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CLSI

CLSI Subcommittee on Antimicrobial Susceptibility Testing

CLSI AST News Update

The CLSI AST **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 to find information about the CLSI AST Subcommittee proceedings.

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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**.

The CLSI AST Subcommittee welcomes your suggestions about any aspect of CLSI documents, educational materials, or this newsletter. Please contact **marketing@clsi.org**.

Inside This Issue:

Free Webinars

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

Interested in becoming a CLSI volunteer? Learn more here.

If you are planning to attend the American Society for Microbiology's (ASM) Microbe 2017 meeting (June 1–5 in New Orleans), check out our "Meet the Experts" session (June 2 at 7:30 AM) on "Volunteer Opportunities With CLSI to Address Antimicrobial Resistance." Two active volunteers from the CLSI AST Subcommittee will explain how they got involved with this committee and how you can get involved, too!

CLSI AST Subcommittee shares ideas with other "like" organizations

Many professional organizations share common goals with those of the CLSI AST Subcommittee. In order for CLSI and other organizations to mutually benefit from ideas and processes related to these goals, the CLSI AST Subcommittee has established formal liaisons with several professional organizations. These include ASM, the College of American Pathologists, Infectious Diseases Society of America, Pediatric Infectious Diseases Society, Society for Healthcare Epidemiology of America, and Society for Infectious Diseases Pharmacists. In addition, representatives from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Susceptibility Testing Manufacturers Association contribute to the CLSI AST Subcommittee processes. Representatives with expertise in antimicrobials from these organizations attend and participate in CLSI AST Subcommittee meetings and aid in dissemination of information regarding CLSI decisions and AST issues.

ASM/CLSI AST Webinar Series—A Complement to the 14th Annual CLSI/APHL AST Update Webinar

ASM and CLSI will jointly sponsor a program entitled "A Comprehensive Course in Antimicrobial Susceptibility Testing," which is geared toward bench-level technologists. This program will consist of a series of approximately 15 webinars, and is scheduled to begin in January 2017 and continue through June 2017.

The series will be divided into three parts. Part 1 (basic level) will highlight fundamentals of susceptibility testing and reporting. Parts 2 and 3 (intermediate level) will cover resistance mechanisms; testing for resistance among various organism groups; and susceptibility testing of fungi, mycobacteria, etc. During Parts 2 and 3, the value of AST reports for antimicrobial stewardship and infection control will be covered. It will not be necessary to sign up for the entire webinar series and laboratories can select particular topics that appeal to them. For details on the program, visit: **https://www.pathlms.com/asm/courses/3662**.

Recently archived CLSI webinars can be accessed on demand. Learn more about program availability. Click here.

Recent archived webinar topics include:

- ***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***
- ***Practical Recommendations for Antifungal Susceptibility Testing and Reporting in Clinical Laboratories: New Drugs, New Breakpoints, New Guidelines***

Upcoming Webinar

14th Annual CLSI/APHL Webinar—This Popular Program Is Back Again!

Wednesday, February 1, 2017
or Thursday, February 2, 2017

CLSI/APHL 2017 AST Update Webinar

Each year, CLSI updates standards for AST. It is imperative for clinical laboratories to incorporate the new recommendations into routine practice to optimize detection and reporting of antimicrobial resistance. CLSI has partnered with the Association of Public Health Laboratories (APHL) for the past 14 years to hold annual webinars that inform users of the important changes in the document.

- Learn about the changes in the NEW M100S, 27th Edition tables ("The CLSI AST Tables") as well as practical tips to help you easily comprehend and implement the new recommendations into your protocols, as appropriate.
- Receive a self-study tool that you and your staff can access, which is designed to reinforce materials presented during the webinar.
- Content is uniquely designed to complement the new multipart ASM/CLSI webinar.

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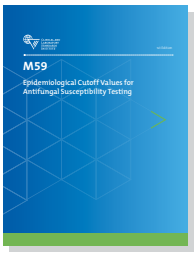
For more information on CLSI webinars, click here.

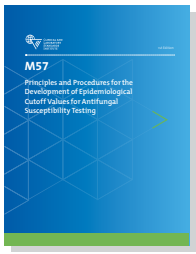
New CLSI Antifungal Susceptibility Testing Documents Published in 2016!

Two brand new documents that focus on antifungal susceptibility testing and use of epidemiological cutoff values (ECVs) are now available. CLSI is using the ECV concept for both fungi and bacteria, and these new documents explain ECVs and how to use them for fungi.



M59—Epidemiological Cutoff Values for Antifungal Susceptibility Testing, 1st Edition

- **Introduces ECVs for various *Candida* spp. for which no clinical breakpoints exist, such as:**
  - Amphotericin B for a variety of *Candida* spp.
  - Itraconazole for *C. glabrata, C. krusei, C. lusitaniae,* and *C. tropicalis*
  - Several agents for *C. lusitaniae* and *C. dubliniensis*
- **Recommends ECVs for various *Aspergillus* spp., such as:**
  - Voriconazole for *A. flavus, A. fumigatus, A. niger,* and *A. terreus*
- **Offers ECVs for various *Candida* spp. for which clinical breakpoints already exist**
  - Used for the purpose of monitoring emergence of resistance



M57—Principles and Procedures for the Development of Epidemiological Cutoff Values for Antifungal Susceptibility Testing, 1st Edition

- **Lists criteria for development of ECVs**
- **Defines an ECV**
- **Provides example reports of how to explain an ECV**
- **Specifies when an ECV should and should not be used**

Those performing antifungal testing will likely find these documents useful as companions to the following:

- ***M27—Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts***
- ***M27S—Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Informational Supplement***
- ***M38—Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi***

Check out material from 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. Past workshops included topics such as Pharmacokinetics/Pharmacodynamics (PK-PD), AST Devices, Biofilms, Antibiotic Stewardship, Antibiotic Surveillance, New Drugs, and Molecular Methods.

The June 2016 workshop, "'Unusual Suspects' – Resistance Concerns and Susceptibility Testing Among Less Common, but Noteworthy Bacteria," included discussions and case studies for some of the less frequently encountered, but still important, bacteria such as *Bacteroides fragilis* group, nontuberculous mycobacteria, *Bacillus, Corynebacterium, Campylobacter,* and *Lactococcus* spp., and the role of AST in treating infections caused by these "unusual suspects." In fact, you might find answers to some questions you have about AST of these organisms within the materials posted for this session, which can be accessed **here**.

The upcoming January 2017 educational workshop topic is "One Health – One Medicine: Linking Human, Animal, and Environmental Health." We have lined up four excellent speakers who have much experience with human and animal pathogens. This workshop will highlight the interplay of human and animal bacteria, and the critical role of AST in monitoring resistance among these bacteria for treatment and other purposes. Please check out details **here**.

Suggestions for future educational workshop topics are always welcome and can be submitted to any of the ORWG members.

Presentations from the June 2016 workshop and past workshops can be found **here**.

Future CLSI AST Subcommittee Meetings!

January 11–17, 2017 – Tempe, Arizona, USA
June 22–27, 2017 – Philadelphia, Pennsylvania, USA

Do you need help with verification of your AST system?

The ORWG presented a webinar entitled "Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems" on August 17, 2016. This webinar is now available on demand for a fee **here**.

Questions that surfaced subsequent to the webinar and answers provided by the presenters can be found **here**. For example, if you have questions about selecting isolates for your verification, and when it might be necessary to calculate essential agreement in addition to categoric agreement, please review the Q&A. The ORWG is also providing a protocol **template** for verifying the revised ertapenem, imipenem, and meropenem breakpoints for the *Enterobacteriaceae* and a **spreadsheet** for recording results.

For a variety of reasons, not all commercial AST systems are cleared by the US Food and Drug Administration (FDA) to report current CLSI-recommended ertapenem, imipenem, and meropenem breakpoints for *Enterobacteriaceae*. Our protocol provides detailed guidance to help you convert to lowered breakpoints. If you have not yet implemented these revised interpretations, the ORWG urges you to consider doing so in order to best detect carbapenem-resistant *Enterobacteriaceae* that are being noted with increasing frequency in the United States.

What's Being Done to Address Current Challenges for Clinical Laboratories With Commercial Antimicrobial Susceptibility Tests?

We all know that antimicrobial resistance is a critical public health dilemma and multiple national initiatives are addressing this issue.[1] As a result, new antimicrobial agents have achieved FDA approval in the past five years. In addition, both CLSI and FDA have updated clinical breakpoints for several β-lactams and the *Enterobacteriaceae, Acinetobacter* spp., and *Pseudomonas aeruginosa* to better predict clinical resistance to these agents. But despite this progress, there remain serious challenges for the clinical laboratory[1]:

- Significant (1–5 years) delays between approval of antimicrobial agents for human use by the FDA and FDA clearance of commercial antimicrobial susceptibility tests (cASTs) for these agents
- Significant (3–6+ years) delays between publication of revised breakpoints for older antimicrobial agents and FDA clearance of cASTs with these breakpoints
- Enhanced FDA regulation of cASTs to allow testing of only organisms that are clinically indicated in the drug package insert (ie, shown to be active *in vivo* during clinical trials)

Despite these challenges, there is some good news! In September 2016, the FDA hosted a workshop on issues associated with cASTs, focusing primarily on the lack of tests for new antimicrobial agents. Clinicians, laboratorians, and representatives from the diagnostic manufacturers, pharmaceutical industry, FDA, and the Centers for Disease Control and Prevention (CDC) came together to identify the roadblocks to coordinated clearance of cASTs at the time new antimicrobial agents are approved for clinical use by the FDA. The FDA has recently developed draft guidance to assist diagnostic

manufacturers and pharmaceutical companies with this effort, which can be found **here**.

Another positive development involves the recent passage in the United States of the 21st Century Cures Act, which includes a provision to remove breakpoints from drug labeling. This may allow FDA clearance of cASTs for organisms that were not specifically evaluated during human clinical trials. Recognizing the need for improved capacity of laboratories to test newer antimicrobial agents, the CDC has established the **Antibiotic Resistance Laboratory Network**.

CLSI has been keeping a close eye on these developments and will discuss them at the January 2017 AST Subcommittee meeting. We are optimistic that with multiple stakeholders working together, there will soon be solutions to some of these challenges facing those working in clinical laboratories and other health care–related disciplines.

[1] Humphries RM, Hindler JA. Emerging resistance, new antimicrobial agents, but no tests! The challenge of antimicrobial susceptibility testing in the current US regulatory landscape. *Clin Infect Dis*. 2016;63(1):83-88.

Special Considerations for Susceptibility Testing of *Streptococcus agalactiae* (Group B *Streptococcus*)

Here is a brief overview of antimicrobial susceptibility testing and reporting for S. *agalactiae*, or "Group B Strep" (GBS). Testing and reporting of antimicrobials differ based on whether GBS are isolated from a **vaginal-rectal source** vs a non-vaginal-rectal source.

When should GBS be tested for susceptibility?

- Test GBS isolates from vaginal-rectal sources for susceptibility upon request from clinicians and/or when penicillin allergy is noted.
- Do not test GBS isolates from vaginal-rectal sources routinely for susceptibility to the penicillins and vancomycin (see below).
- For GBS from sources other than vaginal-rectal, as noted in CLSI M100S, 26th Edition, Table 2H-1, Comment 4, "susceptibility testing of penicillins and other β-lactams approved by the FDA for treatment of β-hemolytic streptococcal infections need not be performed routinely because nonsusceptible isolates are extremely rare in any β-hemolytic streptococcus and have not been reported for *S. pyogenes*."

Which antimicrobials should be tested for GBS?

The CLSI M100S 26th edition (Table 1B) lists the following for *Streptococcus* spp. β-hemolytic group: (**NOTE:** The Table 1B CLSI listing refers to GBS in general and does not provide specific guidance for GBS from vaginal-rectal sources.)

- "Group A, Primary Test and Report": clindamycin, erythromycin, and penicillin or ampicillin.
- "Group B, Optional, Primary Test and Report Selectively": vancomycin and *one* of the following: cefepime or cefotaxime, or ceftriaxone.
  - Note that GBS are also predictably susceptible to vancomycin.
- Additional antimicrobials with interpretive breakpoints that can be selectively tested and reported include the lipopeptides (eg, daptomycin), the lipoglycopeptides (eg, oritavancin and telavancin), the oxazolidinones (eg, linezolid and tedizolid), and the fluoroquinolones.
- Penicillin is a surrogate for the following: amoxicillin, ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefazolin, cefepime, ceftaroline, cephadrine, cephalothin, cefotaxime, ceftriaxone, ceftizoxime, imipenem, ertapenem, and meropenem.
  - If laboratories opt to test GBS and the isolate is susceptible to penicillin, the additional antimicrobials listed above are considered susceptible.
  - Should results for one of these agents (e.g., cefazolin – see below) be requested, a note can be added to the report such as: "Penicillin susceptible GBS are susceptible to cefazolin."

Which antimicrobials should be tested and reported for GBS from vaginal-rectal specimens?

Refer to the 2010 CDC guidelines on prevention of perinatal group B streptococcal disease, which covers testing and reporting of antimicrobials for GBS in pregnant women: **http://www.cdc.gov/groupbstrep/guidelines/guidelines.html.** The CDC guidelines were developed with several professional associations, including ASM and the American Congress of Obstetricians and Gynecologists (ACOG). Highlights include: 1) GBS can colonize the vaginal-rectal tract, leading to invasive neonatal GBS infection as a result of exposure in the genital tract during delivery; 2) vaginal-rectal screening of pregnant women is performed at 35 to 37 weeks gestation for determination of GBS colonization; 3) antimicrobials are administered intrapartum (while the mother is delivering) to women positive for GBS to minimize the risk of the neonate developing the disease; and 4) the antimicrobial of choice is penicillin or ampicillin (cefazolin may be administered to penicillin-allergic women at low risk for anaphylaxis; clindamycin is recommended for penicillin-allergic women at high risk for anaphylaxis; vancomycin should be administered if the isolate is not susceptible to clindamycin or shows inducible clindamycin resistance).

- The clinician should have a means of informing the laboratory if the patient is at high risk for penicillin anaphylaxis.
- Perform testing only if the clinician has indicated a severe penicillin allergy, or if the patient is at high risk for anaphylaxis. In these cases, neither penicillin nor cefazolin can be given to the patient. Clindamycin is the antimicrobial of choice and the isolate must be tested for clindamycin resistance.
- Only ampicillin, penicillin, cefazolin, clindamycin, and vancomycin are specifically recommended for treatment in pregnant women for GBS colonization. See Table 1. It would be misleading to report other agents such as the fluoroquinolones, other cephalosporins, macrolides, and tetracyclines.
- If the clinician wishes to administer cefazolin and asks the laboratory for susceptibility, the laboratory should state that no further testing is necessary since a) penicillin is a surrogate for cefazolin, and b) GBS are predictably susceptible to penicillin. Furthermore, there are no cefazolin breakpoints for β-hemolytic streptococci.

Special Considerations for Susceptibility Testing of *Streptococcus agalactiae* (Group B *Streptococcus*) *(continued)*

- If the laboratory is not aware of a penicillin allergy at the time of testing, options include:
  - **Do not perform susceptibility testing unless the clinician notifies the laboratory that there is a penicillin allergy** (order comments are helpful, such as "Susceptibility testing is not performed on this isolate due to predictable penicillin susceptibility; contact the laboratory for testing if the patient has a severe penicillin allergy."); OR
  - **Test the isolate for inducible clindamycin resistance and report clindamycin. Do not report erythromycin,** as erythromycin is not an effective therapeutic option for intrapartum prophylaxis.

Table 1.

Suggested algorithms for antimicrobial susceptibility testing and reporting for GBS isolates from vaginal-rectal sources (reprinted with permission from College of American Pathologists Proficiency Test Specimen D-12 Final Critique, 2016).

Penicillin Allergy	Recommended Therapy ACOG and CDC Guidelines	Suggested Approach to Susceptibility Testing
No known penicillin allergy	Penicillin	Susceptibility testing is not necessary.
PCN-allergic, low risk for anaphylaxis	Cefazolin	Susceptibility testing is not necessary.
PCN-allergic, high risk for anaphylaxis	Clindamycin (if clindamycin is S) Vancomycin (if clindamycin is R)	Test clindamycin and erythromycin. Report only clindamycin.

PCN = penicillin; **S** = susceptible; **R** = resistant; **ACOG** = American Congress of Obstetricians and Gynecologists; **CDC** = Centers for Disease Control and Prevention

How should GBS isolates from prenatal screens be tested to determine if they are clindamycin susceptible or resistant?

- Both erythromycin and clindamycin must be tested to determine clindamycin results. Testing of erythromycin enables detection of a specific type of clindamycin resistance known as inducible clindamycin resistance (ICR).
- Virtually all clindamycin-resistant GBS are erythromycin resistant; however, some erythromycin-resistant GBS are clindamycin susceptible. The mechanisms and respective susceptibility profiles of clindamycin in erythromycin-resistant GBS isolates are shown in Table 2.

Table 2.

Mechanisms and respective susceptibility profiles of clindamycin in erythromycin-resistant GBS isolates

Mechanism	Resistance Determinant	Erythromycin	Clindamycin
Efflux	Mef	R	S
Ribosome modification	Erm	R	S (requires ICR test to show "R")
Ribosome modification	Erm	R	R (constitutive resistance – always shows "R")

ICR = inducible clindamycin resistance; **R** = resistant; **S** = susceptible.

Special Considerations for Susceptibility Testing of *Streptococcus agalactiae* (Group B *Streptococcus*)

(continued)

- GBS isolates that test erythromycin resistant and clindamycin intermediate or susceptible must be examined for ICR by the D-zone test or another validated method prior to reporting clindamycin results. Methods for testing for ICR (disk diffusion and broth microdilution) are listed in CLSI M100S, 26th Edition in Table 3G. Commercial systems that are FDA cleared specifically for testing of GBS are also acceptable.
- If the ICR test is positive, clindamycin must be reported as resistant on the basis of detection of ICR. Some laboratories may choose to comment that clindamycin may still be used clinically but with caution.
- Do not report erythromycin on GBS from vaginal-rectal sources, since erythromycin is not suggested therapy according to the CDC and other agencies.
- In 2010, the CDC reported that more than 25% of GBS isolates from patients with serious GBS disease (isolated from normally sterile sites) were resistant to clindamycin and almost 50% of isolates were resistant to erythromycin **(http://www.cdc.gov/abcs/reports-findings/survreports/gbs10-suscept.html)**.

Additional Reading:

1 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 26th ed. CLSI supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.

2 Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. *MMWR Morb Mortal Wkly Rep.* 2010;59(RR-10):1-32.

3 American College of Obstetricians and Gynecologists. Committee opinion no. 485: Prevention of early-onset group B streptococcal disease in newborns. *Obstet Gynecol.* 2011;117(485):1019-1027.

4 Phares CR, Lynfield R, Farley MM, et al. Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. *JAMA.* 2008;299(17):2056-2065.

5 Lewis JS, Lepak AJ, Thompson GR, et al. Failure of clindamycin to eradicate infection with beta-hemolytic streptococci inducibly resistant to clindamycin in an animal model and in human infections. *Antimicrob Agents Chemother.* 2014;58(3):1327-1331.

Continuing Conversation About Colistin!

Colistin and polymyxin B are viewed as drugs of last resort for the treatment of patients with infections caused by multidrug-resistant gram-negative bacteria. However, clinicians, pharmacists, and laboratorians alike struggle with how to best use these drugs in practice. In 2016, CLSI and EUCAST reported the findings of a Joint Working Group that addressed global challenges associated with colistin. Identifying reliable testing methods and establishing breakpoints based on CLSI document M23—*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters* were the primary objectives. Polymyxin B was not specifically addressed by this group, as there are few studies that have evaluated polymyxin B. While polymyxin B and colistin molecules are very similar, the PK-PD of the clinical formulations of these two drugs differs significantly, requiring independent studies to evaluate clinical breakpoints.

Breakpoints and ECVs

The CLSI/EUCAST Joint Working Group recommended the following clinical breakpoints, which were approved by the CLSI AST Subcommittee in 2016:

Organism	Susceptible	Resistant
Acinetobacter spp.	≤2 µg/mL	≥4 µg/mL
Pseudomonas aeruginosa	≤2 µg/mL	≥4 µg/mL

Breakpoints for the *Enterobacteriaceae* were considered, but ultimately there were insufficient data to establish a clinical breakpoint for this organism group. As such, an ECV was set, based on minimal inhibitory concentration (MIC) distribution data for isolates of *Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae,* and *Raoultella ornithinolytica.* This ECV should be applied only to these species, as wild-type MIC distributions may be different for other genera and species of *Enterobacteriaceae.*

Continuing Conversation About Colistin! *(continued)*

Organism	ECV	
	Wild-type	Non-wild-type
Enterobacter aerogenes	≤2 µg/mL	≥4 µg/mL
Enterobacter cloacae		
Escherichia coli		
Klebsiella pneumoniae		
Raoultella ornithinolytica		

The ECV allows laboratorians, clinicians, and public health professionals to identify isolates that have colistin MICs above the wild-type distribution (ie, those with acquired and/or mutational resistance mechanisms to colistin, such as *mcr-1*). ECV interpretations ("wild-type" vs "non-wild-type") should not be used for clinical decision making, as neither PK-PD nor clinical data have been evaluated for this drug with any members of the *Enterobacteriaceae*, as are required by CLSI document M23 for establishing a clinical breakpoint.

Testing Methods

Testing colistin is challenging, as the molecule is large and has a propensity to adsorb to testing surfaces (eg, pipettes, polystyrene tubes). The CLSI/EUCAST Joint Working Group evaluated colistin susceptibility testing methods. The findings of the group are as follows:

- Broth microdilution, without surfactant, is the reference method for testing colistin.
- Disk and agar gradient diffusion methods should not be used for testing colistin, as these yield unacceptably high error rates.

In the United States, all commercial colistin testing devices are labeled "research use only" (RUO). FDA clearance of commercial AST devices requires the manufacturer to use only FDA (and not CLSI) breakpoints for interpretation of results. Since the FDA label for colistin does not contain any breakpoints, at this time there is no mechanism to get a commercial AST device FDA cleared for testing colistin. This leaves laboratories with little recourse when requested to test colistin by the clinicians they serve. RUO commercial broth microdilution methods have been shown in some studies to perform acceptably as compared to CLSI reference broth microdilution, and could be considered as testing methods, provided the laboratory appropriately verifies the performance of these, and disclaims results as "RUO." Alternatively, laboratories may consider sending isolates to reference laboratories, after ensuring these laboratories perform a broth dilution method for testing colistin.

To learn more about colistin, check out these references.

- Hindler JA, Humphries RM. Colistin MIC variability by method for contemporary clinical isolates of multidrug-resistant gram-negative bacilli. *J Clin Microbiol*. 2013;15:1678-1684. This study evaluated broth microdilution (BMD) vs agar dilution, Etest, and Sensititre panels for testing colistin, using a collection of MDR gram-negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii*). The study also investigated the impact of adding a surfactant (polysorbate 80) to BMD panels.
- Sader HS, Rhomberg PR, Farrell DJ, Jones RN. Differences in potency and categorical agreement between colistin and polymyxin B when testing 15,377 clinical strains collected worldwide. *Diagn Microbiol Infect Dis*. 2015;83(4):379-381. This study evaluated the MIC distributions for a large collection (>15,000) of gram-negative bacteria for polymyxin B and colistin.
- The following two articles are excellent reviews for colistin and polymyxin B susceptibility testing, including description of the molecules, description of resistance mechanisms, and performance of currently available tests for colistin and polymyxin B.
  - Jerke KH, Lee MJ, Humphries RM. Polymyxin susceptibility testing: a cold case reopened. *Clin Microbiol Newsl*. 2016;38:69-77.
  - Humphries RM. Susceptibility testing of the polymyxins: where are we now? *Pharmacotherapy*. 2015;35(1):22-27.
- In 2016, the CDC released an advisory to health care professionals about detecting colistin resistance, and in particular the *mcr-1* gene. In the advisory, laboratories that test for colistin resistance were instructed to evaluate isolates with MICs >2 µg/mL for the *mcr-1* gene, either in house or by sending isolates to the CDC. This advisory can be found **here**.
- CLSI recently presented a webinar on colistin, which can be accessed for a fee on demand **here**.

The CLSI Anaerobe Working Group and Anaerobe Susceptibility Testing

A team of microbiologists, clinicians, pharmacists, and researchers with experience in anaerobe susceptibility testing and/or treatment of anaerobic infections is responsible for providing recommendations for AST of anaerobes. These recommendations are found in two CLSI documents:

- M11—*Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria* provides recommendations for performing reference broth microdilution and agar dilution susceptibility testing of anaerobes; the current version is M11-A8, published in 2012; the next revision is anticipated to publish in 2017.
- M100 tables related to anaerobe susceptibility testing list agents recommended for testing, breakpoints, quality control ranges, and antibiogram tables (see below).

When should susceptibility tests be performed on anaerobes isolated from clinical specimens?

Susceptibility testing should be considered for anaerobic isolates when empiric therapy cannot be predicted, as for *Bacteroides* spp., *Prevotella* spp., *Fusobacterium* spp., and *Clostridium* spp. when encountered from the following sources/situations:

- Severe infections that may require long-term therapy
- Isolates from brain abscess, endocarditis, osteomyelitis, joint infections, infections of prosthetic devices or vascular grafts, and bacteremia

β-lactamase testing by the rapid nitrocefin-based method should be considered for gram-negative anaerobic isolates and gram-positive anaerobic isolates from sterile sources, with the exception of the *Bacteroides fragilis* group. The *B. fragilis* group of organisms is presumed to be β-lactamase positive, so such testing is not suggested for this group.

NOTE: For those facilities that do not currently perform susceptibility testing on anaerobes, the laboratory should consider sending out isolates to another laboratory for testing. While awaiting susceptibility testing results, β-lactamase testing can be considered, if applicable (see above). Also, the laboratory and clinicians can refer to recent antibiogram data as a source of possible options for empiric therapy. These antibiogram data can be found in the most recent M100S published in 2016.

Recent Evaluations and Anticipated Revisions in Recommendations for Anaerobic AST

Currently, the only anaerobic bacteria for which broth microdilution is an acceptable testing method are those in the *B. fragilis* group. For all other anaerobes, agar dilution is the only CLSI-approved method. In hopes of expanding the bacterial species for which broth microdilution is acceptable, the Anaerobe Working Group is evaluating the performance of broth microdilution vs agar dilution for a variety of anaerobes. To date, broth microdilution results have unfortunately not agreed with those from agar dilution testing for *Clostridium difficile*. The Working Group will continue to evaluate other anaerobic species, and through CLSI documents M11 and M100 will notify laboratories if broth microdilution can be used for anaerobes other than the *B. fragilis* group.

Publication of Anaerobe Susceptibility Data

The Anaerobe Working Group has recently published antimicrobial susceptibility data entitled "Changes in the antibiotic susceptibility of anaerobic bacteria from 2007-2009 to 2010-2012 based on the CLSI methodology" in *Anaerobe* (2016: vol 42, 27-30). Results from agar dilution testing were gathered from four different medical centers, which served as reference testing sites for primarily US laboratories. A few notable changes were observed by comparing the antibiograms between the two time periods. Overall, resistance to metronidazole remained low in most anaerobic bacteria; however, there was a small but significant increase in metronidazole resistance in *B. fragilis*. Furthermore, high rates of clindamycin resistance were noted for anaerobic isolates including, but not limited to, the *B. fragilis* group.

The Anaerobe Working Group looks forward to evaluating new data, providing communications, and making recommendations for anaerobic susceptibility testing to the CLSI AST Subcommittee, as well as through publication of findings. The Working Group welcomes volunteers, questions, comments, or new information in this unique area of susceptibility testing.

Resistance Hot Topic!

Vancomycin-Variable Enterococci: An Unrecognized Threat?

Vancomycin, a bactericidal glycopeptide, inhibits cell wall (peptidoglycan) synthesis in most gram-positive bacteria, including enterococci. Acquired vancomycin resistance in enterococci is often mediated by *vanA*, which confers high-level resistance to vancomycin by substituting the D-alanyl-D-alanine vancomycin (glycopeptide)-binding site in peptidoglycan with D-alanyl-D-lactate. This substitution decreases the affinity of vancomycin for peptidoglycan 1,000-fold and negates its ability to inhibit peptidoglycan synthesis.

A report from Canada in 2014 described a 69-year-old man with a complicated medical history who was admitted to the hospital with *Escherichia coli* sepsis. He was known from a prior admission to be colonized with an *Enterococcus faecium* isolate positive for *vanA*, but susceptible to vancomycin (MIC = 1 µg/mL), and a rectal swab obtained upon admission yielded the same organism. On days 12 to 14 of his hospitalization, he received empiric intravenous vancomycin therapy for suspected recurrent sepsis, and screening rectal swabs taken on days 22 and 24 of hospitalization yielded *vanA*-positive *E. faecium* isolates resistant to vancomycin (MIC = 256 µg/mL) with pulsed-field gel electrophoresis patterns indistinguishable from the vancomycin-susceptible/*vanA*-positive isolates.[1] Based upon this report, the authors proposed the term "vancomycin-variable *Enterococcus* (VVE)" to describe vancomycin-susceptible enterococci containing *vanA* that subsequently become resistant to vancomycin after vancomycin exposure.[1] Similarly, vancomycin-resistant *E. faecium* was isolated from two patients from different wards of a Norwegian hospital following ineffective courses of vancomycin treatment for infections due to vancomycin-susceptible *E. faecium*. Molecular analysis of the initial vancomycin-susceptible isolates revealed the presence of *vanA*.[2] In the Canadian and Norwegian reports, the development of vancomycin resistance arose from different mechanisms, but ultimately resulted in the expression of the *vanA* gene in either a restored vancomycin-inducible fashion or constitutively.[2,3]

Although the overall prevalence of vancomycin-susceptible enterococci containing *vanA* is unknown, dissemination of these isolates within health care facilities has been observed in North America and Europe.[2,4] While the vast majority of VVE are *E. faecium*, a single vancomycin-susceptible/*vanA*-positive *Enterococcus faecalis* isolate has been described.[2] Clearly, VVE may pose a significant problem for infection control practices centered solely on phenotypic-based surveillance methods for vancomycin-resistant enterococci, and may escape detection, leading to uncontrolled dissemination in health care facilities. Treatment of vancomycin-susceptible/*vanA*-positive isolates with vancomycin (or perhaps with other glycopeptides) could promote development of vancomycin resistance and, ultimately, treatment failure.

In conclusion, clinical microbiologists are encouraged to report vancomycin-susceptible/*vanA*-positive enterococcal isolates as vancomycin resistant, as suggested by CLSI in the "Strategies for Reporting Vancomycin Results When Using Molecular and Phenotypic AST Methods for *Enterococcus* spp." table (located on the CLSI website under the "Use of Molecular Assays for Resistance Detection" tab at the following link: **http://clsi.org/standards/micro/microbiology-files/** and to monitor isolates with discordant vancomycin phenotypic and genotypic testing results within their institution. However, it is appreciated that not all laboratories employ both genotypic and phenotypic methods that would enable detection of VVE. Should VVE become widespread, additional recommendations may be necessary. In the meantime, in cases of vancomycin treatment failure for enterococcal infection, testing of subsequent isolates from the patient and possibly use of additional test methods for the isolates should be considered.

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