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CLSI

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 (AST) and reporting. We are listing links to some new educational materials and reminding you where you can find information about the CLSI AST Subcommittee proceedings.

Inside This Issue:

CLSI and the AST Subcommittee During COVID-19

In light of the pandemic, CLSI's June 2020 AST Subcommittee meeting was cancelled.

Specific scheduling modifications for the AST SC include:

1. Virtual meetings of Working Groups and the AST Subcommittee are scheduled for August and September 2020. All are welcome, and free registration for web conferences can be found **here.** It is planned that all meetings will be recorded and made available on the CLSI website.
2. Publishing of M100's 31st edition will occur in Spring 2021 instead of early 2021.
3. The annual AST Update 2021 webinar will be scheduled for late Spring 2021, after the new edition of M100 has published.

CLSI AST Subcommittee Partnerships

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

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

What does the CLSI AST Subcommittee do?

The first edition of the CLSI AST News Update (Vol 1, Issue 1, Spring 2016) described details about the organization and operation of the CLSI AST Subcommittee.

- You can access that Newsletter **here.**
- To learn more about upcoming or past meetings, click **here.**
- CLSI posts meeting minutes and summaries for public access **here.**
- 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.

Volunteers are needed for:

- A new document development committee: M66—Methods for Active Surveillance of Multidrug-Resistant Organisms, 1st Ed. (Deadline August 6)
- A document development committee for a document revision: M56—Principles and Procedures for Detection of Anaerobes in Clinical Specimens, 2nd ed. (Deadline August 17)
- Reviewer positions on the AST and Antifungal Subcommittees. (Deadline August 31)

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

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

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

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

Webinars

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

Recent Webinar

CLSI-SIDP ACCP Annual Webinar

On Demand/Recorded on July 14, 2020 | Access on ProCE's Website **here.**

Speakers:

Audrey Schuetz, MD, MPH, D(ABMM)
Professor of Laboratory Medicine and Pathology
Director of Initial Processing and Media Laboratories
Co-Director of Bacteriology Laboratory
Mayo Clinic College of Medicine and Science
Mayo Clinic, Rochester, Minnesota

Natasha N. Pettit, PharmD, BCPS (AQ-ID)
Clinical Pharmacy Specialist, Infectious Diseases
Clinical Pharmacy Coordinator, ID/Antimicrobial Stewardship
Pharmacy Director, Antimicrobial Stewardship Program
University of Chicago Medicine, Chicago, Illinois

Archived and Free On-Demand Webinars:

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

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

Archives of Retired Breakpoints and Methods

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

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

ASM Microbe 2020 Recordings

ASM members can access sessions from Microbe 2020 held virtually. The CLSI ORWG organized session can be found **here.**

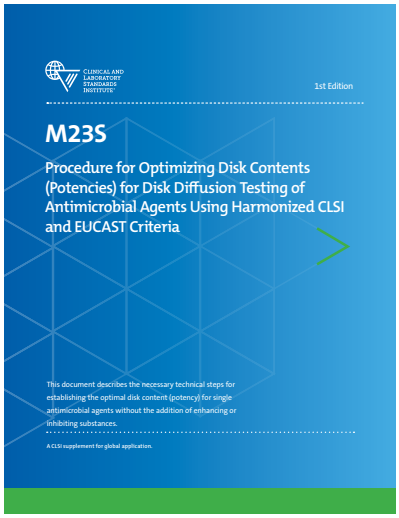
Title: Importance of Reliable Generation and Appropriate Interpretation of AST Results in 2020

#1 Meaningful Reporting of Antimicrobial Susceptibility Test Results
April Bobenchik, PhD D(ABMM)
Associate Director Clinical Microbiology
Lifespan Academic Medical Center, Providence, RI

#2 The Science and the Art of Setting and Revising Breakpoints
James Lewis, PharmD
Supervisor, Infectious Diseases Pharmacy; Co-director Antimicrobial Stewardship
Oregon Health and Science University, Portland, OR

#3 What Does MIC, SDD, S, I and R Mean to the Clinician?
Amy Mathers, MD D(ABMM)
Assoc. Director Clinical Microbiology; Director Antimicrobial Stewardship
University of Virginia Medical Center, Charlottesville, VA

New/Updated CLSI AST Documents Are Here!

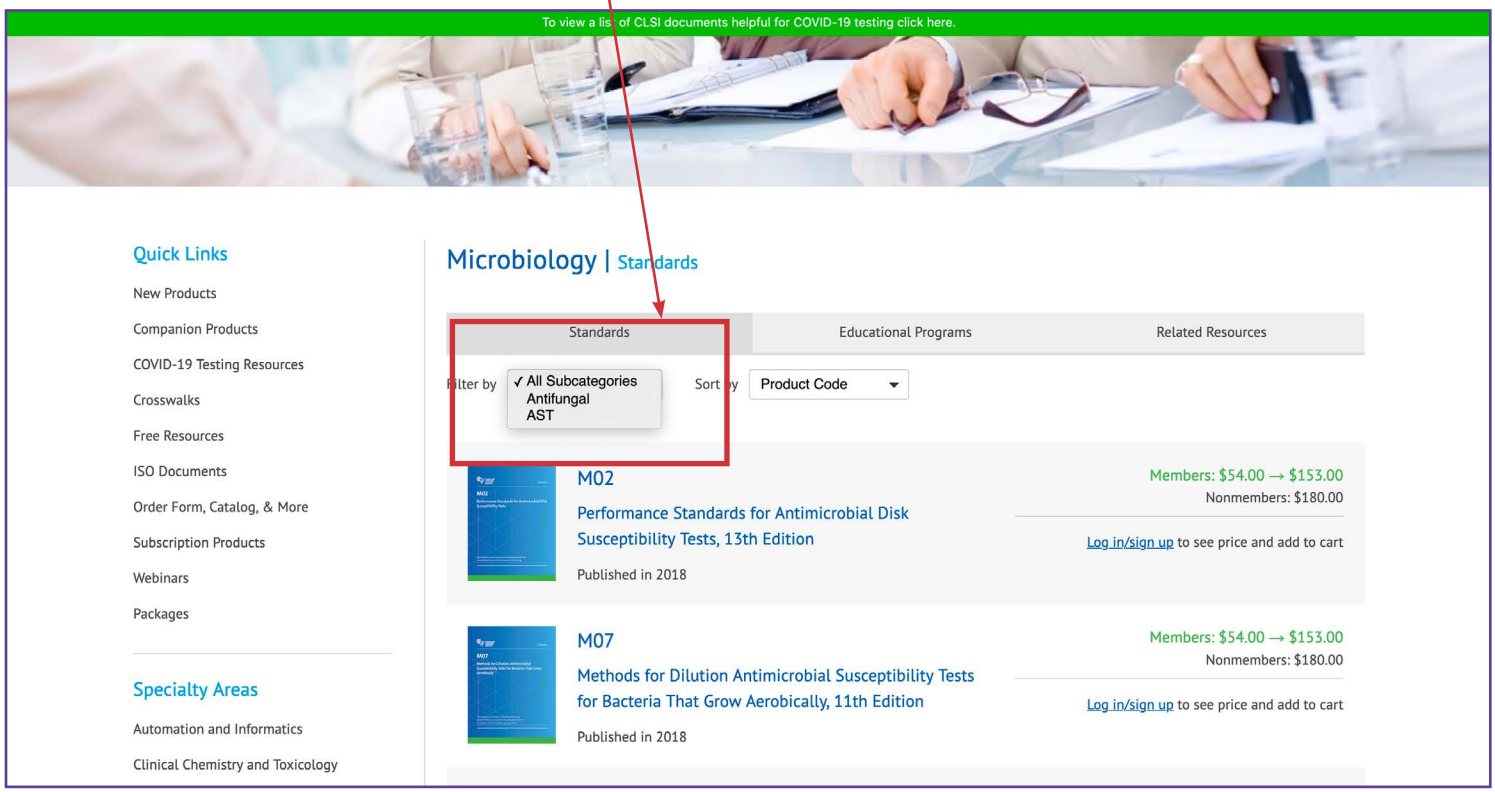


M23S | Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria, 1st Edition

This "NEW" supplement describes the process for selecting the optimal content (potency) of antimicrobial agent to be added to filter paper disks for use with the standard disk diffusion test. It is intended for pharmaceutical manufacturers involved in the development of antimicrobial agents and tests to support evaluation of antimicrobial agent activity. It is also intended for manufacturers of antimicrobial disks and any independent laboratory that supports the development of these disks. M23S is available here.

How do I find CLSI's AST and Antifungal documents?

On the CLSI website, visit here and at the top, select "Filter By." From the drop-down menu you can view just the latest versions of the AST and Antifungal documents.



How do I know I have the latest edition of a CLSI document?

On the CLSI website, you'll only find the most up-to-date editions of each document. To compare it to a version you own, compare the edition number at the end of the title. The edition should match the cover of the version you have; for example, M02 is the "13th Edition" and it will say that in the title on the website listing and on the cover of your document.

New Rationale Documents

CLSI publishes rationale documents that provide the scientific reasons behind the subcommittee's decisions, along with documentation of the standardized data and methods used to determine breakpoints. To access rationale documents, click **here.** To learn more about breakpoint revisions, please check out the archived December 2019 webinar (FREE) "Understanding Breakpoint Decisions: CLSI Rationale Documents" **here.**

The newest rationale documents are:

MR01-Ed2 Polymyxin Breakpoints for Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter* spp., 2nd edition. *(April 2020)*

MR07-Ed1 Cefazolin Breakpoints for Enterobacterales (Systemic Infections) *(March 2020)*

MR08-Ed1 Cefazolin Breakpoints for Enterobacterales (Uncomplicated Urinary Tract Infections) *(March 2020)*

Note: CLSI and the US Food & Drug Administration (FDA) are continuing to make strides in harmonizing breakpoints (referred to as Susceptibility Test Interpretive Criteria [STIC] by FDA) and have now approved meropenem breakpoints for *Acinetobacter* spp. as discussed in MR03. To learn more about FDA-recognized breakpoints, please visit **here.**

Check It Out! Educational Workshops Held at CLSI Meetings

Nicole Scangarella-Oman, GlaxoSmithKline, Collegeville, PA

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

The January 2020 workshop was entitled **"Beyond Susceptible/ Intermediate/Resistant (SIR): Enhancing Laboratory Communication with Reporting Comments."** The objectives of this workshop were to:

- Discuss gaps between breakpoints and clinical practice.
- Convey options for real-time reporting of susceptibility-based comments.
- Discuss mechanisms for communicating susceptibility information beyond S/I/R.
- Discuss the impact of susceptibility comments to clinical practice.

Communication of results from both antibacterial and antifungal susceptibility testing of human isolates was discussed. The challenges of reporting AST results for veterinary practice were also presented.

A pdf of the slides presented at this workshop can be found **here.**

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

Future CLSI AST Meetings

January 23-26, 2021
Arlington (Dallas), Texas, USA

June 26-29, 2021
San Diego, California, USA

Featured Article

COVID-19 and Antimicrobial Resistance (AMR) and Pandemics

Romney M. Humphries, Vanderbilt University Medical Center, Nashville, TN
Graeme Forrest, Oregon Health and Science University, Portland, OR
Janet A. Hindler, Los Angeles County Department of Health, Los Angeles, CA

As the world grapples with the COVID-19 pandemic, many lessons are being learned, not the least of which is how to better prepare for future pandemics. This includes addressing the ongoing antimicrobial resistance (AMR) pandemic, which at a global scale has the potential to eclipse COVID-19. It is estimated hundreds of thousands of patients die globally each year due to complications associated with AMR infections—some projections indicate this number will be 10 million by 2050.[1]

In Part I of this feature, we provide information about use of antimicrobials and AMR during COVID-19. We explore how COVID-19 has impacted one hospital's antimicrobial stewardship program.

In Part II, we discuss the ongoing AMR predicament and what clinical microbiologists can and should do now, based in part on knowledge acquired by clinicians, researchers and public health professionals from COVID-19.

Part I. What Do we Know About Antimicrobial Resistance (AMR) During COVID-19?

COVID-19 is a disease caused by a virus and characterized by cough, fever and shortness of breath. For some patients, particularly the elderly and those with underlying conditions, infection progresses to severe disease, including acute respiratory distress syndrome. These patients often require mechanical ventilation and intensive care hospitalization. Antimicrobials are administered to approximately 75% of COVID-19 patients,[2] many of whom receive broad-spectrum therapy.[3] While at first blush, use of antimicrobials to treat a viral infection seems counter to antimicrobial stewardship efforts, antimicrobial use is supported by national guidelines due to presumed or confirmed bacterial co-infections. Although antimicrobial stewardship is in full force, clinicians may continue antimicrobial therapy when faced with severely ill patients even if bacterial infection cannot be confirmed. One hospital's experience details these challenges, in the Case Study on page 8.

In past viral pandemics (ie, caused by influenza), secondary bacterial pneumonia was a major cause of mortality, affecting roughly 35-50% of patients.[4] In contrast, secondary bacterial infections are rare for COVID-19. A meta-analysis of nine published studies documented 8% of patients hospitalized for COVID-19 experienced bacterial or fungal co-infections.[2] Rates of co-infection ranged from 0–27% across the studies.[3] The reasons for this variability are unclear, but may relate to differences in treatment practices (eg, use of corticosteroids), infection control policies and/or local epidemiology.[3] Additionally, case definitions may play a role. Anecdotal reports from hospitals across the US indicate specimen collection (in particular of respiratory secretions) from patients with COVID-19 may not be performed due to infection control risk, meaning cases of co-infection often remain unconfirmed by the laboratory.

At a higher level, the COVID-19 pandemic has the potential to detrimentally impact global rates of drug-resistant infections.[5] Some factors of the COVID-19 pandemic that could contribute to an increase in AMR include:

1. Widespread use of antimicrobials for patients with COVID-19 for presumptive or confirmed secondary infection.
2. A large new population of patients at risk for AMR infections due to use of mechanical ventilation and prolonged hospitalization. These patients would not traditionally be considered at risk for AMR, as they have had limited prior healthcare exposure.
3. Potential lapses in institutional infection control and antimicrobial stewardship practices while institutions adapt to challenges of the pandemic.
4. Depleted availability of personal protective equipment (PPE) which may reduce use for other infections that would normally require PPE.
5. Use of contact precautions may be limited due to cohorting based on COVID-19 status versus other infections.
6. Closure or limited access to ancillary clinics in favor of telemedicine, driving empiric antimicrobial prescriptions versus those prescribed following diagnostic testing.
7. Individuals in the community resorting to use of leftover antimicrobial prescriptions in their medicine cabinet to self-treat.

COVID-19 and Antimicrobial Resistance (AMR) and Pandemics (*Continued*)

The news is not all bad. Increased vigilance for hand hygiene, other infection prevention activities and continued efforts to educate the public on the appropriate use of antimicrobials may offset some of the above factors. Many healthcare facilities are reviewing or enhancing their strategies for addressing AMR concerns during COVID-19. A few examples include:

1. Monitoring AMR trends in isolates from COVID-19 patients to inform empiric therapy needs at the local level.
2. Adopting rapid diagnostics (eg, respiratory panels for agents other than SARS-CoV-2).
3. Continuing to emphasize best practices for work-up of respiratory specimens (see ORWG **News Update Volume 3 Issue 1** Winter 2018).
4. Strictly enforcing recommended infection control practices for all patients, staff, and visitors in all healthcare facility locations.
5. Ramping up assessment of additional practices that might be implemented to help control transmission of all types of microbes.

Clinicians, microbiology laboratorians, infection preventionists, and public health practitioners must be vigilant in monitoring the potential impact of these factors on the emergence of AMR in individual patients, as well as at institutional and regional levels. Preparedness activities, listed in Part II, can aid the laboratory in addressing the potential challenges associated with changes to AMR related to COVID-19.

References

1 O'Neill J. Tackling Drug-resistant infections globally: final report and recommendations. Review on antimicrobial resistance, Vol. London, United Kingdom: Wellcome Trust, 2016.

2 Rawson TM, Moore LSP, Zhu N, et al. Bacterial and fungal co-infection in individuals with coronavirus: A rapid review to support COVID-19 antimicrobial prescribing. *Clin Infect Dis.* 2020;10.1093/cid/ciaa530.

3 Clancy CJ, Nguyen MH. *COVID-19,* superinfections and antimicrobial development: What can we expect? *Clin Infect Dis.* 2020;10.1093/cid/ciaa524.

4 Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis.* 2008;198:962-70.

5 Rawson TM, Moore LSP, Castro-Sanchez E, et al. COVID-19 and the potential long-term impact on antimicrobial resistance. *J Antimicrob Chemother.* 2020;10.1093/jac/dkaa194.

One Hospital's Reality in the COVID-19 Era

As our hospital fights COVID-19, we have faced several diagnostic and antimicrobial stewardship challenges. COVID-19 infections can rapidly progress from mild respiratory illness to overwhelming pneumonia with sepsis requiring intensive care unit (ICU) monitoring with ventilatory support. While caused by a virus, COVID-19 disease includes pathophysiologic features that are traditional hallmarks of bacterial infection: pneumonia on imaging; increasing C-reactive protein and procalcitonin levels; and elevated peripheral white blood cell counts. This dynamic has resulted in our clinicians using antimicrobials broadly to treat COVID-19 patients.

One early decision was an effort to reduce unnecessary laboratory testing as our hospital prepared for testing surges associated with the pandemic. Among the tests discontinued was routine methicillin-resistant *Staphylococcus aureus* (MRSA) screening for patients prior to admission into the ICU. Our site has a low rate of MRSA infections, and knowledge of a patient's MRSA status through this testing normally resulted in rapid vancomycin de-escalation. Subsequent to eliminating these MRSA screens, vancomycin usage increased by over 50% in the ICU.

The broad-spectrum antimicrobials routinely given to severely ill COVID-19 patients in our hospitals include coverage for *Pseudomonas aeruginosa*. Such treatment was often excessive, as the majority of COVID-19 patients have no risk factors for *Pseudomonas* infection. Compounding this stewardship challenge is the fact that no change in therapy was considered, even after a COVID-19 diagnosis was rendered and bacterial cultures were negative. Markers normally used to help with antimicrobial de-escalation discussions, such as procalcitonin, were elevated in these patients, making de-escalation decisions even more challenging.

Finally, during the pandemic we have noted increases in the daily numbers of sputum, endotracheal aspirates and blood cultures submitted to the laboratory. These specimens were collected in response to patients who developed high fever and shock, which again reflect the disease pathogenicity rather than "bacterial super-infection". Compounding this, as the number of days during which the patient was intubated increased and the patient did not respond to therapy, more cultures were sent with the hope of identifying the reason for the patient's continued decline. Unsurprisingly, these cultures often yielded organisms commonly associated with colonization of the respiratory tract during mechanical ventilation, such as *Stenotrophomonas maltophilia* or *P. aeruginosa*. Because of the patient's symptoms, these were treated as pathogens. By late in the patient's hospitalization, the impact of antimicrobial usage could be seen to drive AMR in these bacteria.

These have been unprecedented times and a lack of data available early on to assist management combined with the severe clinical condition resulted in pressure of testing and antimicrobial usage. When this is eventually over, examining the practices that could reduce overcollection of specimens and treatment with broad-spectrum antimicrobials could be implemented.

COVID-19 and Antimicrobial Resistance (AMR) and Pandemics (*Continued*)

Part II. Preparing for an AMR Pandemic – the Role of the Clinical Microbiology Laboratory

A major lesson learned from COVID-19 is the critical role of laboratory testing, and surge testing capabilities for crisis management. To address the AMR pandemic, there is an urgent need for rapid diagnostics that can determine if a patient has an infection, distinguish viral and bacterial infections, and identify the etiologic agent and its susceptibility profile (if bacterial or fungal).

Much like for COVID-19, each individual in the clinical laboratory plays a role in fighting global AMR. Activities to ensure appropriate use of antimicrobials can occur at the bench, laboratory, institutional and regional level. Unlike COVID-19, which is caused by a single virus, SARS-CoV-2, AMR spans across a variety of microorganisms and affects all antimicrobials. Furthermore, continual evolution of resistance mechanisms, changes in organism prevalence, and introduction of new antimicrobials require continual adaptation. Suggested activities for the laboratory to ensure preparedness for this ongoing AMR pandemic are listed in Table 1.

Table 1. Ongoing "key" activities for clinical microbiology laboratories to ensure preparedness for the AMR pandemic

Key Activity	Why is this important?	Examples
Implement tests to rapidly identify etiology of infection and develop practices to ensure appropriate use of these tests.	Helps clinicians determine if patient has infection and if infection warrants antimicrobial treatment	• Rapid tests for respiratory viruses • Direct tests for Group A *Streptococcus* • Direct molecular assays on positive blood cultures to identify bacteria (or yeast)
Follow current recommendations for AST.	Reliably detect resistant (and susceptible) isolates	• CLSI AST recommendations
Develop a robust quality assurance program for AST beyond routine testing of ATCC® QC strains.	Ensures results are accurate; unusual results are confirmed; all results are reported in an effective and timely manner	• Staff competency • Equipment maintenance • Relationship with vendor of AST supplies/equipment (obtain latest information) • AST system software that flags unusual susceptibility results • Resources identified to assist in confirmation of unusual results (eg, reliable reference laboratory; public health laboratory) • Reporting meets laboratory's established turnaround times
Routinely test/report antimicrobial agents used by clinicians; adopt selective/ cascade reporting protocols, as appropriate.	Guides use of antimicrobial agents appropriate for organisms encountered and deemed appropriate for patient population served; encourages us of narrower-spectrum agents first	• Ongoing relationship with antimicrobial stewardship program (ASP) and regularly scheduled review of panel of agents tested / reported routinely or selectively
Use up-to-date breakpoints to interpret disk diffusion and/or MIC values.	Out-of-date breakpoints may miss important clinical resistance	• Current CLSI breakpoints
When new antimicrobial agents become available, determine if/when/how these will be tested in-house or at a reference laboratory.	Guides use of additional agents that may be appropriate for MDR isolates	• Newer beta-lactam combination agents: Ceftazidime-avibactam Imipenem-relebactam Meropenem-vaborbactam • Cefiderocol • Eravacycline • Lefamulin • Omadacycline

COVID-19 and Antimicrobial Resistance (AMR) and Pandemics (*Continued*)

Table 1. *(Continued)*

Key Activity	Why is this important?	Examples
Report AST results in a clear and concise manner and within a reasonable timeframe for clinicians.	Identifies resistance and susceptibility clearly and minimizes chance for misinterpretation of results; enables timely administration of appropriate agents	• Ongoing relationship with ASP to review reports • Regularly solicit and review feedback from stakeholders
Develop a system to identify and immediately notify leadership and clinicians of results that are most significant (eg, pan-resistant isolates).	Enables timely detection and management of isolates that might be untreatable and pose the greatest threat to humankind	• Written guidelines to "catch" and act on those results that are most critical; regularly review with all staff
Communicate AST results of epidemiological interest in a clear, concise, and timely manner to infection preventionists and public health practitioners; implement surveillance for MDR as appropriate.	Enables efficient containment of resistant isolates in the institution and in the community	• Ongoing relationship with Infection Prevention to establish reportable criteria • Screening tests for VRE, MRSA, CRE, *Candida auris,* etc. • Daily log of specific resistant isolates encountered • Submit results/isolates for MDR as defined by the public health laboratory • Submit institutional antibiogram data to stakeholders as requested

Abbreviations: CRE, carbapenem-resistant Enterobacterales; MRSA, methicillin-resistant *Staphylococcus aureus*; MDR, multidrug resistant; VRE, vancomycin-resistant enterococcus.

Select Resources for Table

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

CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.* 3rd ed. CLSI guideline M45. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.

CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems.* 1st ed. CLSI guideline M52. Wayne, PA; Clinical and Laboratory Standards Institute; 2015.

For additional CLSI documents go **here.**

Publications and Websites:

Messacar K, Parker SK, Todd JK, Dominguez SR. Implementation of rapid molecular infectious disease diagnostics: the role of diagnostic and antimicrobial stewardship. *J Clin Microbiol.* 2017;55(3):715-723. DOI: 10.1128/JCM.02264-16

Morency-Potvin P, Schwartz DN, Weinstein RA. Antimicrobial stewardship: how the microbiology laboratory can right the ship. *Clin Microbiol Rev.* 2017;30(1):381-407. DOI: 10.1128/CMR.00066-16.

FDA Susceptibility Test Interpretive Criteria (STIC) website https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria. Accessed July 13, 2020.

CDC/FDA Antimicrobial Resistance (AR) Bank for isolates (can be used for verification studies) https://www.cdc.gov/drugresistance/resistance-bank/index.html. Accessed July 13, 2020.

Case Study

Learning About Veterinary AST From Lady Glittersparkles

Robert Bowden, Beth Israel Deaconess Medical Center, Boston, MA
Claire Burbick, Washington Animal Disease Diagnostic Lab, Washington State University, Pullman, MA

Just as the CLSI subcommittee on AST develops standards that support the judicious use of antimicrobial agents in human medicine, the subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) develops standards for performing AST on bacteria isolated from animals and releases VET documents (listed in Table 1) that closely parallel the CLSI "M" series of AST documents.

Table 1. CLSI Veterinary AST Documents

Document Code	Document Title
VET01	Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 5th Edition. 2018
VET02	Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents, 3rd Edition. 2008
VET03	Methods for Antimicrobial Broth Dilution and Disk Diffusion Susceptibility Testing of Bacteria Isolated From Aquatic Animals, 2nd Edition. 2020
VET04	Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated From Aquatic Animals, 3rd Edition. 2020
VET05	Generation, Presentation, and Application of Antimicrobial Susceptibility Test Data for Bacteria of Animal Origin, 1st Edition. 2011
VET06	Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated From Animals, 1st Edition. 2016
VET08	Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 4th Edition. 2018
VET09	Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings, 1st Edition. 2019

CLSI VAST Documents

VET01 and VET03 describe methodology for performing AST (broth dilution, disk diffusion, and agar dilution), while VET04, VET06, and VET08 provide tables with quality control parameters and veterinary-specific clinical breakpoints for dogs, cats, cattle, horses, pigs, poultry, and fish. Other important groups, such as birds, sheep, goats, camelids, reptiles, amphibians, and wild animals, currently lack breakpoints due to limited clinical and pharmacologic data, though antimicrobials are used to treat these animal groups and are, in some cases, necessary for endangered species facing population-limiting bacterial or fungal infections. For small companion animals, however, VAST has determined veterinary-specific breakpoints for a broad variety of antimicrobials. Many of these agents, and many of the pathogens commonly infecting dogs and cats, will be quite familiar to colleagues working in human medicine. Furthermore, a new educational document, VET09, exists to aid veterinarians and clinical laboratories in understanding important concepts in AST of veterinary isolates.

To illustrate an approach to AST of veterinary isolates, and to examine the similarities and differences between AST workup in veterinary and human labs, we will look at the case of a canine patient presenting at a small private veterinary clinic.

Learning About Veterinary AST From Lady Glittersparkles (*Continued*)

The Case

An 11-year-old female spayed German Shepherd, Lady Glittersparkles, is brought to a primary care veterinary clinic with the owner reporting that the patient has exhibited a two-day history of increased urinary frequency and straining, along with several accidents in the house. Lady has a history of controlled diabetes mellitus and chronic kidney disease.

A cystocentesis (ie, removal of fluid from the bladder) is performed and sent to the diagnostic laboratory at the state's veterinary academic teaching hospital for urinalysis and culture.

Veterinary microbiology testing is most often done at commercial reference labs, veterinary teaching hospitals associated with veterinary colleges, or state-supported veterinary diagnostic laboratories. Very few clinics perform microbiology testing in-house.

Lady receives an empiric 10-day prescription for the veterinary formulation of oral amoxicillin-clavulanate at the labeled dosage for dogs of 13.75 mg/kg every 12 hours.[1] Longer treatment durations remain common practice in veterinary medicine, as there is little veterinary-specific evidence for short course therapy, and practitioners' awareness of this evolution in human medicine is limited.[2] Beta-lactams and fluoroquinolones are the most common agents prescribed for urinary tract infections (UTIs) in dogs, and drugs commonly utilized in human medicine, like nitrofurantoin and trimethoprim-sulfamethoxazole, are infrequently used. Currently, no veterinary breakpoints exist for nitrofurantoin or trimethoprim-sulfamethoxazole.[3] Many veterinarians are unfamiliar with the former and are reluctant to use the latter particularly in certain large breed dogs such as shepherds due to concerns about their genetic predisposition for idiosyncratic reactions, though the degree of risk remains controversial.

Small animal veterinary medicine relies almost exclusively on oral agents or long-acting single-dose subcutaneous injectables due either to the difficulty or expense of outpatient parenteral antimicrobial therapy (OPAT) administration, or the difficulty in administering oral medication to unruly pets. Furthermore, as expenses are paid out of pocket by owners, the type and extent of diagnostic testing that may be performed and cost of prescriptions must also be taken into consideration. For all these reasons, veterinary fluoroquinolones (enrofloxacin, orbifloxacin, marbofloxacin, and pradofloxacin) or third-generation cephalosporins (cefpodoxime) are frequently utilized, especially for cats because of the greater ease afforded by once-daily dosing. A long-acting, injectable 3rd-generation cephalosporin, cefovecin, is also frequently used to treat UTIs in cats when oral administration is not possible. (**Note:** these descriptions are not intended as a position statement on antimicrobial selection or stewardship but are included to provide insight into several of the factors surrounding testing and use of antimicrobial agents which are unique to veterinary medicine.)

A standard quantitative culture is performed at the microbiology laboratory, where 1 µL of urine is inoculated on each half of a blood/MacConkey agar biplate, and 10 µL is plated to a separate blood agar plate. After overnight incubation at 35°C in 5% CO_2, a single colony morphology is observed and quantitated as >100,000 CFU/mL. The isolate is a gram-negative rod that is beta-hemolytic, lactose-fermenting, indole-positive, and oxidase-negative, allowing for an at-the-bench identification of *E. coli.*

For less straightforward identifications, many veterinary laboratories still rely on biochemical methods, though acquisition and utilization of MALDI-TOF MS is increasing.

Learning About Veterinary AST From Lady Glittersparkles (*Continued*)

As is true for humans, *E. coli* is the most common cause of UTI in both dogs and cats. Other species commonly implicated in UTIs will also be familiar, including staphylococci (most often *S. pseudintermedius* in dogs and *S. felis* in cats), enterococci, and somewhat less frequently, *Proteus mirabilis* and *Klebsiella pneumoniae*.

One very notable difference between human and veterinary AST is that the FDA does not regulate AST devices for veterinary use, nor does it set veterinary breakpoints.

Veterinary AST is most often performed using commercial broth microdilution MIC methods with panels designed specifically for veterinary medicine.

Disk diffusion testing is less common as there are no disk diffusion breakpoints for many of the agents that might be tested in a veterinary laboratory. This is usually due to an absence of disk correlate data at the time breakpoints were established or the result of poor correlation between MIC and disk methods. In the current case, the laboratory tests the isolate using a commercial Gram-negative broth microdilution panel for companion animals, yielding the results shown in Table 2.

Table 2. AST results for *E. coli* urine isolate

Organism: E. coli Quantity: >100,000 CFU/mL		
Antimicrobial Agent	**MIC (µg/mL)**	**Interpretive Category**
Amikacin	4	S
Ampicillin	8	S
Amoxicillin-clavulanate	4/2	S
Cefazolin	2	S
Cefovecin	1	S
Cefpodoxime	≤ 1	S
Cephalexin	8	S
Enrofloxacin	≤0.12	S
Gentamicin	≤1	S
Marbofloxacin	≤0.12	S
Nitrofurantoin	≤16	S
Trimethoprim-sulfamethoxazole	≤0.5/9.5	S

Note: Additional agents tested on the panel but not reported include (µg/mL): ceftazidime ≤1 S, chloramphenicol 8 S, imipenem ≤1 S, piperacillin-tazobactam 2/4 S

For most of these agents, canine-specific breakpoints are published in Table 2A of VET08-Ed4. Agents of interest for which canine-specific breakpoints have not been published are generally interpreted using human breakpoints from M100. These are reproduced in VET08 and designated as human breakpoints, with the recognition that they may be less predictive of clinical outcomes as compared to veterinary-specific breakpoints. They include chloramphenicol, imipenem, nitrofurantoin, and trimethoprim-sulfamethoxazole. Many veterinary laboratories routinely suppress results for chloramphenicol and carbapenems, and their use is prohibited in some countries, although these agents may be reported and considered for use in some countries when few other options exist. Additionally, canine-specific breakpoints for ceftazidime and piperacillin-tazobactam are listed as secondary agents, to be selectively reported. Table 3 shows an example of how veterinary-specific and human breakpoints are presented in VET08-Ed4.

Learning About Veterinary AST From Lady Glittersparkles (*Continued*)

Table 3. Excerpt from CLSI VET08-Ed4

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	Disk Content	Zone Diameter Breakpoints and Interpretive Categories (nearest whole mm)			MIC Breakpoints and Interpretive Categories (µg/mL)			Comments
					S	I	R	S	I	R	
Dogs											
A	UTI	Cefovecin	*E. coli* *P. mirabilis*	30 µg	≥24	21–23	≤20	≤2	4	≥8	
Cats											
A	UTI	Cefovecin	*E. coli*	30 µg	≥24	21–23	≤20	≤2	4	≥8	
Humans											
		Trimethoprim-sulfamethoxazole	*Enterobacteriaceae*	1.25/23.7 µg	≥16	11–15	≤10	≤2/38	–	≥4/76	

For cefovecin, there are dog-specific and cat-specific breakpoints. At this time, human breakpoints (denoted with grey shading) must be applied for interpreting trimethoprim-sulfamethoxazole on veterinary isolates, as veterinary breakpoints have not been established.

Summary

This case is simple but intended to demonstrate the value of CLSI VET documents in providing veterinary laboratories with standards that assure high quality AST results are produced. It also shows the overarching similarities between the work of the human and veterinary subcommittees on AST.

The *E. coli* encountered here is pan-susceptible, and the empiric therapy is likely to bring resolution. Increasingly, though, many cases are less straightforward. Advances in veterinary medicine over the past 20 years enable treatments that often parallel interventions and therapies seen in human medicine. Orthopedic surgery, radiation oncology, and dermatology are just a few of the specialties that have greatly extended and improved quality of life for veterinary patients. As is true in human medicine, however, opportunistic infections and resistance phenotypes that were previously rare are now encountered with more regularity in small animal medicine, including oxacillin-resistant staphylococci causing skin, urinary tract, and even prosthetic joint infections in dogs,[4] multidrug-resistant *Pseudomonas aeruginosa* from chronic ear infections in dogs, and ESBL-producing Enterobacterales in multiple species. These challenges highlight the necessary work of VAST as it continues to develop veterinary-specific criteria that assist appropriate selection of antimicrobial agents in veterinary medicine.

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Practical Tips

Applying Susceptibility Interpretations to the *Candida parapsilosis* species Complex

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As DNA sequence- and MALDI-TOF-based identification of yeasts becomes widespread, we have learned much about the prevalence of cryptic species within species complexes. One of the more common *Candida* species, *Candida parapsilosis*, is part of a three species complex including *C. orthopsilosis* and *C. metapsilosis*. Both *C. orthopsilosis* and *C. metapsilosis* are cryptic species. From a worldwide surveillance program collected between 2006 and 2016, the *C. parapsilosis* species complex isolates were comprised of 95.5% *C. parapsilosis*, 3.2% *C. orthopsilosis*, and 1.3% *C. metapsilosis*.[1] A conundrum that arises in the clinical laboratory is that there are separate epidemiological cutoff values (ECVs) for each of the three species,[2] but there are only breakpoints for *C. parapsilosis*.[3] How should the laboratory report results of antifungal susceptibility tests of C. *parapsilosis* complex isolates?

If a laboratory is able to perform species identification within the species complex:

- *C. parapsilosis* breakpoints should only be used for *C. parapsilosis* sensu stricto isolates.
- For isolates identified as *C. metapsilosis* or *C. orthopsilosis,* the species-specific ECVs should be used with a comment that ECVs are not a predictor of clinical outcome but may help identify non-wild type isolates. Other suggestions of interpretive comments regarding ECVs are available within the CLSI M59 document.[2]

If a laboratory is able to identify *C. parapsilosis* but cannot distinguish the other species within the complex, the *C. parapsilosis* breakpoints can be used. When the original *C. parapsilosis* breakpoints were set, it is likely that a low number of cryptic species within this complex were included. If laboratories cannot distinguish between the complex and are applying the *C. parapsilosis* breakpoints, it may be prudent to include a comment such as the following: "*Candida parapsilosis* is part of a species complex but the other species cannot be distinguished by the procedures used in this laboratory. While the prevalence of these other species is low, the predictive clinical response when applying the C. *parapsilosis* breakpoints used here to them is unknown."

For antifungals with no breakpoints or ECVs of *C. parapsilosis* or other members of the species complex, such as flucytosine, no interpretation should be reported. It is not acceptable to use an ECV from a closely related species for interpretation.

Table 1. *Candida parapsilosis* breakpoints and species specific epidemiological cutoff values for the *C. parapsilosis* species complex[2,3]

Antifungal	Breakpoints (µg/ml)	Epidemiological cutoff values (µg/ml)		
	C. parapsilosis	*C. parapsilosis* sensu stricto	*C. orthopsilosis*	*C. metapsilosis*
Fluconazole	≤2 S; ≥8 R	2	2	4
Itraconazole	N/A	0.5	0.5	1
Posaconazole	N/A	0.25	0.25	0.25
Voriconazole	≤0.12 S; ≥1 R	No ECV	0.125	0.06
Anidulafungin	≤2 S; ≥8 R	4	2	0.5
Caspofungin	≤2 S; ≥8 R	1	1	0.25
Micafungin	≤2 S; ≥8 R	2	1	1
Amphotericin B	N/A	1	2	1

Abbreviations: N/A, not available; R, resistant; S, susceptible.

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Hot Topic

Cefiderocol and Lefamulin: What Do Clinical Microbiologists Need to Know?

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Three new antimicrobial agents were approved by the US Food and Drug Administration (FDA) for treating bacterial infections in the past year: cefiderocol, imipenem-relebactam, and lefamulin. To help familiarize clinical microbiology laboratories with these agents, the present and next issue of the News Update will provide a snapshot of the role of these new agents. Cefiderocol and lefamulin will be discussed here, and the next issue will focus on imipenem-relebactam.

Table 1. Basic features of cefiderocol[1] and lefamulin[2]

	Cefiderocol	Lefamulin
Trade Name	Fetroja®	Xenleta™
Manufacturer	Shionogi, Inc.	Nabriva Therapeutics US, Inc.
Drug Class	Cephem (Sub-class: siderophore cephalosporin)	Pleuromutilin
Route of Administration	Intravenous	Intravenous, Oral
FDA approval date	November, 2019	August, 2019
FDA approved for treatment of infections	Complicated urinary tract infection, including pyelonephritis in patients ≥18 yo with limited or no alternative treatment options	CABP in adults
Organisms for which clinical efficacy has been demonstrated as listed in the FDA drug label	*Escherichia coli* *Enterobacter cloacae* complex *Klebsiella pneumoniae* *Proteus mirabilis* *Pseudomonas aeruginosa*	*Chlamydophila pneumoniae* *Haemophilus influenzae* *Legionella pneumophila* *Mycoplasma pneumoniae* *Staphylococcus aureus* (methicillin-susceptible) *Streptococcus pneumoniae*
Additional organisms for which activity has been demonstrated *in vitro* as listed in the FDA drug label	*Stenotrophomonas maltophilia* Carbapenem resistant (CR)- Enterobacterales CR-*Pseudomonas aeruginosa* CR-*Acinetobacter baumannii*	*Haemophilus parainfluenzae* *Moraxella catarrhalis* *Staphylococcus aureus* (methicillin-resistant) *Streptococcus agalactiae* *Streptococcus pyogenes* *Streptococcus* viridans group (several species)
Inactive against	Most gram-positive bacteria and anaerobic bacteria	Enterobacterales, *Pseudomonas aeruginosa* and other nonfermenters
Treatment Strategy	Multidrug resistant gram-negative bacteria including some carbapenem-resistant strains and non-fermenters	An alternative to current agents (eg, beta-lactams, macrolides, fluoroquinolones) for treating CABP

Abbreviation: CABP, community acquired bacterial pneumonia.

Cefiderocol

1. What is cefiderocol? Is it like any other antimicrobial agent currently tested?

Cefiderocol is a sidero- (iron), phore (bearing) cephalosporin. Cefiderocol has a cephalosporin core that is conjugated to a catechol moiety that chelates iron. The iron-cefiderocol complex is actively transported by bacterial iron-transport systems across the outer membrane. Once in the periplasmic space, cefiderocol inhibits penicillin binding protein 3 (PBP3), resulting in cidal activity.[1,3] Cefiderocol has increased stability against hydrolysis by ESBL, AmpC and carbapenemase enzymes compared to other cephalosporins.[1,3]

Cefiderocol and Lefamulin: What Do Clinical Microbiologists Need to Know? (*Continued*)

2. Should cefiderocol be tested routinely? When might a laboratory be asked to test cefiderocol?

Cefiderocol is used selectively for treatment of infections due to multidrug resistant (MDR) gram-negative organisms. Most laboratories will test cefiderocol on special request, either in-house or at a reference laboratory. Some laboratories may reflexively test cefiderocol on specific MDR gram-negative organisms.

3. How should cefiderocol be tested? Are there any unique testing considerations?

CLSI reference disk diffusion and broth microdilution MIC methods can be used for testing cefiderocol:
Disk Diffusion: unsupplemented Mueller-Hinton agar (MHA)[4]; FDA-cleared disks are available from two manufacturers (see Table 2).
Broth Microdilution: special, iron-depleted cation-adjusted Mueller-Hinton broth (CAMHB); recommendations for preparation of this medium are provided in CLSI M100.[5]
Thermo Scientific™ Sensititre™ is the only FDA-cleared MIC method for cefiderocol (see Table 2).

Table 2. Testing options for cefiderocol and lefamulin

Antimicrobial	Disk manufacturer (disk content)	Gradient diffusion	Broth microdilution
Cefiderocol	Hardy Diagnostics[a] (30 µg) Liofilchem[b] (30 µg)	Not available	Thermo Scientific™ Sensititre™[a]
Lefamulin	Hardy Diagnostics (20 µg)[c] Liofilchem[b] (20 µg) (5 µg for use with EUCAST breakpoints)	Liofilchem[c]	Not available

[a]FDA cleared for testing *Escherichia coli, Enterobacter cloacae* complex, *Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa*

[b]Research use only in the USA

[c]FDA cleared for testing *Streptococcus pneumoniae, Staphylococcus aureus* (methicillin-susceptible) and *Haemophilus influenzae*

4. How should cefiderocol results be interpreted? Why are there different breakpoints from various organizations?

Breakpoints provided by FDA, CLSI, and EUCAST are listed in Table 3. There are some distinct differences.

CLSI set "INV" or "investigational" breakpoints for cefiderocol in January 2019 based on MIC distribution and PK/PD data for Enterobacterales, *P. aeruginosa, Acinetobacter* spp., and *S. maltophilia*. No clinical outcome data were available at the time. The CLSI INV breakpoint designation is used for antimicrobials that have not yet been FDA approved. Cefiderocol was submitted to FDA in late 2019. FDA reviewed the same PK/PD and MIC distribution data as CLSI, but additionally reviewed clinical trial patient outcome data which had become available in the interim. FDA set breakpoints for Enterobacterales and *Pseudomonas aeruginosa* which differed from those of CLSI. They did not recognize *Acinetobacter* spp. or *S. maltophilia* breakpoints, based on the results of the clinical studies, which showed elevated mortality rates for patients infected with these organisms.[6]

CLSI will reevaluate cefiderocol breakpoints soon. In the meantime, it is suggested that clinical laboratories use the FDA MIC and disk diffusion breakpoints when testing cefiderocol. If testing for *Acinetobacter* spp. or *S. maltophilia* is requested by an infectious diseases physician, laboratories should discuss the pros and cons of using CLSI INV breakpoints with the provider, prior to testing.

EUCAST published cefiderocol breakpoints in April 2020 and did not establish intermediate breakpoints ("I" implies susceptible, increased exposure, per EUCAST definitions). Nor did they set criteria for an ATU (area of technical uncertainty) which is a EUCAST term to warn laboratories of uncertain interpretation of some values obtained by MIC or disk diffusion testing. EUCAST did not set breakpoints for *Acinetobacter* spp. or *S. maltophilia*, although they did recognize epidemiological cut-off values for these organisms.

Cefiderocol and Lefamulin: What Do Clinical Microbiologists Need to Know? (*Continued*)

5. What are expected AST results for cefiderocol?

Data demonstrate >80% of carbapenem-resistant (CR) Enterobacterales, CR-*P. aeruginosa*, CR-*A. baumannii* and *S. maltophilia* are susceptible to cefiderocol by CLSI breakpoints.[1,7] Cefiderocol is active against most isolates of Enterobacterales that produce metallo beta-lactamases (eg, NDM, VIM, IMP), making cefiderocol an attractive option to treat these often pan-drug resistant isolates.[6,7]

A recent study showed the MIC_{90} values for MDR *A. baumannii* and *S. maltophilia* were 1 and 0.25 µg/mL, respectively. This means that at least 90% of the isolates tested would be categorized as susceptible to cefiderocol using the CLSI INV breakpoints for these species. The MIC_{90} for 27 isolates of CR-*P. aeruginosa* was 0.5 µg/mL, suggesting most CR-*P. aeruginosa* are susceptible to cefiderocol.[3]

Data from over 700 carbapenemase-producing isolates of Enterobacterales (including both serine and metallo beta-lactamase producers) demonstrated approximately 80% of isolates had MICs ≤2 µg/mL[3] which is susceptible according to breakpoints published by all three organizations.

Table 3. FDA, CLSI and EUCAST breakpoints for cefiderocol[5, 8-9]

	FDA Breakpoints						CLSI INV Breakpoints						EUCAST Breakpoints			
	MIC (µg/mL)			DD (mm)[a]			MIC (µg/mL)			DD (mm)[a]			MIC (µg/mL)		DD (mm)[a]	
Organism	S	I	R	S	I	R	S	I	R	S	I	R	S	R	S	R
Enterobacterales[b]	≤2	4	≥8	≥18	14-17	≤13	≤4	8	≥16	≥16	12–15	≤11	≤2	>2	≥22	<22
P. aeruginosa	≤1	2	≥4	≥25	19-24	≤18	≤4	8	≥16	≥18	13-17	≤12	≤2	>2	≥22	<22
Acinetobacter spp.	-	-	-	-	-	-	≤4	8	≥16	≥15	11-14	≤10	IE[c]	IE	-	-
S. maltophilia	-	-	-	-	-	-	≤4	8	≥16	≥17	13-16	≤12	IE[c]	IE	-	-

Abbreviations: DD, Disk Diffusion; IE, Insufficient evidence to set clinical breakpoints; I, Intermediate; INV, investigational; R = Resistant; S, Susceptible.
[a]Disk content = 30 µg
[b]Enterobacterales FDA breakpoints are for *E. coli, K. pneumoniae, P. mirabilis* and *E. cloacae* complex only
[c]EUCAST epidemiological cut-off value is ≤0.25 µg/mL for *Acinetobacter baumannii* and 0.06 µg/mL for *S. maltophilia*

Lefamulin

1. What is lefamulin? Is it like any other antimicrobial agent that we are currently testing?

Lefamulin belongs to the pleuromutilin class of antimicrobial agents. It is bactericidal and inhibits protein synthesis by preventing tRNA positioning in the bacterial ribosome. Retapamulin is the only other pleuromutilin approved for use in humans and is used topically to treat skin infections such as impetigo.[10] Tiamulin and valnemulin are pleuromutilins approved for use in veterinary medicine.[11]

Lefamulin is used for treatment of patients with CABP. Use of traditional agents for CABP, such as macrolides, penicillin and fluoroquinolones is hampered by emerging resistance[12] and safety concerns (ie, fluoroquinolones).[13] As such, alternative agents such as lefamulin are needed for some cases of CABP.

2. Should lefamulin be tested routinely against bacterial pathogens associated with CABP (eg, respiratory isolates)? When might a laboratory be asked to test lefamulin?

CABP is often treated empirically and >99.0% of the bacteria associated with CABP are susceptible to lefamulin. As such, routine AST is not indicated.[12] If antimicrobial resistance is suspected, testing can be performed.

3. How should lefamulin be tested? Any unique testing considerations?

CLSI reference disk diffusion and broth microdilution MIC methods can be used for testing lefamulin:
Disk Diffusion: unsupplemented MHA; supplemented MHA as appropriate for fastidious species;[4] FDA-cleared disks are available from a single manufacturer (see Table 2).
Broth Microdilution: CAMHB; supplemented CAMHB as appropriate for fastidious species.[5]
Liofilchem MTS is the only FDA-cleared MIC method for cefiderocol (see Table 2).

Cefiderocol and Lefamulin: What Do Clinical Microbiologists Need to Know? (*Continued*)

4. How should lefamulin results be interpreted? Why are there only susceptible breakpoints?

Disk diffusion and MIC breakpoints for lefamulin are currently only available from FDA[8] and these can be used for interpreting and reporting results. Only "susceptible" breakpoints have been assigned (see Table 4) as nearly all isolates examined to date have MICs below the susceptible breakpoint. A result for lefamulin other than susceptible is classified as "nonsusceptible" and should be investigated.

Table 4. FDA breakpoints for lefamulin

Bacteria	FDA Breakpoints[a]					
	MIC (µg/mL)			DD (mm)[b]		
	S	I	R	S	I	R
Staphylococcus aureus[c]	≤0.25	-	-	≥23	-	-
Streptococcus pneumoniae	≤0.5	-	-	≥17	-	-
Haemophilus influenzae	≤2	-	-	≥17	-	-

Abbreviations: DD, disk diffusion; I, intermediate; S, susceptible; R, resistant.
[a]Isolates with MIC or disk diffusion results other than susceptible should be confirmed.
[b]Disk content = 20 µg
[c]Methicillin-susceptible isolates only
Clinical breakpoints are forthcoming from CLSI and EUCAST.

5. What are expected AST results for lefamulin?

Lefamulin is highly active against common bacterial pathogens that cause CABP.[11] In one study, nearly all *S. pneumoniae* (including penicillin and/or macrolide and/or fluoroquinolone resistant strains), *Staphylococcus aureus* (including methicillin resistant strains) and *Haemophilus influenzae* (including beta-lactamase producing strains) were susceptible using FDA breakpoints. Lefamulin was highly active against *Moraxella catarrhalis* as well.[12]

Developing a Strategy for AST of Cefiderocol and Lefamulin

Cefiderocol is an attractive option for treating some infections caused by MDR gram-negative organisms. It would be prudent for clinical laboratories to identify a strategy for testing this agent now, as limited options exist for in-house testing and few reference laboratories offer testing. In contrast, lefamulin testing is unlikely necessary at this time, given the rare incidence of resistance.

When implementing AST for cefiderocol or any new agent, a verification study is needed. Questions for the clinical microbiology laboratory to consider when a new antimicrobial agent becomes available are listed in Table 5. Previous editions of **CLSI AST News Update** (Vol. 1, Issue 2, Winter 2016; Vol. 4 Issue 2 June 2019) addressed verification study design. Detailed guidance for verification of ASTs is available in CLSI M52.[14] Verification studies may include 30 or perhaps fewer clinical isolates if the method is already in use for other agents in a laboratory. Laboratories must take into consideration a variety of factors when planning the verification to include:

- Any unique considerations for testing the agent by a particular method.
- Experience with the method and expertise of the laboratory in performing AST.
- The extent to which QA protocols in place in the laboratory might identify any subtle problems with the AST.
- Availability of strains with varying degrees of resistance or susceptibility.
- Any other information gleaned from the literature or colleagues who have tested the agent.

Although the CDC FDA Antimicrobial Resistant (AR) Bank often contains isolates that can be used for verification of ASTs for newer drugs,[15] at this time no strains are available for cefiderocol or lefamulin. It is possible that the manufacturer of the drugs may provide assistance.

Cefiderocol and Lefamulin: What Do Clinical Microbiologists Need to Know? (*Continued*)

Table 5. Questions for the clinical microbiology laboratory to consider when a new antimicrobial agent becomes available

#	Question
1	Does your antimicrobial stewardship program(ASP) recognize a need for antimicrobial susceptibility testing (AST) of the new agent for patients served by your laboratory? Will this antimicrobial be available on formulary?
2	Does the projected testing volume warrant consideration for testing in-house?
3	What testing methods are available?
4	Can testing be done by a method currently used in your laboratory?
5	Are there studies that demonstrate reliability of the method(s) that might work for your laboratory? a. Any reports of difficulties in testing? b. Any special requirements (eg, special QC strains)?
6	Does your laboratory have resources to implement in-house testing? a. Staffing? b. Organisms and system to perform verification studies, including identification of a CLIA-certified reference laboratory that can arbitrate discordant results? c. Quality control plan - IQCP and QC in use? d. IT support to enable reporting through electronic health record (EHR) and for billing?
7	Is the new agent tested by the reference laboratory(s) that serves your laboratory? a. What is the turnaround time?
8	What option would be most cost efficient for the laboratory?
9	What option would be most cost efficient for patient management in your facility?
10	What option would be acceptable for patient care considering turnaround time for in house versus send out testing?

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Cefiderocol and Lefamulin: What Do Clinical Microbiologists Need to Know? (*Continued*)

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For additional information on cefiderocol and lefamulin, ASM members can access ASM Virtual Journal Club that discussed this topic: Available on demand: Journal club on May 12, 2020 **here.**

In Memoriam

Mary Jane Ferraro, PhD, MPH

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Dr. Mary Jane Ferraro, an eminent clinical microbiologist, died on January 18, 2020 after a prolonged illness. She was 72. Dr. Ferraro's work with CLSI spanned 30 years, during which her steadfast commitment, technical expertise, and strong leadership impacted the practice of clinical microbiology, most notably in the realm of improvements to the standards and methods used for antimicrobial susceptibility testing. Dr. Ferraro served as the first woman chairholder of the CLSI AST Subcommittee and went on to chair the CLSI Area Committee on Microbiology, to serve as Vice Chairholder of the CLSI Microbiology Expert Panel, and to serve on the CLSI Board of Directors.

A native of Pittsfield, Massachusetts, Dr. Ferraro received her PhD degree in Medical Sciences-Microbiology from Boston University School of Medicine and then earned her MPH through a Harvard School of Public Health post-doctoral fellowship program designed to train directors of large infectious diseases laboratories. In 1976, she was recruited to the Massachusetts General Hospital as an Assistant Director of the Bacteriology Laboratory, and by 1984, she had become the Director of the MGH Clinical Microbiology Laboratories. During her 40-plus year career at the MGH, Dr. Ferraro was known for her dedication to excellence. She was meticulous, tenacious, and had an uncanny knack for quickly identifying the root cause of almost any problem. She trained and mentored numerous microbiologists and fellows, many of whom have gone on to serve as leaders in clinical microbiology. Dr. Ferraro became Professor of Pathology in 2004 and Professor of Medicine in 2006, making her one of only a small number of women to have ever held dual professorships at Harvard Medical School.

In Memoriam

Dr. Ferraro contributed her expertise and vision to numerous national and international committees, as a consultant to various organizations and as an invited speaker at scientific meetings worldwide. She authored many research publications, review articles, and book chapters and served as co-editor of the Clinical Microbiology Newsletter, section editor of the Manual of Clinical Microbiology, and editorial board member of the Journal of Clinical Microbiology and Diagnostic Microbiology & Infectious Disease. Dr. Ferraro was a Fellow of the American Academy of Microbiology and of the Infectious Diseases Society of America. She received numerous other honors, including the CLSI Russell J. Eilers Memorial Award in 2001, the CLSI John V. Bergen Excellence Award in 2006, and the American Society for Microbiology Sonnenwirth Award for leadership in clinical microbiology in 2018.

In addition to her impressive professional accomplishments, Dr. Ferraro made the time to nurture wonderful friendships – for example, through longstanding Friday evening get-togethers over the phone with friends who had moved to other cities (virtual cocktail hours ahead of their time!). She and her husband, Robert C. Moellering, Jr., an outstanding infectious diseases physician-scientist who passed away in 2014, travelled the world together and shared a love for Italy, opera, and fine wines.

Dr. Ferraro will be greatly missed by her family, friends, and colleagues. She dedicated her career to the care of patients with infectious diseases through the practice of clinical microbiology, improving the lives of innumerable individuals. She left us a legacy of excellence, and the impact of her professional contributions, mentorship, and friendship will be long lasting.

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