**Answer #1:** If the MIC is ≥8 µg/mL, you can report the result as resistant. However, you would have to use an alternate method to test cefazolin on isolates with MICs ≤4 µg/mL.

**Question #2:** Why have the cephalothin breakpoints for prediction of oral cephalosporins for uUTI been removed from the CLSI document?

**Answer #2:** Cephalothin was found to be a poor predictor of susceptibility to certain oral cephalosporins, with high rates of overcalling resistance.<sup>3</sup> Thus, CLSI removed the cephalothin surrogacy claim for oral cephalosporins from the Table 2A of the M100 document in 2016.

## Cefazolin, Urine, and *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*: Entertaining Solutions for Antimicrobial Susceptibility Testing and Reporting (Continued)

**Question #3:** Can the oral cephalosporin prediction rules apply to *Klebsiella oxytoca*, *Citrobacter koseri*, and other unlisted *Enterobacteriaceae*?

**Answer #3:** No. However, there were insufficient data for these other species of *Enterobacteriaceae* at the time that cefazolin surrogacy was created. Remember, many species within the *Enterobacteriaceae* family are intrinsically resistant to cefazolin and other narrow-spectrum cephalosporins. Please see Appendix B Intrinsic Resistance Tables in M100. A CLSI **Rationale Document** explaining establishment of the cefazolin uUTI surrogacy for oral cephalosporins and a summary of the data are provided in the Rationale Document on the CLSI website.<sup>4</sup>

**Question #4:** Does the oral cephalosporin prediction rule apply to *K. pneumoniae* complex, or just *K. pneumoniae*?

**Answer #4:** This is not really known. Many of the data gathered were based on identification that was not necessarily examined at the level of the various species that make up the *K. pneumoniae* complex.

### References:

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## Burning Question

# What Should Clinical Laboratorians Know About Gonorrhea in 2018?

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## Background

In the US, despite campaigns to encourage safe sex practices, the prevalence of gonococcal infections is on the rise. In 2016, there were 468,514 cases of gonorrhea reported in the US, representing an increase of 18.5% since the year before.<sup>1</sup> The most common presentation in men is urethritis and in women, cervicitis. However, these presentations are not specific to gonococcal infections. Moreover, up to 80% of infected women are asymptomatic, or they experience mild symptoms that can be mistaken for a bladder or vaginal infection.<sup>2</sup> Nonetheless, gonococcal infections can have severe sequelae. Therefore, confirmation of potential or suspect cases with laboratory testing is essential. Currently, the preferred test of choice for most specimen sources utilizes nucleic acid amplification (NAAT).<sup>3</sup>

## Treatment of Gonorrhea and the Emergence of Resistance

In the 1930s, sulfonamides were the first class of antibiotics used for treatment of gonococcal infections; however, resistant isolates began to emerge and treatment failures were encountered. Penicillin then became the drug of choice for several decades until isolates harboring altered penicillin binding proteins in the chromosome and plasmid-mediated  $\beta$ -lactamases appeared. Subsequently, tetracycline, spectinomycin, the fluoroquinolones, and cefixime, have all been used, and emerging resistance has followed (see Table 1).<sup>4,5</sup> In fact, the Centers for Disease Control and Prevention (CDC) reported that over a quarter of all *N. gonorrhoeae* strains are resistant to some combination of penicillin, tetracycline, and ciprofloxacin; over 5% of strains are resistant to all three antimicrobials.<sup>6</sup>

**Table 1. Chronology of Recommended Treatments for *Neisseria gonorrhoeae***

Antimicrobial Agent(s)	Year Resistance Detected	Mechanism of Resistance	Year Agent No Longer Recommended for Empiric Therapy in the US
Penicillin	1976	$\beta$ -lactamase	1987
	1983	CMRNG (chromosomally-mediated resistant <i>N. gonorrhoeae</i> ), altered PBP, permeability	1987
Fluoroquinolone	Early 1990s	Acquisition of <i>gyrA</i> and <i>parC</i> mutations	2007
Cephalosporins (eg, cefixime and ceftriaxone)	Early 2000s	Mosaic <i>penA</i> encoding modified PBP and overproduction of an efflux pump	2012 (cefixime alone)

PBP, penicillin-binding protein

**Table 2. Current recommended empiric therapy for uncomplicated gonococcal infections of the cervix, urethra, and rectum**

Ceftriaxone 250 mg IM + Azithromycin 1 gm PO
-------------------------------------------------

NOTES: (1) If ceftriaxone is not available, a single oral dose of cefixime PLUS a single oral dose of azithromycin or 7 days of oral doxycycline can be used. (2) If the patient is cephalosporin-allergic, a single oral dose of azithromycin can be considered. Complete antimicrobial therapy recommendations are available at [www.cdc.gov/std/tg2015/gonorrhea.htm](http://www.cdc.gov/std/tg2015/gonorrhea.htm).

## What Should Clinical Laboratorians Know About Gonorrhea in 2018? (Continued)

Since 2010, after cefixime was determined to be unsuitable for empirical treatment of uncomplicated gonococcal infections, the regimen recommended by the CDC has been ceftriaxone 250 mg IM and azithromycin 1 g PO (see Table 2).<sup>7</sup> However, according to the Gonococcal Isolate Surveillance Project (GISP), a collaborative project between select sexually transmitted disease (STD) clinics in the US, public health authorities, GISP regional laboratories, and the CDC, resistance to these agents has also emerged. MICs for cefixime and ceftriaxone are creeping up although MICs still remain in the susceptible category, according to CLSI breakpoints.<sup>8</sup> *Neisseria gonorrhoeae* isolates with cefixime and/or ceftriaxone MICs in the CDC “alert” range (defined by the CDC as cefixime MIC  $\geq$  0.25  $\mu\text{g}/\text{mL}$  or ceftriaxone MIC  $\geq$  0.125  $\mu\text{g}/\text{mL}$ , both of which are lower than CLSI clinical breakpoints in order to detect increased MICs before the organism develops full resistance) may be associated with treatment failure.<sup>9</sup> The percentage of urethral *N. gonorrhoeae* isolates tested as part of GISP with reduced azithromycin susceptibilities (defined as MIC  $\geq$  2.0  $\mu\text{g}/\text{mL}$ ) increased from 0.6% in 2013 to 2.5% in 2014.<sup>8</sup> Of particular concern is the recent appearance of isolates highly resistant to ceftriaxone, currently the only remaining option for first-line empiric treatment of gonorrhea. These strains with ceftriaxone MICs ranging from 1-4  $\mu\text{g}/\text{mL}$  (susceptible breakpoint  $\leq$  0.25  $\mu\text{g}/\text{mL}$ ) have been reported in Japan, France, and Spain.<sup>4</sup>

Most recently in March 2018, Public Health England reported a case of treatment failure for pharyngitis associated with an isolate resistant to ceftriaxone (MIC 0.5  $\mu\text{g}/\text{mL}$ ) and non-wild type for azithromycin (MIC  $>$ 256  $\mu\text{g}/\text{mL}$ ).<sup>10</sup> In this case, the patient had a sexual encounter in Southeast Asia one month prior to symptom onset. Treatment with high dose ceftriaxone (1 g) and spectinomycin failed to eradicate the pharyngeal infection. Although there are no breakpoints for ertapenem, the ertapenem MIC was very low at 0.032  $\mu\text{g}/\text{mL}$  and the patient was successfully treated with a three-day course of intravenous ertapenem. The travel history to Southeast Asia suggests that what we are seeing now may be the tip of the iceberg, and there may be many more clinical isolates resistant to ceftriaxone and/or azithromycin among travelers that have thus far gone unreported.

### What can laboratories do to help detect emerging resistance in *N. gonorrhoeae*?

Prior to 2013, routine antimicrobial susceptibility testing (AST) of gonococcal isolates was not recommended by the CLSI, as there was predictable susceptibility to the antimicrobial agents used for empiric therapy. However, due to emerging resistance, CLSI modified guidelines and now recommends that **culture and AST of *N. gonorrhoeae* should be considered in cases of treatment failure.**<sup>11</sup>

Standard recommendations for AST of *N. gonorrhoeae* are provided in CLSI documents M02 (disk diffusion) and M07 (MIC testing). CLSI M100-S28 provides breakpoints, and antimicrobials recommended for testing include, at a minimum: ceftriaxone, cefixime, ciprofloxacin, and tetracycline.<sup>11</sup> Notably, though disk diffusion can be used, agar dilution MIC-based methods are optimal for AST of *N. gonorrhoeae* isolates, in light of the MIC creep observed for some antimicrobial agents.

Although NAAT is currently the diagnostic test of choice and validation and maintenance of routine AST competence may seem unfeasible, clinical laboratories should at least retain the capability to perform culture and AST of *N. gonorrhoeae*. In a 2014 CAP DEX-A survey, of the 197 participants who identified *N. gonorrhoeae* as the challenge organism from a patient’s joint fluid, less than 10% provided AST results on the isolate. Interestingly, the majority of laboratories reported results of  $\beta$ -lactamase testing which is only predictive of susceptibility to the penicillins and therefore no longer useful or necessary to perform for treatment purposes.<sup>12</sup> Culture for *N. gonorrhoeae* should be considered in unusual cases, such as disseminated gonococcal infections from which specimen sources (blood, joint fluids, etc.) would be non-validated for NAAT testing. Clinical laboratories should also attempt to recover isolates from patients who fail empiric treatment so the isolate could be referred to a reference laboratory, local public health or GISP laboratories, or the CDC for phenotypic AST.

Due to the widespread use of NAATs as diagnostic tests for gonorrhea and the subsequent lack of an isolate on which to perform conventional susceptibility testing, molecular assays to predict susceptibility to antimicrobials in *N. gonorrhoeae* have been developed, some of which could be performed in-line with existing molecular diagnostic workflow.<sup>13,14</sup> Although these molecular assays could be used as a tool for epidemiological surveillance of antimicrobial resistance in *N. gonorrhoeae*, most of them are still in development and not currently offered in most reference and public health laboratories. Moreover, these tests may have limited clinical utility, since their performance usually depends on local prevalence of genetic elements conferring antimicrobial resistance in areas where the assay will be implemented.

In the United States, most suspected treatment failures are likely to be re-infections rather than actual treatment failures.<sup>7</sup> However, in the US, there remains a gap in the understanding of the relationship between MIC breakpoints and likelihood of treatment failure. Therefore, in addition to offering culture and AST, laboratorians should promptly report *N. gonorrhoeae* isolates that meet laboratory-based criteria for suspect or probable cephalosporin-resistant isolates to their state or local health departments, as well as to the ordering clinician (see Table 3).<sup>6</sup> Moreover, isolates obtained from all patients with possible treatment failure or possible cephalosporin-resistant infection should be stored at the local laboratory and/or public health laboratory in case further testing is needed.

## What Should Clinical Laboratorians Know About Gonorrhea in 2018? (Continued)

**Table 3. Laboratory Criteria for Suspect and Probable Cases of Cephalosporin-Resistant Isolates of *N. gonorrhoeae***

<p><b>Suspect Case</b> - pre- or post-treatment isolate demonstrates: Cefixime MIC <math>\geq 0.25</math> <math>\mu\text{g/mL}</math> OR Ceftriaxone MIC <math>\geq 0.125</math> <math>\mu\text{g/mL}</math></p> <p><b>Probable Case</b> - pre- or post-treatment isolate demonstrates: Cefixime MIC <math>\geq 0.5</math> <math>\mu\text{g/mL}</math> OR Ceftriaxone MIC <math>\geq 0.25</math> <math>\mu\text{g/mL}</math></p>
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## Hot Topic

It's Enough to *mec* You Crazy!

Lars Westblade, Weill Cornell Medicine

Not long after the introduction of methicillin, a penicillinase-stable penicillin, methicillin-resistant *Staphylococcus aureus* (MRSA) was reported.<sup>1</sup> The primary mechanism of methicillin resistance in staphylococci is through production of penicillin-binding protein (PBP) 2a, an alternative PBP encoded by *mecA* that has low affinity for almost all  $\beta$ -lactams.<sup>2,3</sup> The *mecA* gene is carried on a mobile element: the staphylococcal cassette chromosome *mec*, which is integrated into the chromosomal *orfX* gene, and can spread between staphylococci.<sup>4,5</sup>

In 2011, a novel PBP2a-encoding *mecA* homologue, *mecC* (formerly *mecA*<sub>LGA251</sub>) was reported in *S. aureus* isolates obtained from cattle and humans in Europe.<sup>6</sup> Subsequently, *mecC*-encoding MRSA isolates have been described in a variety of host species and are capable of causing human infection.<sup>7,8</sup> The low homology (70% nucleotide identity) of *mecC* to *mecA* resulted in significant diagnostic challenges in detecting *mecC*-harboring MRSA, especially with *mecA*-specific genotypic and PBP2a-based tests.<sup>6,9,10</sup> Recently, the performance of several phenotypic methods for detecting *mecC* MRSA was assessed.<sup>10</sup> When tested on three commercial automated antimicrobial susceptibility testing (AST) platforms, “cefoxitin resistant/oxacillin susceptible” was the most frequent phenotypic pattern observed, and cefoxitin disk diffusion and oxacillin broth microdilution assays categorized 100% and 61.3% of *mecC* positive isolates as MRSA, respectively. These data led the authors to conclude cefoxitin as the superior marker for *mecC*-harboring MRSA. Importantly, the five commercially available MRSA chromogenic screening media assayed in the study detected all *mecC*-encoding isolates as presumptive for MRSA.<sup>10</sup>

If that wasn't enough to make you crazy, earlier this year *mecB* (formerly *mecA*<sub>m</sub>), originally identified in *Micrococcus caseolyticus*<sup>11</sup>, was detected in a single *S. aureus* isolate obtained through routine MRSA screening in a German hospital.<sup>12</sup> The isolate was recovered from chromogenic MRSA screening agar and identified as MRSA by automated AST, but conflicting negative *mecA/mecC* and PBP2a tests ultimately led to the detection of *mecB*-encoded methicillin resistance. Because *mecB* has only 60.1% and 60.7% nucleotide identity to *S. aureus mecA* and *mecC*, respectively,<sup>12</sup> this likely explains the negative *mecA/mecC* and PBP2a tests. In fact, *mecB* shares the highest nucleotide identity (68.7%) with another *mec* homolog, *mecD*, which results in elevated MIC values to the  $\beta$ -lactams (including the anti-MRSA cephalosporins), implying resistance, in *M. caseolyticus*.<sup>12,13</sup> Fortunately, *mecD* has yet to be detected in *S. aureus*, although, as discussed in the report describing *mecD*, there is potential for *mecD* acquisition by staphylococci. AST revealed the *mecB* positive *S. aureus* isolate displayed MIC values of 32  $\mu\text{g}/\text{mL}$  and 4  $\mu\text{g}/\text{mL}$  to cefoxitin and oxacillin, respectively, indicating resistance. However, like *mecC*-harboring MRSA isolates, the *mecB*-encoding MRSA isolate exhibited low ceftaroline MIC values, implying susceptibility.<sup>12,14</sup> Of some concern, the *mecB* gene was found to be plasmid-encoded, which could lead to accelerated spread of *mecB*-mediated  $\beta$ -lactam resistance.

In summary, although the prevalence of *mecB* and *mecC* in *S. aureus* appears to be very low compared to *mecA*, this might be underestimated because of diagnostic challenges associated with their detection. Therefore, clinical microbiologists should maintain a suspicion for *mecB/mecC* (and perhaps *mecD*)-mediated  $\beta$ -lactam resistance in the setting of elevated MIC values to cefoxitin or oxacillin and negative tests for *mecA* and/or PBP2a, and are encouraged, for epidemiologic purposes, to work with their Public Health Laboratory to investigate the mechanism of methicillin resistance further. Interestingly, the single *mecB*-harboring MRSA isolate and multiple *mecC*-encoding MRSA isolates exhibited elevated cefoxitin MIC values compared to oxacillin.<sup>10,12</sup> Therefore, this phenotypic pattern in the absence of a positive result for *mecA*/PBP2a may provide a diagnostic clue to the presence of *mecB/mecC*.

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## It's Enough to *mec* You Crazy! (Continued)

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