CLSI

CLSI Subcommittee on Antimicrobial Susceptibility Testing

CLSI AST News Update

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

Members:

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What does the CLSI AST Subcommittee do?

The first edition of this newsletter 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.**

Interested in becoming a CLSI volunteer? Learn more here.

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

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 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)

Susceptibility Testing Manufacturers Association (STMA)

Updated CLSI AST Documents – *What's new?*

M100S 27th ed. Major changes in *"Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Seventh Informational Supplement"* include:

- CLSI now uses only the term "breakpoint"; discontinued use of "interpretive criteria"
- Clarified testing/reporting for:
  - Colistin/polymyxin with *P. aeruginosa* and *Acinetobacter baumannii*
  - Atypical (poor growing) *S. aureus*
  - Coagulase-negative staphylococci and oxacillin
- Expanded definition/discussion of ECVs and added ECVs for:
  - Colistin – Enterobacteriaceae
  - Azithromycin – *Neisseria gonorrhoeae*
- Added mCIM for detecting carbapenemases in Enterobacteriaceae
- Quality control/Quality assurance
  - Modified disk diffusion QC ranges:

    Cefepime - *P. aeruginosa* ATCC 27853

    Meropenem - *E. coli* ATCC 25922
  - Modified MIC QC ranges:

    Meropenem - *P. aeruginosa* ATCC 27853

    Tedizolid - *S. aureus* ATCC 29213
  - Added QC ranges for several new drugs that are in development (not yet available for human use)
  - Expanded MIC Troubleshooting Guide
- Added suggestions for confirmation of resistant antimicrobial results for *B. fragilis*
- Deleted tetracycline as intrinsic "R" for *M. morganii*

More information on availability of M00 including the Read Only web version can be found <u>here.</u>

The M100 document cover shows:

CLINICAL AND LABORATORY STANDARDS INSTITUTE

27th Edition

M100

Performance Standards for Antimicrobial Susceptibility Testing

This document includes updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02-A12, M07-A10, and M11-A8.

An informational supplement for global application developed through the Clinical and Laboratory Standards Institute consensus process.

Archive of Retired Breakpoints

An archive of breakpoints removed from M100 since 2010 together with the rationale for their removal is now available **<u>here.</u>**
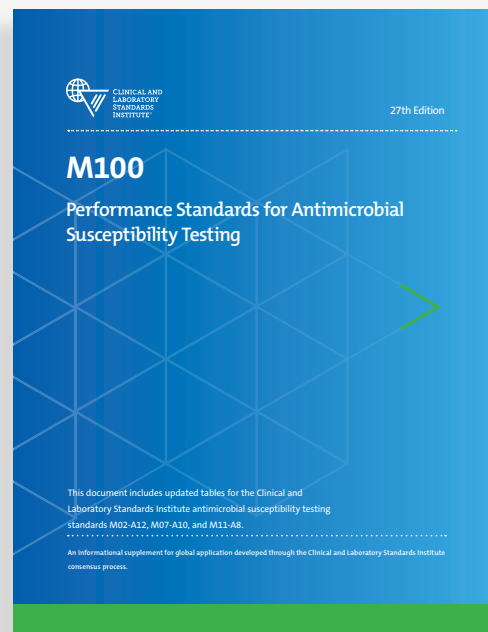
Webinars

For information on webinars please go **here.** Recently archived CLSI webinars can be accessed on demand. Learn more about program availability **here.**

On-Demand Webinars:
- *Practical Recommendations for Antifungal Susceptibility Testing and Reporting in Clinical Laboratories: New Drugs, New Breakpoints, New Guidelines*
- *Facts and Fiction about Colistin from Clinical and Public Health Perspectives*
- *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*
- *Navigating CLSI Document M100: Antimicrobial Susceptibility Testing Made Easy*
- *CLSI 2017 Antimicrobial Susceptibility Testing Update*

Free Webinars

Webinars are available free of charge six to twelve months after the scheduled event for CLSI members. Please contact CLSI for more information about accessing these on-demand webinars.

Upcoming Webinars (registration details will be posted soon!)

October 17, 2017
3rd Annual CAP-CLSI Webinar – Digging Deeper into AST Challenges

> **Angella Charnot-Katsikas, MD**
> *University of Chicago, Chicago, Illinois*

> **Romney M. Humphries, PhD D(ABMM)**
> *UCLA Health, Los Angeles, California*

October 19, 2017
5th Annual CLSI-SIDP Webinar – Merging Microbiology and Stewardship: CLSI updates on antimicrobial susceptibility testing and advances in detecting and managing *Clostridium difficile*

> **Jennifer Dien-Bard, PhD D(ABMM)**
> *Children's Hospital, Los Angeles, California*

> **Dhara Surati, PharmD, BCPS**
> *University of Houston, Houston, Texas*

ASM/CLSI 2017 AST Webinar Series

ASM and CLSI have been coordinating a webinar series entitled "A Comprehensive Course in Antimicrobial Susceptibility Testing" which is geared towards bench level technologists. This program is being presented in three parts with a series of 5 or 6 talks in each part. The first two parts have been completed and presentations are available on demand for a fee. Click on the links below to learn more:

Part I. Fundamentals of Susceptibility Testing, Reporting, and Test Validation

Part II. Mechanisms of Resistance, Antimicrobial Stewardship, and Infection Prevention

Part III. Special Antimicrobial Susceptibility Tests - scheduled to be presented Fall 2017

Check It Out! Educational Workshops held at CLSI meetings

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 next workshop to be held Saturday June 24, 2017, in Philadelphia is entitled "New and Successful Approaches to Antimicrobial Stewardship: The Role of the Microbiology Laboratory." Clinical microbiology laboratories play a critical role in implementing, influencing, and executing successful antimicrobial stewardship programs with the ultimate goal of improving patient care. Some of the topics to be highlighted during this workshop include: antimicrobial stewardship in the hospital, long-term care and outpatient settings, the role of rapid diagnostics and interpretive reporting, and the role of antibiograms in antimicrobial stewardship.

The January 2017 Workshop was focused on "One Health – One Medicine"- Linking Human, Animal and Environmental Health (see article in this Newsletter on page 4).

PowerPoint presentations from past workshops can be found **here.**

Future CLSI AST Meetings!

June 25-27, 2017
Philadelphia, Pennsylvania, USA

January 28-30, 2018
Dallas, Texas, USA

June 3-5, 2018
San Diego, California, USA

The One Health Initiative

The One Health Initiative is a movement that was launched in 2006 and is "a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals, and the environment." CLSI has been involved in the One Health Initiative in a practical way for 25 years through its antimicrobial susceptibility testing (AST) subcommittees that establish testing standards for the human and veterinary laboratory testing sectors. Bacteria isolated from animals may become zoonotic and cause disease in people, either via foodborne transmission (eg, *Salmonella* or *Campylobacter*), direct contact (eg, *Staphylococcus pseudintermedius*), or possibly through environmental contamination. Likewise, many common human pathogens are anthroponotic (i.e., carried by humans but then transferred to animals; an example is *Cryptosporidium*) and may produce colonization and disease in domestic and companion animals. In recognition of the 25th anniversary of the Veterinary Antimicrobial Susceptibility Testing (VAST) Subcommittee, an educational workshop featuring four speakers was held during the January 2017 CLSI Committee Week. Each speaker discussed the shared interests between the VAST and Human AST Subcommittees and between human and animal laboratory medicine testing.

Dr. Jeff Watts, Zoetis Animal Health, outlined key recommendations from the WHO (World Health Organization), OIE (World Organization for Animal Health) and FAO (Food and Agricultural Organization) which address responsible antibiotic use guidelines and national antimicrobial resistance monitoring (AMR) programs with harmonized AST methodology. Responsible use guidelines require the identification and AST of animal pathogens as for human pathogens. The VAST methods are described in VET01-A4 (Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Fourth Edition) and breakpoints are listed in VET01S-Ed3. These documents encompass both companion and food animal species, the pathogens for many major diseases, and antibiotics used to treat them. Dr. Watts discussed the value that national AMR programs may find in the VET05-R document (Generation, Presentation, and Application of Antimicrobial Susceptibility Test Data for Bacteria of Animal Origin; A Report), which emphasizes the use of CLSI methods to ensure comparability of cumulative AST data. A recent blog on the One Health Commission website amplified the key role of CLSI AST methodology in comparing AST data across institutions and can be accessed **here.**

Dr. Mark Papich, Professor of Veterinary Pharmacology at North Carolina State University, reviewed how the unique pharmacokinetics in animals require careful consideration to properly establish appropriate clinical breakpoints, which may not be the same as those set for humans. He noted all current clinical breakpoints for veterinary medicine can be found in VET01S-Ed3 and VET03/VET04-S2 (only applicable to aquatic animals). Access to species-specific clinical breakpoints is a key component to the foundations of "Responsible Use" by veterinarians, since reliance on human clinical breakpoints could result in inappropriate use, emergence of resistance and/or therapeutic failure.

Dr. Tom Fritsche, Marshfield Clinic, Marshfield, Wisconsin, related his One Health – One Lab experience in creating a single lab entity for performing human and animal microbiology testing by integrating mass spectrometry and broth microdilution AST for all specimens. Cross-training of technical staff, partnership with in-house veterinary pathologists, and a common laboratory information system were key factors in successfully streamlining laboratory operations and improving client satisfaction. Benefits realized from this One Health lab approach has included recognition of new pathogens common to both humans and animals, and comparative antibiogram analyses for detecting emerging resistance in both populations.

The final speaker, Dr. Ron Miller, FDA Center for Veterinary Medicine, shared a glimpse of the future comparing Whole Genome Sequencing (WGS) data with phenotypic MIC data for key food-borne bacteria. An exceptionally high correlation was determined between the genotypic and phenotypic data. The new concept of a Genotypic Cutoff Value (GCV) was explored as a counterpart to validate and confirm more traditional MIC distribution analyses used to establish epidemiological cutoff values (ECVs).[1]

The presentations collectively provided insight into the overlap between the human and animal laboratory sectors and how awareness and cooperation can benefit overall health. Slides from this educational workshop can be found **here.**

Reference

[1]   Tyson GH, et al. Establishing genotypic cutoff values to measure antimicrobial resistance in *Salmonella*. *Antimicrob Agents Chemother.* 2017;61(3): pii: e02140-16. PMID 27993845.

What is The Antibiotic Resistance Laboratory Network (ARLN)?

In August 2016, the Centers for Disease Control and Prevention (CDC) launched a significant new effort that will dramatically impact our understanding of the landscape of antimicrobial resistance (AR) in the US. This new venture will provide an avenue to combat persistent and emerging resistance in targeted microorganisms identified as critical AR threats. The new Antibiotic Resistance Laboratory Network, or the ARLN, is the structure for great things to come.

The CDC has responded to the March 2015 **National Strategy for Combating Antibiotic-Resistant Bacteria,** a National Action Plan put forth by the White House that called for "...creation of a regional public health laboratory network." CDC's insightful planning and rapid implementation of a simple yet robust network of laboratories is ambitious and necessary. To allow for faster identification and response to outbreaks, this network provides public health laboratories in all 50 states and 5 cities/territories with funding and a framework to fortify connections with clinical laboratory partners and to start building testing capacity for carbapenem resistant Enterobacteriaceae (CRE) and carbapenem resistant *Pseudomonas aeruginosa* (CRPA) isolates from their jurisdictions; in most cases, this capacity never existed. This effort includes performing susceptibility testing as well as characterizing resistance mechanisms, such as KPC, NDM, and OXA-48-like genes in Enterobacteriaceae and KPC, NDM, and VIM in *P. aeruginosa*.

Adding to this solid base of 55 state/local laboratories, are the seven ARLN regional laboratories. The seven regional laboratories listed on the map accept referral isolates with new or unusual resistance from state laboratories within their region. They have the capacity to perform additional characterization and recognize emerging or novel resistance mechanisms such as *mcr-1* and detect other, difficult-to-treat microorganisms such as *Acinetobacter*. The goal of the regional labs is to support rapid detection of existing and emerging resistance more effectively, by closing the gap between hospital-based laboratory testing and the need for pertinent information to guide infection control and prevention across the spectrum of care. Thus, a responsibility of the regional labs is to perform outbreak detection in the form of rapid rectal swab testing for carbapenemase-producing bacteria. Hospital and other healthcare facilities' infection control personnel now have a resource for laboratory testing that is required to investigate possible outbreaks of carbapenemase-producing (CP) CRE and CRPA. This testing is rapid, taking no longer than 2 days, but mostly same-day, so that infection prevention and control measures can be implemented to stop transmission of CP-CRE or CP-CRPA.
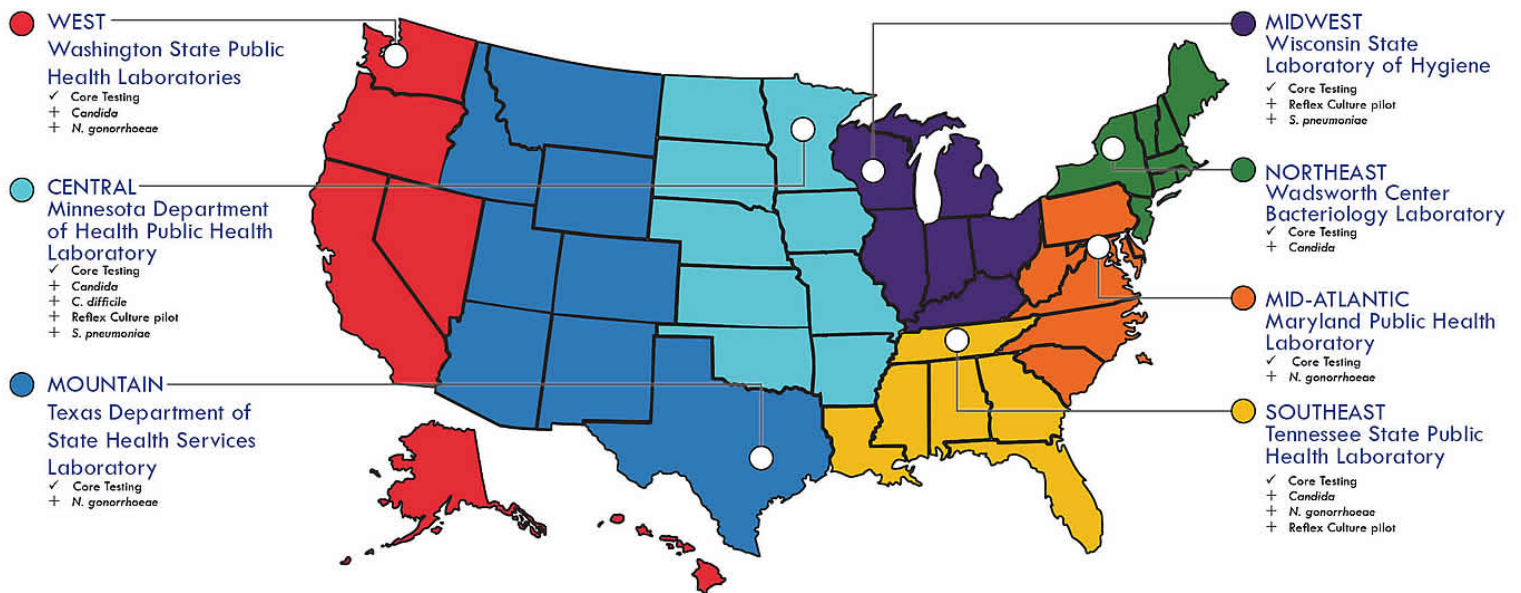
Select regional laboratories perform additional resistance testing of *Neisseria gonorrhoeae*, *Candida* species, and *Streptococcus pneumoniae*. Beginning in August 2017, all regional laboratories will characterize certain *Candida* species such as *C. glabrata* that show unusual resistance patterns (eg, resistance to an echinocandin) and boost efforts to detect and prevent the emergence of *Candida auris*.[1] Also in August 2017, two laboratories will be funded to perform whole genome sequencing to track and detect emergence of drug-resistant *Mycobacterium tuberculosis*.

The ARLN regional laboratories are also tasked with providing consistent and substantial communication, coordination and consultation for the healthcare system within their regions about laboratory testing and AR. They also help CDC to track all pertinent organisms detected, with the promise of impactful pathogen-specific solutions. An important product of this work is the **FDA-CDC AR Isolate Bank.** This repository will continue to grow through the submission of isolates from the ARLN regional laboratories, so that researchers, diagnostic device developers, antimicrobial drug manufacturers, and clinical laboratories will have well-characterized pathogens to support their work of combatting AR. In addition, as the ARLN matures, regional laboratories will provide AR training to state/local ARLN and clinical laboratories.

Finally, it is necessary that the ARLN regional laboratories be nimble, not only to build capacity and expertise for CRE and CRPA, but to be able to turn on a dime to respond to the next "nightmare bacteria" lurking in our health care hallways. Will that be another mechanism of resistance such as *mcr-1,* or an emerging resistant yeast like *Candida auris?* Only time will tell, but with the capacity and expertise that is being brought to the fore, there will be more actionable data, quicker detection of emerging threats leading to faster implementation of control measures, and a growing knowledge of AR, ultimately ensuring a healthier public. More information on ARLN can be found **here.**

What is the Antibiotic Resistance Laboratory Network (ARLN)? (*Continued*)

CDC Antibiotic Resistance Laboratory Network: 7 Regional Labs

WEST
Washington State Public
Health Laboratories
✓ Core Testing
+ Candida
+ N. gonorrhoeae

CENTRAL
Minnesota Department
of Health Public Health
Laboratory
✓ Core Testing
+ Candida
+ C. difficile
+ Reflex Culture pilot
+ S. pneumoniae

MOUNTAIN
Texas Department of
State Health Services
Laboratory
✓ Core Testing
+ N. gonorrhoeae

MIDWEST
Wisconsin State
Laboratory of Hygiene
✓ Core Testing
+ Reflex Culture pilot
+ S. pneumoniae

NORTHEAST
Wadsworth Center
Bacteriology Laboratory
✓ Core Testing
+ Candida

MID-ATLANTIC
Maryland Public Health
Laboratory
✓ Core Testing
+ N. gonorrhoeae

SOUTHEAST
Tennessee State Public
Health Laboratory
✓ Core Testing
+ Candida
+ N. gonorrhoeae
+ Reflex Culture pilot

ARLN consists of:

- Seven regional laboratories that will perform testing for new and unusual resistance threats and perform real-time CRE colonization testing for outbreak response
- 55 state/local public health laboratories that will perform CRE and CRPA testing for mechanisms of resistance
- Clinical laboratories that are contributing isolates of AR interest to state/local and regional ARLN laboratories

How will clinical laboratories benefit from ARLN?

- They will have an accessible ARLN laboratory to which they can send isolates of CRE and CRPA for resistance mechanism testing and will receive results in a timely manner.
- They will have AR experts in their state to consult with AR questions.
- They will have a laboratory which can perform CRE colonization testing to assist infection prevention personnel in their hospitals to investigate potential outbreaks of CRE.
- They will be contributing to and have access to state/regional/national aggregate reports of AR data collected for the organisms of interest (CRE, CRPA, *Candida, S. pneumoniae*, etc.) that are tested in the ARLN laboratories.

Reference

1   Vallabhaneni S, et al. Epidemiology and risk factors for echinocandin nonsusceptible *Candida glabrata* bloodstream infections: data from a large multisite population-based candidemia surveillance program, 2008-2014. *Open Forum Infect Dis.* 2015;2(4):ofv163.

A New Phenotypic Test for Detection of Carbapenemases – The Modified Carbapenem Inactivation Method (mCIM)

Several phenotypic methods for detecting carbapenemase production are described in CLSI M100S 27th Ed, Table 3D. The newest of these is the modified carbapenem inactivation method or mCIM.[1]

When might mCIM be used?

If there is a need to know if a carbapenem resistant Enterobacteriaceae (CRE) isolate is a carbapenemase producer, the mCIM is an option. As a reminder, it is not necessary to perform a test for carbapenemase when reporting patient results using the current CLSI carbapenem breakpoints. However, because genes encoding for carbapenemase production are generally located on highly transmissible plasmids, CRE that are resistant due to this mechanism versus alternative mechanisms (eg, other β-lactamases and porin changes) are often considered a greater threat for transmission between patients. Consequently, a laboratory may be asked to determine if a CRE isolate is a carbapenemase producer for infection control or epidemiological purposes.

How does mCIM work?

When a meropenem disk is placed in a suspension of carbapenemase-producing bacteria and incubated for a few hours, the carbapenem in the disk will be hydrolyzed by the carbapenemase. When the disk is then transferred to a plate that has just been inoculated with a meropenem-susceptible *E. coli* and the plate is incubated overnight, there will be no zone or a very small zone of inhibition around the meropenem disk. In contrast, if the test bacterium is not a carbapenemase producer, the meropenem disk will retain its activity and be available to inhibit growth of *E. coli* as evidenced by a zone of inhibition around the disk.
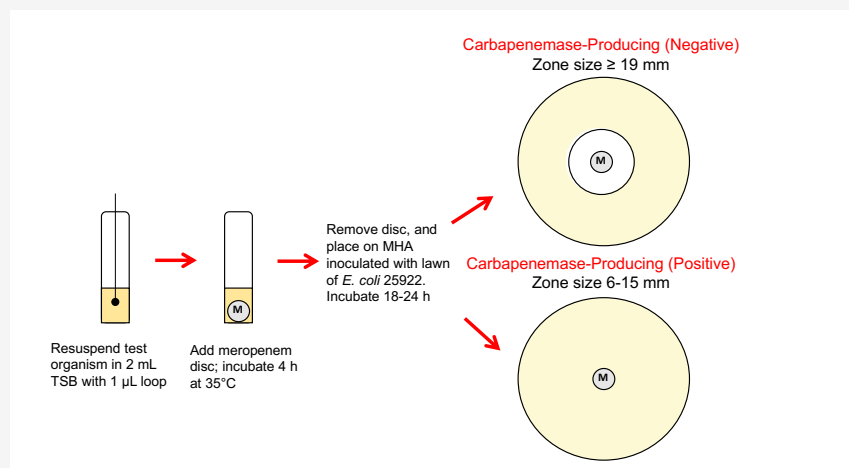
How is mCIM actually performed?

1. A 1-µL loopful of test bacteria from an overnight agar plate is transferred to a tube containing 2 mL of trypticase soy broth (TSB) and the suspension is vortexed. (Figure 1)

2. A standard 10-µg meropenem disk is added to the suspension.

3. The TSB - disk suspension is incubated for 4 hours at 35°C in ambient air.

4. Just prior to completion of the 4 hour incubation cycle, a 0.5 McFarland suspension of *E. coli* ATCC® 25922 is prepared and inoculated onto a MHA plate following the routine disk diffusion procedure as described in CLSI M02.

5. The meropenem disk is removed from the TSB suspension using a 10-µL loop, taking care to remove excess liquid from the disk.

6. The meropenem disk is immediately placed on the MHA plate that has been inoculated with *E. coli* ATCC® 25922.

7. The plate is incubated at 35°C in ambient air.

8. Following overnight incubation, the zone of inhibition around the meropenem disk is measured.

9. Results are interpreted as follows (Figure 2):

    Carbapenemase positive - zone 6-15 mm or presence of colonies within a 16-18 mm zone.

    Carbapenemase negative - zone ≥ 19 mm.

    Indeterminate - zone 16-18 mm (unable to confirm if isolate is or is not a carbapenemase producer)

Figure 1. Overview of mCIM Procedure

Resuspend test organism in 2 mL TSB with 1 µL loop

Add meropenem disc; incubate 4 h at 35°C

Remove disc, and place on MHA inoculated with lawn of *E. coli* 25922. Incubate 18-24 h

Carbapenemase-Producing (Negative)
Zone size ≥ 19 mm

Carbapenemase-Producing (Positive)
Zone size 6-15 mm

A New Phenotypic Test for Detection of Carbapenemases (*Continued*)

What else should we know about mCIM?

The mCIM test is simple to perform with minimal hands-on time (< 5 min per isolate when testing multiple isolates) and uses laboratory supplies that are readily available. In fact, there are several advantages of mCIM over other phenotypic tests previously described for carbapenemase production (see Table). The mCIM has > 99% sensitivity and > 99% specificity for detection of carbapenemase producing Enterobacteriaceae. For additional details on performing mCIM (including QC recommendations), please see M100S 27th Ed.[1] For results on the performance of mCIM as demonstrated in a controlled multicenter study, please see a recent publication.[2]

Does a verification need to be performed prior to implementation of mCIM?

Yes, a limited verification of at least 30 isolates (15 carbapenemase positive, 15 carbapenemase negative) needs to be performed to measure accuracy and precision of the assay in your laboratory. Characterized isolates (known carbapenemase producers/non-producers) from your own institution should be used, if possible. For laboratories lacking these isolates or those desiring a greater variety of carbapenem resistance mechanisms than available in house, a source for well-characterized isolates is included in the "Gram-negative Carbapenemase Detection Panel" from the **FDA-CDC Antimicrobial Resistance Isolate Bank.**

What about use of mCIM for Pseudomonas aeruginosa and Acinetobacter spp.?

The mCIM test has been shown to be reliable for detection of carbapenemase production in *P. aeruginosa* and a slight modification of the above-mentioned procedure will be included for *P. aeruginosa* in M100S in 2018. Unfortunately, the mCIM as described here is not reliable for detecting carbapenemase production in *Acinetobacter baumannii* complex.

mCIM Key Points

- mCIM is a new phenotypic method for detection of carbapenemase producing Enterobacteriaceae and described in detail in CLSI M100S 27th ed.

- mCIM uses readily available laboratory reagents.

- Results from mCIM are available within 24 hours following testing of isolated colonies suspicious for carbapenemase production.

- mCIM is not reliable for testing *Acinetobacter* spp.

References

[1]   CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 27th ed. CLSI document M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

[2]   Pierce VM, et al. The modified carbapenem inactivation method (mCIM) for phenotypic detection of carbapenemase production among Enterobacteriaceae. *J Clin Microbiol*. 2017;Epub Apr 5.

Figure 2. mCIM positive and negative results for carbapenemase production

Zone 6 mm = positive   **Zone 22 mm = negative**

A New Phenotypic Test for Detection of Carbapenemases (*Continued*)

Table. Comparison of Modified Hodge Test, Carba NP, and mCIM

	Modified Hodge Test	Carba NP	mCIM
Reagents	Uses readily available reagents	Requires special reagents, not routinely used in clinical laboratories	Uses readily available reagents
Materials Cost	< $1 per test	$2-10 per test	< $1 per test
Time to Result	24 hours, requires overnight incubation	2 hours	24 hours, requires overnight incubation
Interpretation	Subjective interpretation	Subjective interpretation	Subjective interpretation but less problematic
Strengths	Simple to perform	Rapid results Detects carbapenemases in Enterobacteriaceae, *P. aeruginosa*, and *Acinetobacter*	Good sensitivity for detection of Ambler class A, B and D carbapenemases in Enterobacteriaceae
Limitations	False positives with some *Enterobacter* spp. possessing AmpC enzymes and porin alterations False negatives with NDM-1 carbapenemases	Poor sensitivity for detection of OXA-48 carbapenemases	Poor sensitivity and specificity for carbapenemases in *Acinetobacter*

Carbapenems and Enterobacteriaceae: *What is the difference between CRE vs CP-CRE vs non-CP-CRE?*

Discussing "carbapenem resistant Enterobacteriaceae" (CRE) can be confusing, especially for laboratories that are being asked to identify the resistance mechanism. When determining if a carbapenem might be a therapeutic option for a patient, it is not necessary to know the resistance mechanism, and reporting results from disk diffusion or MIC tests using current CLSI carbapenem breakpoints is sufficient. However, for epidemiological or infection control purposes, laboratories may be asked to identify the type of carbapenemase produced by a CRE isolate. See CLSI M100 Tables 3A-3D[1] and also a recent publication[2] that address these issues. The table below (modified from the publication) summarizes terminology including the acronyms used to describe carbapenem resistance mechanisms that can be applied to *E. coli, Klebsiella* spp. and other members of the family Enterobacteriaceae.

Organism	Acronyms	Definition	Laboratory Identification	Mechanisms of Carbapenem Resistance
Carbapenem Resistant Enterobacteriaceae	**CRE**	Encompasses organisms from the family Enterobacteriaceae that are resistant to carbapenems regardless of the mechanism.	"R" result from a carbapenem disk diffusion or MIC test interpreted with current CLSI breakpoints.[a]	All carbapenem resistance mechanisms (i.e., production of a carbapenemase OR production of an AmpC or ESBL with altered permeability due to porin mutations or efflux pumps)
Carbapenemase Producing Enterobacteriaceae	**CP-CRE**	Encompasses organisms from the family Enterobacteriaceae that are resistant to carbapenems due to the production of a carbapenemase enzyme.	Positive result from a carbapenemase test.[b]	Production of a carbapenemase (i.e., KPC, NDM, OXA-48-like)
Non Carbapenemase Producing Carbapenem Resistant Enterobacteriaceae	**Non CP-CRE**	Encompasses organisms from the family Enterobacteriaceae that are resistant to carbapenems due to mechanisms other than carbapenemase production.	"R" result from a disk diffusion or MIC test interpreted with current CLSI breakpoints AND negative result for carbapenemase.	Production of an AmpC or ESBL with altered permeability due to porin mutations or efflux pumps.

[a]   "R" to ertapenem, doripenem, imipenem, and/or meropenem

[b]   Carbapenemase tests include: Modified Hodge Test, carba NP, mCIM, molecular assays.

References

[1]   CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 27th ed. CLSI document M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

[2]   Gniadek TJ, et al. Carbapenem-resistant non-glucose-fermenting gram-negative bacilli: the missing piece to the puzzle. *J Clin Microbiol.* 2017;54:1700-10.

Newer Developments in Antifungal Susceptibility Testing - Species-Specific Breakpoints for *Candida* spp.

In November 2016, the ORWG presented the webinar "Practical Recommendations for Antifungal Susceptibility Testing and Reporting in Clinical Laboratories: New Breakpoints, New Drugs, New Guidelines." Below are some highlights from the program that can be accessed through the CLSI webinar archives **here,** under Microbiology webinars.

In 2012, the CLSI Subcommittee on Antifungal Susceptibility Testing published species-specific clinical breakpoints in the M27-S4 supplement.[1] The new breakpoints replaced those previously listed in M27-S3[2] that included all species in the *Candida* genus (eg, did not differentiate among species). See Table below. Additionally, with M27-S4 the "non-susceptible" interpretive category previously assigned to the echinocandins has been eliminated. Finally, M27-S4 no longer lists clinical breakpoints for itraconazole which has been assigned epidemiological cutoff values (ECVs) that are listed in M59 1st Ed.[3] There are currently insufficient data to set breakpoints or ECVs for flucytosine.

| Antifungal Agent	Obsolete M27-S3*	Current M27-S4**
Echinocandins		
anidulafungin	X	X
caspofungin	X	X
micafungin	X	X
Azoles		
fluconazole	X	X
itraconazole	X	
posaconazole		
voriconazole	X	X
Other		
flucytosine	X	

*   One set of breakpoints for all *Candida* spp. grouped together
** Species-specific breakpoints for *Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis, Candida tropicalis* and for *Candida guilliermondii* (echinocandin breakpoints only)

Increasing awareness that MIC distributions for *Candida* species versus an antifungal agent can be different among species has contributed to the assignment of species-specific clinical breakpoints. This is illustrated in the Figure below, where caspofungin MIC distributions are displayed for all *Candida* spp. isolates grouped together, and for five species listed separately, collected worldwide during 2014 and 2015 as part of the SENTRY Antifungal Surveillance Program.[4] The distributions are remarkably distinct for each of the five species and it would be difficult to establish a single breakpoint that could be applied for all *Candida* species. For example, *C. albicans* shows a modal MIC value of 0.015 µg/mL and *C. parapsilosis* displays a modal MIC value of 0.25 µg/mL. These differences reflect the MICs for the wild type strains (strains without an acquired resistance mechanism).

Newer Developments in Antifungal Susceptibility Testing - Species-Specific Breakpoints for *Candida* spp. (*Continued*)

Figure. Caspofungin MIC Distributions Displayed for All *Candida* Spp. and Individually for Five Different Species of *Candida*.*
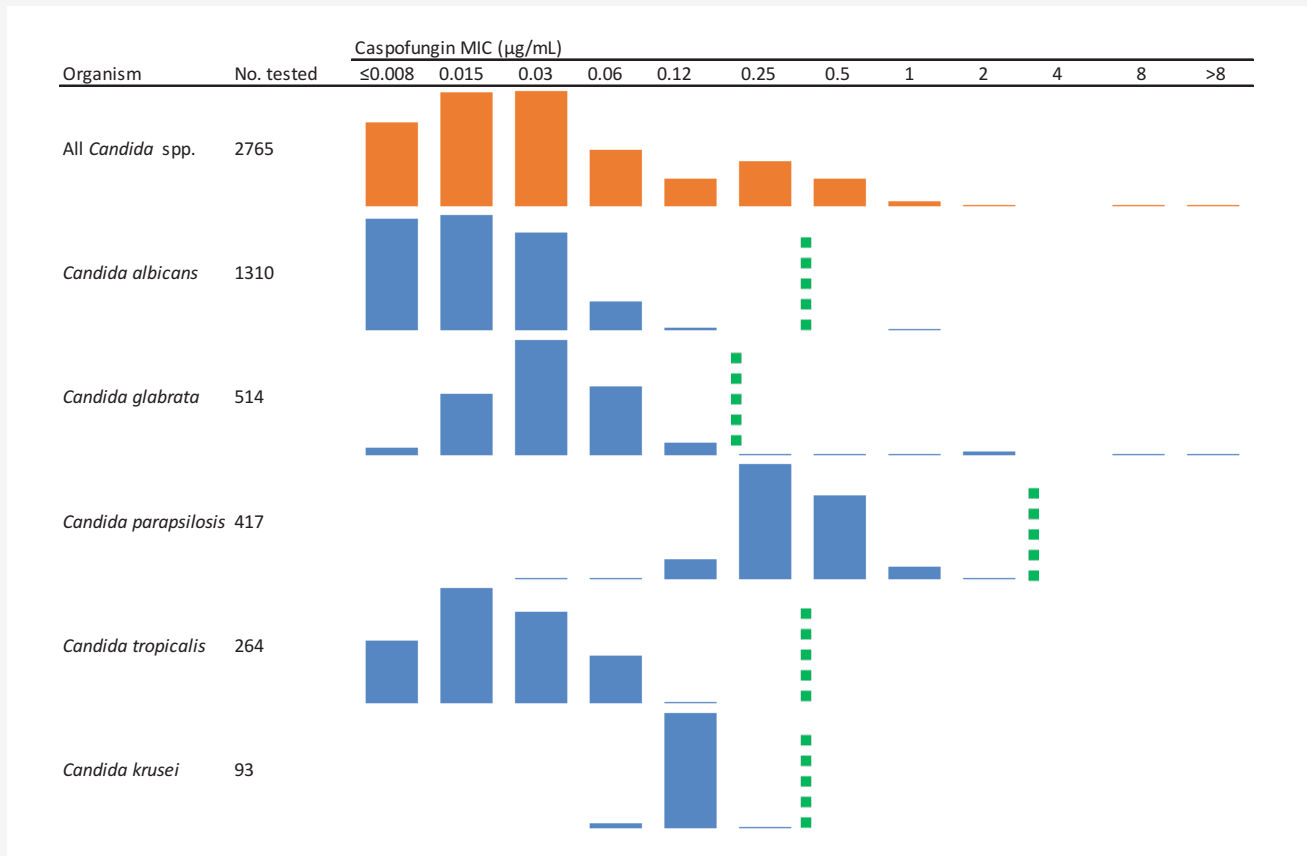
* The new susceptible breakpoints are shown as green vertical dotted lines.

Use of a single breakpoint for all *Candida* spp. could not always differentiate isolates with and without resistance mechanisms as these might be expressed differently among species. For example, *C. glabrata* isolates displaying mutations at the target site of caspofungin activity exhibit caspofungin MIC values as low as 0.25 μg/mL and it is unlikely that these isolates would respond to caspofungin therapy. However, if one were to use breakpoints from M27-S3, many *C. glabrata* isolates with resistance mechanisms would be categorized as susceptible to caspofungin (MIC ≤ 2 μg/ml was susceptible). The current revised breakpoints (μg/ml) for caspofungin and *C. glabrata* are: ≤ 0.12 susceptible; 0.25 intermediate; ≥ 0.5 resistant.

The MIC distributions for anidulafungin, micafungin and the azoles are similarly distinct among the five *Candida* species, and the application of a single breakpoint for *Candida* spp. with these antifungal agents could also impact the accurate detection of resistance.

MIC distributions are not the only factor to consider when creating clinical breakpoints as PK/PD and clinical outcome data must also be evaluated. The Subcommittee on Antifungal Susceptibility Testing studied the most recent clinical outcome data, resistance mutation data (eg, *fks1/fks2* mutations associated with echinocandin resistance), and PK/PD data as part of the review of the antifungal breakpoints. For instance, published literature has shown clinical treatment failure to be associated with *fks1/fks2* mutations for *Candida* isolates for which the caspofungin MICs measured below the M27-S3 susceptible breakpoint.[5] Additionally, new PK/PD data demonstrated that the highest MICs for which the PD target would be achieved is approximately 0.25 μg/mL for anidulafungin, 1 μg/mL for caspofungin, and 0.5 μg/mL for micafungin using current dosing regimens, which are well below the former M27-S3 breakpoint of 2 μg/mL that was assigned to all echinocandins and all *Candida* spp.

Newer Developments in Antifungal Susceptibility Testing - Species-Specific Breakpoints for *Candida* spp. (*Continued*)

Despite the rationale behind the new breakpoints, according to supplemental questions from the 2016 College of American Pathologists (CAP) Fungal proficiency testing survey, approximately one-third of subscribing laboratories still used the obsolete M27-S3 guidelines. While a minority (25/107; 23%) of these laboratories stated using the old M27-S3 guidelines in conjunction with the current M27-S4 guidelines, the majority of these laboratories (82/107, 77%) reported using solely the old guidelines. It is important for clinical laboratories to discontinue using M27-S3.

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The 21st Century Cures Act and the Future of AST

Current Challenges:

Over the past few years, clinical microbiologists and clinicians have all encountered frustrating scenarios when it comes to antimicrobial susceptibility testing (AST) using commercial, automated devices.

Challenge	Examples
Inexorable delays between the time a breakpoint is updated by CLSI and the time automated ASTs are updated and FDA-cleared with new breakpoint	Enterobacteriaceae and carbapenems, cephems (updated in 2010, however many systems still do not have clearance)
Newer drugs unavailable on commercial antimicrobial susceptibility test systems most commonly used in clinical laboratories	Ceftazidime-avibactam Ceftolozane-tazobactam
Inability to test drugs on automated AST systems against organisms that do not have a clinical indication in the drug label but are clinically used for that organism	Daptomycin and *E. faecium* Meropenem and *Acinetobacter* spp.
Lack of any FDA-cleared tests for antimicrobial agents that do not have FDA breakpoints	Colistin

Many of these challenges are in part related to the fact that in the United States, two organizations set breakpoints: FDA and CLSI. FDA breakpoints are listed in the drug prescribing information (package insert or drug label), which can be found at **www.dailymed.nlm.nih.gov**, whereas CLSI breakpoints are listed in **CLSI's M100S document.**

Per US law, FDA can only approve an AST system using FDA breakpoints, and *only for those organisms listed in the drug labeling to have activity both* in vitro *and in clinical infections*. This latter piece is an important consideration, as FDA may have assigned breakpoints for the Enterobacteriaceae, but if no or few infections caused by *Enterobacter aerogenes* were encountered during the clinical trial (eg, as is the case for ceftazidime-avibactam), an AST system cannot be cleared by FDA for this particular species. These restrictions have led to the challenges listed in the table and much frustration for diagnostic manufacturers who cannot market tests that are analytically precise, have a CLSI (or even FDA) breakpoint and are critical to patient care.

The Good News!

The 21st Century Cures Act, which was passed in November 2016, and was signed into law by President Obama on December 13th, 2016, has the potential to provide solutions to these challenges (Sec. 3044). In particular, Sec 3044 of the act includes updates to Sec. 511 of the Federal Food, Drug and Cosmetic Act (21 USC 360a), to allow FDA to recognize breakpoints established by breakpoint-setting organizations, like CLSI, providing they uphold certain standards to mitigate potential conflicts of interest and maintain transparency in decision making processes. Furthermore, the intent is for all breakpoints to be listed on a website established by FDA, and breakpoints will be removed from drug prescribing information (eg, package insert or drug label). Diagnostics manufacturers can then submit data to FDA to have their AST system cleared using breakpoints listed on the new FDA website.

Importantly, this allows a streamlined process for FDA to recognize breakpoints established by CLSI (or other breakpoint-setting organizations), as this website must be updated at minimum every 6 months. Furthermore, it will provide more transparency regarding which breakpoints must be used by diagnostic manufacturers for FDA clearance. Manufacturers will be able to request FDA clearance for drugs that currently lack FDA breakpoints (eg, colistin). Finally, because the breakpoints will no longer be associated with the 'indications for use' listed in the drug package insert, many are hopeful that ASTs for drug/bug combinations without an FDA indication for use could be cleared by FDA.

The Timeline:

The intended timeline for these changes is rapid: FDA must establish the breakpoint website by November 2017. The drug manufacturers will have until November 2018 to delete breakpoints from their drug prescribing information. While promising, there remain some unanswered questions, including which breakpoint setting organization(s) will be recognized by FDA, and how quickly diagnostic manufacturers will be able to update their systems. If the delay between FDA updating Enterobacteriaceae carbapenem and cephem breakpoints and the diagnostic manufacturers updating these on their AST systems is any indication, it may be several years before these changes will have a positive impact on the clinical laboratory. On the flip side, many manufacturers of AST systems have a global presence and may be able to rapidly assemble and/or supplement the data that was submitted to non-US agencies for clearance in other countries, for submission to FDA.

The 21st Century Cures Act and the Future of AST (*Continued*)

...to find current FDA Breakpoints and clinical indications listed in "Prescribing Information" (aka "Drug Label" or "Package Insert"), proceed as follows:

1. Go to: **https://dailymed.nlm.nih.gov/dailymed/.**

2. Enter a drug name and you will be directed towards one or more links to the Prescribing Information for the drug. If your search doesn't yield results, try using the drug's trade name. There is a separate link for each manufacturer of the drug. This means many links for generic drugs (ceftriaxone has 4 pages of links!). Select any of these to access the drug's Prescribing Information. Eg, daptomycin.

3. Once inside the "Prescribing Information" document, search for "Clinical Pharmacology" section and "Microbiology" Subsection. Scroll through this subsection to find breakpoints and QC ranges.

4. Under "Microbiology," check out the "Activity" subsection where it states something like "Drug X has been shown to be active against most isolates of the following microorganisms both ***in vitro* and in clinical infections.** The drug manufacturer has clinical data supporting positive outcomes for these species only." For daptomycin and enterococci, you will see *Enterococcus faecalis* (VSE only) listed. This is sometimes called the "A" list.

5. And, then check out a second list of organisms, introduced with something like "at least 90% of the following bacteria exhibit an ***in vitro*** MIC less than or equal to the susceptible breakpoint for Drug X against isolates of similar genus or organism group. However, the efficacy of Drug X in treating clinical infections due to these bacteria has not been established in adequate and well-controlled clinical trials." For daptomycin and enterococci, you will see *E. faecalis* (VRE) and *Enterococcus faecium* listed. This is sometimes called the "B" list.

Commercial manufacturers can currently seek FDA approval for only organisms in the "A" list for their AST!

Notes:

1. For most antimicrobial agents, there are multiple manufacturers of the drug and there are separate "Prescribing Information" documents for each product.

2. Some manufacturers of these antimicrobial agents have updated their breakpoints in the "Prescribing Information" and others have not. FDA keeps track of those which have updated breakpoints **here.**

3. Many patients are prescribed drugs for organisms that are not on list A or list B ("off label"). Some estimate up to 40% of critically ill patients are prescribed antibiotics off-label, and clinicians rely on AST results to help guide the decision to use these antibiotics.

Hot Topic

Class D (OXA) Carbapenemases: the Achilles Heel of Carbapenemase Detection Tests

Carbapenems play an essential role in healthcare. Due to their broad activity and relative stability against β-lactamases, these agents are often reserved for treatment of multi-drug resistant organisms in critically ill patients. A primary mechanism of resistance to carbapenems in gram-negative bacteria is production of carbapenemases—enzymes that confer resistance by hydrolyzing the essential four-membered β-lactam ring of the carbapenem molecules, rendering them inactive. Carbapenemases belong to one of three groups based on their molecular structure: Ambler class A, B, and D.[1,2] Class A and D enzymes have serine residues at their active sites, while class B enzymes require zinc ions for activity. Historically, class D enzymes were thought to be mainly restricted to members of the genus *Acinetobacter*; however, it is now apparent that these enzymes are a significant cause of carbapenem resistance in other genera, including members of the family Enterobacteriaceae.

Class D (OXA) Carbapenemases: the Achilles Heel of Carbapenemase Detection Tests (*Continued*)

The first class D enzymes described were shown to confer resistance to penicillin and oxacillin (hence the prefix OXA). Subsequently in the 1980s, OXA enzymes that hydrolyze carbapenems were reported in *Acinetobacter* species, especially *Acinetobacter baumannii*. In the early 2000s, a novel enzyme, termed OXA-48, was isolated from a carbapenem resistant *Klebsiella pneumoniae* strain obtained from a patient in Istanbul, Turkey.[3] OXA-48 and its variants (collectively often termed OXA-48-like β-lactamases) found in Enterobacteriaceae are listed in Table 1. These β-lactamases hydrolyze narrow-spectrum β-lactams (aminopenicillins and ureidopenicillins) and weakly hydrolyze carbapenems, but spare extended-spectrum cephalosporins (eg, ceftriaxone, ceftazidime, cefepime) and are not inhibited by clavulanic acid or the metal ion chelator EDTA. However, in the presence of permeability defects or other β-lactamases, OXA-48-like β-lactamases can impart high levels of carbapenem resistance.[4]

OXA-48 like β-lactamases are now widespread in Enterobacteriaceae, including *K. pneumoniae* and *E. coli*. They are endemic in many European and North African countries. Sporadic outbreaks/occurrences of clustered cases have been documented in the US,[1] but given their weak activity against carbapenems, which sometimes results in MICs or zone size values in the susceptible range, their prevalence in the US is probably underestimated.

It can be difficult to identify bacteria harboring OXA-48-like β-lactamases in isolates of Enterobacteriaceae when tested with antimicrobial susceptibility testing systems routinely used in clinical laboratories. Current CLSI-recommended carbapenemase detection tests including the Modified Hodge test (MHT), the Carba NP and the modified carbapenem inactivation method (mCIM), that are based on the hydrolysis of a carbapenem can be falsely negative for isolates that produce OXA-48-like β-lactamases (Table 2).[6-11] In contrast, nucleic acid-based assays targeting OXA-48-like genes are generally reliable.[12] However, some commercial or laboratory-developed molecular assays may not detect all OXA-48-like β-lactamase genes due to sequence variations between them.[13] Thus, the sensitivity of nucleic acid-based assays could be a shortcoming in the setting of changing epidemiology or emergence of novel enzymes. Finally, with the use of "direct from positive blood culture" assays, laboratories may be faced with the detection of an OXA-48-like β-lactamase gene in an isolate belonging to the family Enterobacteriaceae without phenotypic resistance to the carbapenems. In these situations, CLSI recommends that clinical laboratories utilizing current Enterobacteriaceae carbapenem breakpoints should interpret the agents as they test (i.e., if the MIC value corresponds to a susceptible interpretation, report as such).

Plasmid-mediated Ambler class D OXA-48-like β-lactamases have arisen as a significant cause of transmissible carbapenem resistance in members of the family Enterobacteriaceae and can be difficult to detect using routine antimicrobial susceptibility tests and phenotypic tests for carbapenemases. If an isolate demonstrates a susceptibility profile of resistance to narrow-spectrum β-lactams (eg, ampicillin, cefazolin) and shows intermediate or resistant results for carbapenems (using current CLSI breakpoints) but is susceptible to extended-spectrum cephalosporins (eg, ceftriaxone, ceftazidime, cefepime), performance of a molecular or phenotypic assay for carbapenemase should be considered. However, the limitations of phenotypic assays in detecting OXA-48 like β-lactamases must be taken into consideration. Optimally, performance of a molecular assay for OXA-48-like β-lactamases could be performed, particularly if there is any evidence of a potential outbreak. Subsequently, if an isolate is confirmed as a carbapenemase producer using a phenotypic assay or positive for an OXA-48-like gene using a molecular assay, the laboratory should follow their protocol for notification of the Infection Prevention team to help avert transmission of significant resistance genes.

Table 1. OXA-48-like β-lactamases found in Enterobacteriaceae

OXA-48	OXA-244
OXA 162	OXA-245
*OXA-163	OXA-370
OXA-181	*OXA-405
OXA-204	OXA-436
OXA-232	OXA-484

* Lack significant hydrolytic activity against carbapenems but have enhanced activity against extended-spectrum cephalosporins as compared to other OXA-48-like β-lactamases.

Adapted from references 2 and 5.

Class D (OXA) Carbapenemases: the Achilles Heel of Carbapenemase Detection Tests (*Continued*)

Table 2. Diagnostic sensitivity of CLSI-recommended phenotypic carbapenemase detection tests for Class A, B, and D enzymes

Ambler Class (Carbapenemase)	Diagnostic Sensitivity[1]		
	MHT	Carba NP	mCIM
A (KPC)	87.5 to 98	84 to 100	98 to 100
B (MBL)	12 to 94	94 to 100	98 to 100
D (OXA-48-like)	93 to 100	38.5 to 86	85 to 100

[1]Extracted from references 6-12

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In Memoriam – Dr. Paul Schreckenberger

The Clinical Microbiology community mourns the loss of an extraordinary legend in the sudden passing of Paul Charles Schreckenberger, PhD D(ABMM) F(AAM), on November 29, 2016. His significant contributions made him a giant in the field of microbiology.

Paul received a B.S. from the State University of New York at Buffalo in 1970 and an M.S. from the University of Minnesota in 1974. He relocated to Chicago and became the supervisor of the Bacteriology Laboratory at the University of Illinois (UIC) hospital in 1977 and completed his PhD at UIC in 1989. He served as the Director of the UIC Clinical Microbiology Laboratory until he "retired" in 2005 and subsequently became the Director of the Clinical Microbiology and Molecular Pathology Laboratory and a Professor of Pathology at Loyola University Medical Center where he worked until the time of his passing.

During Paul's career that spanned over 40 years, his contributions were vast. He was instrumental in developing a new lab identification system for non-fermenting gram-negative bacilli that is still in use today. He was the lead author of Koneman's Color Atlas and Textbook of Diagnostic Microbiology and had just finished the 7th edition in 2016, an accomplishment in which he took great pride. He authored numerous publications, abstracts and posters and was a leading contributor to a study which contested the long held belief that urine is a sterile body fluid. Paul was a dynamic speaker and a sought after lecturer, worldwide.

Paul served on many advisory boards and committees, all who looked to him for future direction in clinical microbiology. He was a dedicated volunteer on the CLSI Subcommittee for AST for many years, often speaking out to ensure the role of the bench tech was taken into consideration in AST SC decisions. In 2008, he was elected to Fellowship in the American Academy of Microbiology. In 2010, he was awarded the Illinois Society for Microbiology Pasteur Award in recognition for having made outstanding contributions in the field of microbiology. He was awarded Teacher of the Year three times by the Loyola pathology residents.

Dr. Paul Schreckenberger

Paul was a passionate teacher, a mentor, a boss, a respected researcher, a leader, a friend and a compassionate human being. His interests were unlimited and he would challenge anyone to a lively discussion about history, politics, music (he played the guitar and sang!) and so much more. He enjoyed sports and got to see his beloved Chicago Cubs win the 2016 World Series.

Paul will always be remembered as leading the charge and pushing the envelope to improve clinical microbiology. He was so proud of his lab and the clinical microbiology profession, and his contributions to the field and impact on patient's lives are immeasurable. Paul's spirit will live on in everyone whose lives he touched. He will be greatly missed. Paul is survived by his wife Ann, his son Dr. Adam Schreckenberger, and the late Laura and Scott.

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