



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.

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 INF D 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](#).

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

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Webinars

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

Upcoming Webinar

CLSI 2019 Antimicrobial Susceptibility Testing Update

Wednesday, February 26, 2020 | 1:00–2:30 PM Eastern (US) Time

Thursday, February 27, 2020 | 3:00–4:30 PM Eastern (US) Time

Moderator

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

Presenters:

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

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

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:

- Understanding Breakpoint Decisions: CLSI Rationale Documents (FREE, December 2019)
- CLSI-CAP Annual Webinar: “Rational Approach to Antibacterial and Antifungal Breakpoints” (November 2019)
- Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings (July 2019)
- *CLSI-SIDP ACCP Annual Webinar; “Merging Microbiology and Stewardship: Making the most of 2019 CLSI updates on antimicrobial susceptibility testing for gram positive and negative bacteria in your stewardship activities” (June 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)

* This webinar was not hosted by CLSI, but can be purchased on demand [here](#).

Upcoming Presentation at ASM Microbe 2020, Chicago, IL

If you will be attending the meeting, please check out:

Date/Time: June 22, 2020 | 8:15-10:15 AM

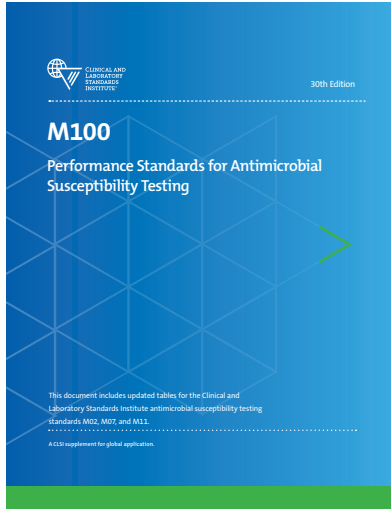
Title: Importance of Reliable Generation and Appropriate Interpretation of AST Results in 2020

Archive of Retired Breakpoints

An archive of breakpoints removed from M100 since 2010 together with the rationale for their removal is available [here](#).

Similarly, an archive of methods removed from M100 since 2017 is available [here](#).

New/Updated CLSI AST Documents Are Here!



M100 | Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition

Major changes include:

New Breakpoints:

- Cefiderocol investigational disk diffusion breakpoints for *Enterobacterales*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., and *Stenotrophomonas maltophilia*
- Colistin and polymyxin B MIC breakpoints for *Enterobacterales* (formerly ECV)
- Daptomycin MIC breakpoints for *Enterococcus faecium* only

Revised Breakpoints:

- Colistin and polymyxin B MIC breakpoints for *P. aeruginosa* and *Acinetobacter* spp.
- Daptomycin MIC breakpoints for *Enterococcus* spp. other than *E. faecium*

New Recommendations:

- Colistin agar test for *Enterobacterales* and *P. aeruginosa*
- Colistin broth disk elution test for *Enterobacterales* and *P. aeruginosa*
- Mueller-Hinton fastidious agar (MH-F) as an alternative medium for disk diffusion testing of *Streptococcus pneumoniae*
- Preparation of iron-depleted CAMHB for testing cefiderocol and reading MICs for cefiderocol

Expanded/Updated Recommendations:

- Definition of “intermediate” (I) and addition of “I[^]” interpretive category for several agents that have the potential to concentrate at an anatomical site
- Definition of “susceptible-dose dependent” interpretive category
- Definitions for Test/Report Groups in Table 1
- Issues that should be considered when testing/reporting colistin and polymyxin B
- Major update and reformatting of Appendix A that suggests those AST results that should be confirmed prior to reporting

Revised Recommendations:

- Zone diameter criteria that suggests a confirmatory MIC test should be performed for ceftazidime-avibactam and *Enterobacterales*
- Disk diffusion QC range for *E. coli* ATCC® 25922 and ciprofloxacin
- Minocycline and levofloxacin now in Test/Report Group A for *S. maltophilia*

Revised Terminology:

- Coagulase-negative staphylococci (CoNS) now referred to as “Other *Staphylococcus* spp.” as more CoNS species (eg, *Staphylococcus pseudintermedius*) are being addressed by name in M100
- The family *Enterobacteriaceae* now listed as the order *Enterobacterales*. **See details on page 14** of this News Update

VET09 Report | Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings, 1st edition (July 2019)

- Provides veterinarians with the information needed to successfully acquire and interpret antimicrobial susceptibility test results
- Promotes common understanding between the veterinarian and veterinary microbiologists by providing example culture and susceptibility reports
- Provides animal species-specific guidance on applying breakpoints to interpret susceptibility test results

For additional information, see archived July 2019 webinar [“Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings.”](#)

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:

MR03 | Meropenem Breakpoints for *Acinetobacter* spp. (September 2019)

MR04 | Azithromycin Breakpoint for *Neisseria gonorrhoeae* (May 2019)

MR05 | Ceftaroline Breakpoints for *Staphylococcus aureus* (November 2019)

MR06 | Daptomycin Breakpoints for Enterococci (September 2019)

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](#).

Future CLSI AST Meetings

January 26–28, 2020

Tempe, Arizona, USA

January 23-26, 2021

Arlington (Dallas), Texas, USA

June 26-29, 2021

San Diego, California, USA

June 14–16, 2020

Baltimore, Maryland, USA

Check It Out! Educational Workshops Held at CLSI Meetings

Nicole Scangarella-Oman, GlaxoSmithKline



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

The June 2019 workshop, held in Dallas, TX, was entitled "To MIC or Not to MIC; That Is the Question. Molecular Characterization of Antimicrobial Resistance (AR) for Healthcare in 2019." This workshop included presentation and discussion of three clinical cases from the perspectives of a clinical microbiologist, a public health microbiologist, and a clinician.

Speakers discussed:

- Molecular testing and reporting for patient diagnosis.
- Molecular testing for surveillance and investigation in outbreak settings.
- Use of molecular test results in guiding patient management.

In addition, incorporation of results from molecular AR tests into annual cumulative antibiograms was addressed.

The complete set of slides presented for all cases can be found [here](#).

Featured Article

Ensuring Reliable Antimicrobial Susceptibility Test (AST) Results When Using an Automated AST System

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Introduction

Timely and accurate antimicrobial susceptibility testing (AST) is one of the principal tasks of the clinical microbiology laboratory. In a 2016 survey of clinical laboratories in California, 84/89 (94.4%) of the laboratories surveyed performed AST exclusively with an automated method.¹ As compared to manually performed ASTs, workflow may be significantly improved when using automated AST systems. Objective interpretation of endpoints coupled with the utilization of result assessing software help to generate reliable and consistent results with shortened turnaround times.

When using an automated AST system, it is important to understand the key processes that must be strictly adhered to in order to ensure the system performs as intended. This article will focus on several of these processes, including: preparing the inoculum, recognizing results that are likely reliable vs those that are unusual and require confirmation, and maintaining QC standards.

Inoculum Preparation, Panel Inoculation, and Purity Check

Appropriate processing of the inoculum is critical for obtaining reliable AST results. As with any AST method, colony age and appearance must be carefully evaluated before colonies are picked for testing. Various commercial automated AST systems have developed methods to help standardize the remaining inoculation steps by performing or simplifying one or more of the following: inoculum standardization, inoculum dilution, and AST panel inoculation. Laboratories should perform all of inoculum preparation steps precisely according to the AST system manufacturer's guidelines. It may be tempting to slightly alter one or more of the recommended steps in order to further streamline a laboratory's workflow. However, prior to implementation of any modification of test instructions as provided by the manufacturer, a validation must be performed.^{2,3} Examples of such modifications to inoculum preparation include:

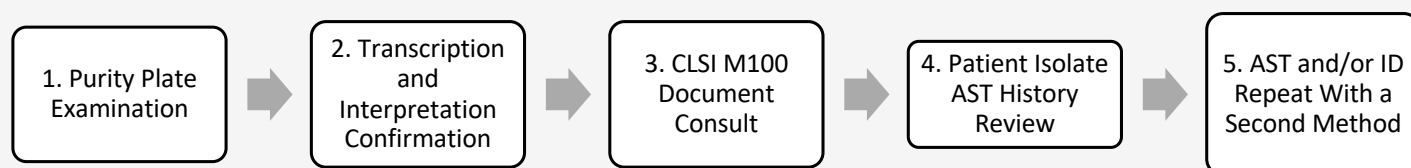
- Picking colonies from agar plates that fall outside the recommended incubation requirements (eg, age of colonies).
- Picking colonies from nonvalidated agar medium types.
- Holding the inoculum suspension longer than recommended prior to AST panel inoculation.

Once the inoculum is prepared and standardized, the panel must be inoculated and incubated within the manufacturer's defined timeline. A critical step to ensure reliability of AST results involves performance of a purity check of the inoculum as testing a contaminated inoculum can lead to erroneous results. The purity plate is prepared by subculturing an aliquot from the inoculum suspension to a nonselective agar plate (eg, blood agar plate [BAP]) following AST panel inoculation. It is important that the subculture yields a sufficient number of isolated colonies. For example, if subculturing from a suspension containing 5×10^5 CFU/ml, a subculture using a 1 μ l loop will reveal approximately 500 colonies, which is usually adequate to detect contamination. CO₂ incubation of the purity plate may improve detection of contaminating organisms.

Ensuring Reliable AST Results and Confirming Unusual Results

Once AST is completed, the technologist must carefully review the purity plate and all results generated prior to releasing the final report. Each laboratory must develop a protocol that describes both typical AST results for a given species with a list of appropriate reportable drugs, as well as those AST results that are atypical/unusual and need further action to be taken before reporting. CLSI provides guidance for results that should be confirmed prior to release in CLSI M100, Appendix A.⁴ A suggested sequential workflow for follow up action for confirming unusual results is shown below in Figure 1.

Figure 1. Sequential Workflow for Confirming Unusual AST Results



Ensuring Reliable Antimicrobial Susceptibility Test (AST) Results When Using an Automated AST System (*Continued*)

1. Purity Plate Examination

The purity plate should be examined with both transmitted and reflected light while looking for inconsistencies in colony morphologies including varying colors, sizes, colony edges, and textures that suggest the organisms inoculated into the AST might have been contaminated. In the case of rapid AST results (<16 hours), purity plate growth may not be sufficient to detect contamination by the time AST results are available. If an unusual AST result is present, results should not be released until the purity plate is examined following at least 16 hours incubation, unless otherwise stated by the manufacturer. When it is deemed necessary to take additional steps to confirm an unusual AST pattern, it is often wise to re-incubate the purity plate for a second day. This may reveal subtle contamination due to slower growing organisms such as *Stenotrophomonas maltophilia*. Monitoring the rate of contaminated purity plates could be considered as an AST QA monitor.

2. Transcription and Interpretation Confirmation

Most laboratories that use automated AST systems transmit results to the laboratory information system (LIS) using an interface. However, if AST results are manually transcribed at any stage in the process, a thorough check of all inputted values may help resolve an unusual result due to a transcription error.

Software provided with automated AST and LIS systems usually includes a feature that allows application of expert rules to AST results generated on individual isolates. Some of these may be required and others are optional. They generally flag atypical/inconsistent results, suggest confirmatory tests, enable suppression of certain agents based on the overall phenotype, and add comments to reports. Expert rules can override “S” or “I” results to “R” consistent with current CLSI AST reporting recommendations. Some AST systems provide optional expert rules that edit “S” or “I” results to “R” based on a phenotype that suggests a specific resistance mechanism. Close collaboration with the institution’s antimicrobial stewardship program (ASP) is highly encouraged to determine which of the optional expert rules available with an automated AST system or LIS are appropriate in their institution.

3. CLSI M100 Document Consult

It is critical for the bench technologist to become familiar with the current CLSI M100 document that contains recommendations for AST and reporting of microbial species most commonly tested for antimicrobial susceptibility. Table 1 (below) provides an overview of select tables and appendices within M100 that are particularly useful to help ensure the reliability of AST results reported on a particular isolate and are often referenced when generating expert rules as discussed above.

Table 1. Select Tables in CLSI M100 That Can Help Ensure Reliable Reporting of AST Results

Table or Appendix	Brief Overview of Content
Table 1A and 1B Suggested Routine and Supplemental Reporting of Specific Agents including Urine Source Restrictions	<ul style="list-style-type: none"> Lists agents appropriate for reporting on select species and body sites Provides guidance for application of antimicrobial agent suppression rules Important reminder: <ul style="list-style-type: none"> Unexpected resistance must be reported even if suppression rules are in place (eg, report carbapenem resistance even if an isolate of <i>Enterobacterales</i> is susceptible to extended-spectrum cephalosporins).
Table 2A-2J Aerobic Organism Specific Breakpoints	<ul style="list-style-type: none"> Lists breakpoints and drugs appropriate to report on a given species Important reminders: <ul style="list-style-type: none"> Breakpoints from a different organism group must not be borrowed if none are listed for the organism group in question. In rare instances, breakpoints absent from CLSI Tables 2A-2J may be available from FDA.⁵ CLSI M45 document provides guidance for testing and breakpoints for fastidious and other infrequently encountered organisms.⁶

Ensuring Reliable Antimicrobial Susceptibility Test (AST) Results When Using an Automated AST System (Continued)

Table 1 (Continued)

Table	Brief Overview of Content
Appendix A Suggestions for Correlating Organism Identification with AST Results and Identification of Novel Resistance	<ul style="list-style-type: none"> Lists results that should be confirmed before reporting. Confirmation approach will vary depending on the type of resistance and frequency of occurrence of the resistance in a specific facility. Lists results that require notification of infection preventionists and public health authorities. <p>Important reminder:</p> <ul style="list-style-type: none"> Appendix A has been updated in the new M100 30th edition.
Appendix B Intrinsic Resistance	<ul style="list-style-type: none"> Provides intrinsic resistance profiles of commonly encountered species <p>Important reminder:</p> <ul style="list-style-type: none"> If an organism-drug result mismatch is observed, a problem with organism identification or AST must be considered.
Appendix H Correlation of Molecular AST and Phenotypic AST	<ul style="list-style-type: none"> Provides recommendations for reporting when there is a mismatch between molecular and phenotypic results when both methods are used for AST of an isolate.

Abbreviations: AST, antimicrobial susceptibility testing, FDA, US Food & Drug Administration.

4. Patient Isolate AST History Review

Prior to repeating an AST to confirm unusual AST results, it is prudent to check the patient history and note any AST results reported for the same species previously recovered from the patient. If the isolate with the unusual phenotype had been previously reported (and presumably confirmed), it is unnecessary to perform additional testing to confirm again.

5. AST and/or ID Repeat With a Second Method

Depending on the type of unusual AST result(s) encountered, it may be necessary to repeat the organism identification and/or confirm AST results with a second method. For example, any result other than susceptible (S) for antimicrobial agents with only non-susceptible (NS) breakpoints should be confirmed. A case in point would be a penicillin NS result for a beta-hemolytic streptococcus which should be retested using a CLSI reference method if an automated method repeatedly reports the same highly unusual penicillin-NS result. If a second method is not available in the laboratory, send-out testing to a reference laboratory that uses a CLSI reference method should be considered.

Prompt Notification to Infection Preventionists and Public Health Authorities

CLSI M100 Appendix A lists those types of resistance that have not been reported to date or are highly unusual. When such results are encountered, laboratories are asked to notify infection preventionists within their facility and public health authorities so effective measures can be taken to contain the spread of unusual resistance. Although it is important to confirm the unusual results using a CLSI reference method, it is best to inform the infection preventionists of a “suspect” unusually resistant isolate as promptly as possible. The laboratory director should be consulted in these cases to determine how best to handle communication of results to all appropriate stakeholders.

The Value of Routine AST QC

Routine performance of QC is essential for checking reagent quality and instrument function and may also serve to check for technical consistency between different individuals performing the AST. Manufacturers of automated AST systems provide recommendations for routine QC to include testing of specific QC strains, which are usually the same as those recommended by CLSI. It is important to use the QC ranges established by the manufacturer as in some cases they may differ slightly from CLSI QC ranges. Some QC strains must be tested routinely (daily or weekly) and others are considered supplemental. Supplemental QC strains can be used to assess a new test system, staff competency, and training. Although routine QC is essential and valuable, it cannot be assumed that every AST result generated on a patient’s isolate is accurate just because QC is “in control.” AST results on a patient’s isolate may be erroneous or misleading due to: 1) a contaminated test, 2) transcription errors, 3) technical problems with the test, 4) reporting inappropriate drugs for the species and/or body site, or 5) failure to report unexpected resistance.

Ensuring Reliable Antimicrobial Susceptibility Test (AST) Results When Using an Automated AST System (*Continued*)

Conclusion

Ensuring reliable AST results is an ongoing process and involves much more than routine testing of QC strains. The minimum questions that must be considered prior to reporting any AST result are listed in Table 2.

Table 2. Essential Questions to Help Ensure Reporting Reliable AST Results on a Patient's Isolate

Prior to releasing AST results on a patient's isolate, answer the following questions:

- Do organism identification and AST results correlate?
- Are drugs reported appropriate for the species/specimen source?
- Are results from similar drugs (drug class) reasonable?
- Are any "R" results unusual for the species and warrant confirmation?
- Was this species isolated from another culture on this patient (eg, same day, different body site or previous day)? Were AST results the same?
- Were appropriate comments added to the report?

If any "R" results are unusual for the species:

- Check transcription.
- Reexamine "test" and purity plate.
- Check previous isolates on patient (recent and/or previous hospitalizations).

IF the above are not revealing, THEN (as appropriate):

- Repeat ID and AST.
- Confirm AST (and possibly ID) with second method.
- Get assistance from reliable reference and/or public health laboratory.

Abbreviations: AST, antimicrobial susceptibility testing; ID, identification; R, resistant.

Management should work closely with the bench technologists to establish standard operating procedures that explain how to perform testing precisely according to the manufacturer's recommendations, minimize contaminating ASTs; detect and confirm unusual results; report highly significant results in a timely manner to all stakeholders; ensure that competency is assessed and maintained; and determine whether the laboratory AST practices are meeting the institution's goals.

References

- 1 Humphries RM, Kircher S, Ferrell A, *et al*. The Continued Value of Disk Diffusion for Assessing Antimicrobial Susceptibility in Clinical Laboratories: Report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. *J Clin Microbiol*. 2018;56(8):437-18.
- 2 Patel, JB, Sharp S, Novak-Weekley S. Verification of Antimicrobial Susceptibility Testing Methods: A Practical Approach. *Clinical Microbiology Newsletter*. 2013;35(13):103-109.
- 3 CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. 1st ed. CLSI guideline M52. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
- 4 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.
- 5 FDA. FDA-Recognized Antimicrobial Susceptibility Test Interpretive Criteria. <https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria>. Accessed 1/14/20.
- 6 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.

The Importance of Being Specific: *Staphylococcus* Species-Specific Breakpoints for Methicillin (Oxacillin) Resistance

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A 60 year-old-female presented to the emergency department (ED) with a short history of a severe ecthyma-like lesion (ie, crusted sores beneath which were ulcers) on her forearm. Initially, a small red mark appeared and rapidly developed into a large eschar (ie, black-colored dead tissue). A specimen for culture was collected using an Eswab collection and transport device (Copan Diagnostics, Inc., Murrieta, CA). Specimen Gram stain revealed gram-positive cocci in clusters (2+) and white blood cells (2+). A pure culture (3+) of white-colored β -hemolytic colonies was observed after incubation for ~24 h at 35°C in an atmosphere of 5% carbon dioxide on tryptic soy agar with 5% sheep blood (Becton, Dickinson and Company, Franklin Lakes, NJ) (See Figure 1). Colony Gram stain showed gram-positive cocci in clusters, and catalase and Staphaurex™ latex agglutination (Remel, Lenexa, KS) tests were positive. Therefore, the isolate was reported as *Staphylococcus aureus*. Antimicrobial susceptibility testing (AST) was performed using broth microdilution (Pos MIC Panel Type 34, MicroScan WalkAway, Beckman Coulter, Inc., Brea, CA) and interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations.¹ AST results are tabulated below (see Table 1).

Table 1 Initial AST Results

Antimicrobial Agent	Minimal Inhibitory Concentration, $\mu\text{g/mL}$ (Interpretation) ¹
Oxacillin	>2 (Resistant)
Cefoxitin ²	≤4 (Susceptible)
Clindamycin	>4 (Resistant)
Daptomycin	≤0.25 (Susceptible)
Erythromycin	>4 (Resistant)
Gentamicin	>8 (Resistant)
Levofloxacin	>4 (Resistant)
Linezolid	1 (Susceptible)
Rifampin	≤1 (Susceptible)
Tetracycline	>8 (Resistant)
Trimethoprim-Sulfamethoxazole	≤0.5/9.5 (Susceptible)
Vancomycin	0.5 (Susceptible)

¹ Interpreted using the Clinical and Laboratory Standards Institute M100, 29th edition.

² Cefoxitin was tested as a surrogate for determining methicillin (oxacillin) resistance.

Based upon a discrepancy between the cefoxitin (susceptible, ≤4 $\mu\text{g/mL}$) and oxacillin (resistant, >2 $\mu\text{g/mL}$) MIC interpretations, the clinical laboratory scientist (CLS) performed a cefoxitin disk diffusion (30 μg disk) test. The cefoxitin zone of growth inhibition was >=22 mm, implying susceptibility to methicillin (oxacillin) and other β -lactam agents when interpreted using *S. aureus/Staphylococcus lugdunensis* breakpoints (see Table 2). At this point, the CLS raised the case to the microbiology director who recommended confirming the identity of the organism using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The isolate was identified as *Staphylococcus pseudintermedius* and the report was corrected to methicillin (oxacillin)-resistant *S. pseudintermedius* (MRSP) based upon oxacillin MIC interpretations for *S. pseudintermedius*. (see Table 2). Interestingly, the isolate tested resistant to several antimicrobial classes (eg, fluoroquinolone, macrolide, and tetracycline), a phenotype observed previously for MRSP.² The patient made a full recovery.

The Importance of Being Specific: *Staphylococcus* Species-Specific Breakpoints for Methicillin (Oxacillin) Resistance (Continued)

Table 2. Oxacillin and Cefoxitin Breakpoints Used for Detecting Methicillin (Oxacillin) Resistance Among Various Species of *Staphylococci*¹

Organism Group	Oxacillin				Cefoxitin ²			
	Zone Diameter Breakpoints (mm)		MIC Breakpoints (µg/mL)		Zone Diameter Breakpoints (mm)		MIC Breakpoints (µg/mL)	
	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
<i>S. aureus/S. lugdunensis</i>	N/A	N/A	≤2	≥4	≥22	≤21	≤4	≥8
<i>S. epidermidis</i>	≥18	≤17	≤0.25	≥0.5	≥25	≤24	N/A	N/A
<i>S. pseudintermedius</i>	≥18	≤17	≤0.25	≥0.5	N/A	N/A	N/A	N/A
<i>S. schleiferi</i>	≥18	≤17	≤0.25	≥0.5	N/A	N/A	N/A	N/A
Other species ³	N/A	N/A	≤0.25	≥0.5	≥25	≤24	N/A	N/A

¹ Clinical and Laboratory Standards Institute M100, 30th edition

² Cefoxitin is tested as a surrogate for determining methicillin (oxacillin) resistance in some species of staphylococci; oxacillin and NOT cefoxitin results should be reported

³ Other *Staphylococcus* species, excluding *S. aureus*, *S. lugdunensis*, *S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi*

Abbreviations: MIC, minimal inhibitory concentration; N/A, not applicable

Staphylococcus pseudintermedius, a coagulase-positive member of the *Staphylococcus intermedius* group, is a zoonotic pathogen where human infection is presumed to be due to contact with animals, especially dogs,^{3,4} although contact with animals is not always recorded.⁵ In this case, the patient was a dog owner. As observed here, *S. pseudintermedius* can be misidentified as I using phenotypic methods.⁶ There is variability in the accuracy of AST methods for detecting *mecA*-mediated methicillin (oxacillin) resistance in *S. pseudintermedius*, and methods and breakpoints employed for *S. aureus* can result in very major errors if applied to *S. pseudintermedius*; ie, *S. pseudintermedius* isolates that contain methicillin (oxacillin) resistance determinants (namely, *mecA* which encodes penicillin-binding protein 2a [PBP2a]) may demonstrate methicillin (oxacillin) susceptible results using *S. aureus/S. lugdunensis* breakpoints.⁷ As such, the CLSI M100-S30 document clearly specifies *S. pseudintermedius*-specific methods and breakpoints for detecting methicillin (oxacillin) resistance (see Table 2).⁸ These include oxacillin MIC methods, such as broth microdilution, and oxacillin disk diffusion. As expected, and in agreement with the oxacillin MIC result, the isolate tested resistant using oxacillin disk diffusion (1 µg disk; zone size, <=17 mm; See Figure 2). Although cefoxitin is a reliable surrogate for detecting methicillin (oxacillin) resistance in some staphylococcal species, neither cefoxitin MIC nor cefoxitin disk diffusion methods perform reliably for *S. pseudintermedius*.

Figure 1. Colony morphology of the *Staphylococcus pseudintermedius* isolate after growth on tryptic soy agar with 5% sheep blood (Becton, Dickinson and Company, Franklin Lakes, NJ) at 35°C in an atmosphere of 5% carbon dioxide for ~24 h.

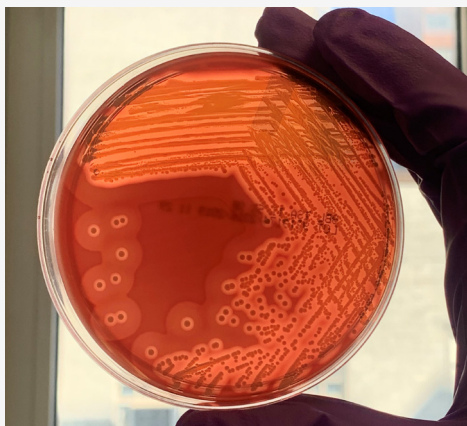
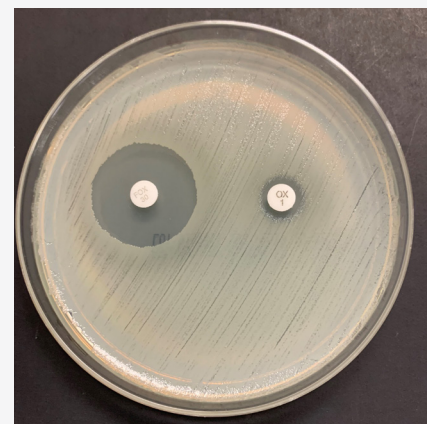


Figure 2. Cefoxitin and oxacillin disk diffusion testing of the methicillin (oxacillin)-resistant *Staphylococcus pseudintermedius* isolate. The cefoxitin (FOX) zone size is >=22 mm and the oxacillin (OX) zone size is <=17 mm. The discrepancy between these two β-lactams is not unusual for *S. pseudintermedius* and cefoxitin methods are not recommended for this species.



The Importance of Being Specific: *Staphylococcus* Species-Specific Breakpoints for Methicillin (Oxacillin) Resistance (Continued)

The necessity for accurate identification of *S. aureus* and *S. lugdunensis* and application of species-specific breakpoints for oxacillin and ceftiofur is well established. However, development of species-specific breakpoints for other *Staphylococcus* species is more recent. A CLSI ad hoc group is in the process of systematically evaluating the performance of methods and breakpoints for detecting *mecA/C*-mediated methicillin (oxacillin) resistance in staphylococci. These studies have resulted in the implementation of additional species-specific breakpoints (see Table 2).⁸ Notably, *Staphylococcus schleiferi* behaves in the same manner as *S. pseudintermedius* with respect to AST and identical oxacillin and ceftiofur breakpoints are used for both species. However, *Staphylococcus epidermidis* has been shown to perform differently during susceptibility testing and not all oxacillin and ceftiofur tests are reliable for this species. In conclusion, it is becoming increasingly apparent that species-specific AST methods and breakpoints are required for reliable detection of methicillin (oxacillin) resistance in staphylococci and these are likely to expand. The clinical microbiology laboratory must use accurate methods for identifying staphylococci to the species level when clinically relevant.

Clinical Pearls

- Identify staphylococci to the species level using accurate identification platforms (eg, MALDI-TOF MS) when clinically relevant so species-specific methods and breakpoints can be used to reliably detect methicillin (oxacillin) resistance.
- Perform additional identification and/or susceptibility tests on staphylococcal isolates using reliable methods in cases of discrepant methicillin (oxacillin) and ceftiofur results.
- Use the current version of CLSI M100 that contains the most up-to-date recommendations for detecting methicillin (oxacillin) resistance in staphylococci.
- If using a commercial AST system, verify the capabilities of that system for detecting methicillin (oxacillin) resistance for specific staphylococcal species.

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A Short Lesson in Fungal Taxonomy: What is CLSI's Stance?

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Fungal taxonomy is a confusing subject for clinical and public health microbiologists, and a frequently posed question is, “Why do the names keep changing?”

In the past, fungal taxonomy was based primarily on morphology; fungi which resembled each other on routine morphologic assessments (eg, slide culture) were grouped together within the same genus. However, reliance on morphology alone has been found to be inaccurate, due in part to the fact that many fungi have two growth phases which are often morphologically different: an asexual (anamorph) and a sexual (teleomorph) growth phase. The anamorph phase is the form of the organism most commonly seen growing on microbiologic media in the laboratory. The teleomorph phase is often attained by fungi when growing in their natural state, such as within the environment (eg, soil). Thus, the same organism can look dramatically different depending upon the environment within which it is grown and this has led to the assignment of two different names to a single fungus. One example is *Candida krusei* (anamorph) and *Pichia kudriavzevii* (teleomorph); both refer to of the same organism.

Clinical microbiology reference books mostly use the anamorph name because it is the name most familiar to clinicians. Sequencing and some matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) databases use the teleomorph name, because that is the name often assigned by fungal taxonomists. Molecular phylogeny, which is the grouping of organisms based on DNA sequencing, has revealed similarities among organisms which differ morphologically, and has revealed differences among morphologically comparable organisms. For example, phylogenetic studies support the renaming of *Bipolaris* spp. which are now classified with *Curvularia*, despite significant morphologic differences between these two genera.¹

Finally, for some fungi, DNA sequencing has revealed that a single species may in fact consist of multiple “cryptic” species. Cryptic species are those that appear morphologically similar but can only be distinguished by DNA sequencing (and sometimes MALDI-TOF MS). An example of this is *Cryptococcus gattii* which has been divided into four species based on molecular typing: *Cryptococcus gattii* (formerly *Cryptococcus gattii* molecular type VG1), *Cryptococcus deuterogattii* (formerly *Cryptococcus gattii* VGII), *Cryptococcus bacillisporus* (formerly *Cryptococcus gattii* VGIII), and *Cryptococcus tetragattii* (formerly *Cryptococcus gattii* VGIV).² This example is clinically relevant as the cryptic species display distinct epidemiological cutoff values (ECVs), but unfortunately they can only be distinguished by DNA sequencing.³ The CLSI Subcommittee on Antifungal Susceptibility Tests has not yet established guidance for laboratories that do not have the ability to distinguish between these closely related species.

The CLSI Subcommittee on Antifungal Susceptibility Tests has chosen to use the more common, clinically recognized anamorph names and to avoid teleomorph names until (and if) they become more clinically established in the literature (see Table 1). This CLSI Subcommittee suggests that laboratories use the anamorph names for reporting. In the clinical laboratory, isolates may be resulted by an instrument using either the anamorph or teleomorph name, as there is no guidance from the Food and Drug Administration (FDA) as to which fungal name (anamorph or teleomorph) to report. Suggested steps are listed below:

- If the anamorph name is resulted, laboratories should use that name for reporting.
- If the teleomorph name is resulted, laboratories may choose to only report the anamorph name, or to report the anamorph name, followed by the teleomorph name in parentheses. Failure to report the name of a fungus in a clinically meaningful format may lead to a missed opportunity for treatment, if the clinician fails to recognize the organism name.

A Short Lesson in Fungal Taxonomy: What is CLSI's Stance? (Continued)

Current and past names can be searched in MycoBank.⁵ After navigating to the website, use the function “Fungal Name Search” to search the fungus of interest. Once the species page comes up, old names can be found under “Synonymy” and other names can be found under either “anamorph synonyms” or “teleomorph synonyms.” Fungal names can be confusing enough for clinicians. Laboratories can play a key role in ensuring clinical teams receive results using names that are recognized and can be acted upon.

Table 1. Examples of Genus and Species Names Used in CLSI Supplements M59 and M60 are on the Left While the Alternative Names are Shown on the Right^{3,4}

Anamorph name (used by CLSI)	Teleomorph name
<i>Candida krusei</i>	<i>Pichia kudriavzevii</i>
<i>Candida guilliermondii</i>	<i>Meyerozyma guilliermondii</i>
<i>Candida lusitanae</i>	<i>Clavispora lusitanae</i>
<i>Scedosporium</i>	<i>Pseudallescheria</i>
<i>Penicillium</i>	<i>Talaromyces, Eupenicillium*</i>
<i>Aspergillus</i>	<i>Neosartorya, Eurotium, Emericella*</i>
<i>Fusarium</i>	<i>Gibberella*</i>
<i>Curvularia, Bipolaris</i>	<i>Cochliobolus</i>
<i>Trichophyton, Microsporum</i>	<i>Arthroderma</i>
<i>Blastomyces, Histoplasma</i>	<i>Ajellomyces</i>
<i>Scopulariopsis</i>	<i>Microascus</i>
<i>Candida</i>	<i>Wickerhamomyces, Kluyveromyces, Kodamaea, Yarrowia, Debaromyces*</i>

*There are other teleomorph names for these genera; only the most commonly encountered are listed here.

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Oh No, *Enterobacterales*?? More Nomenclature Changes!

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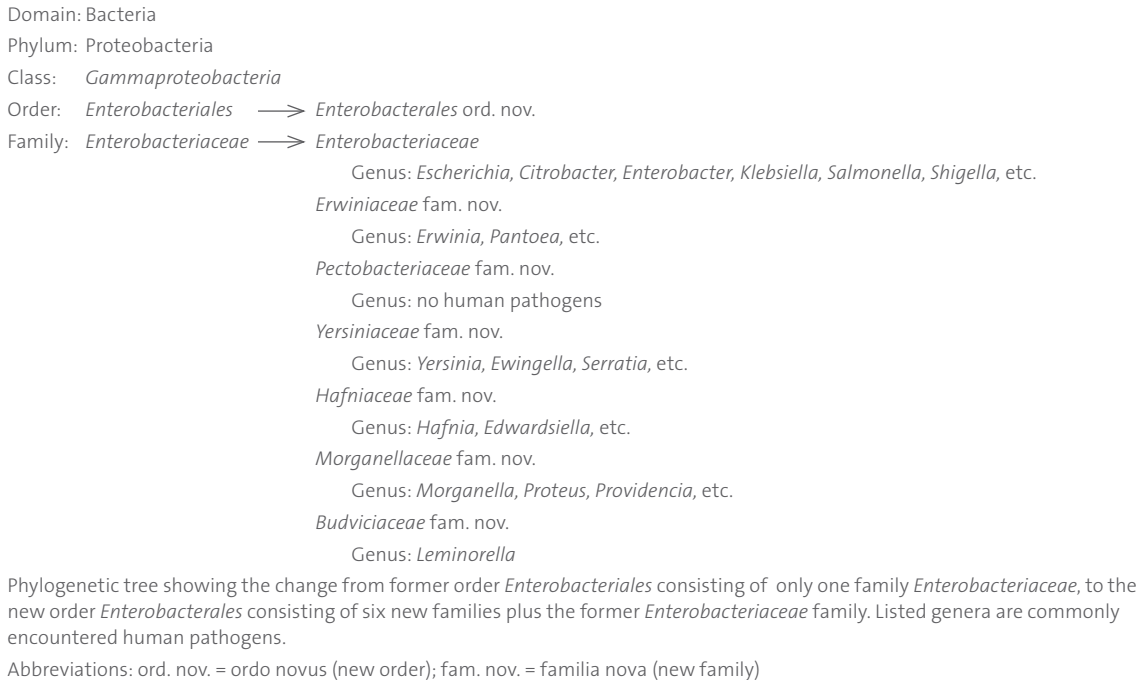
Two nomenclature changes that you may have come across recently are: 1) the change from *Enterobacter aerogenes* to *Klebsiella aerogenes* and 2) the change from referring to the family *Enterobacteriaceae* to the new order *Enterobacterales*. These changes are reflected in the newest edition of the Manual of Clinical Microbiology, 12th ed., chapter 40¹ as well as in the 2019 European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables.²

So why the change?

In 2017 the International Journal of Systematic and Evolutionary Microbiology (IJSEM) published a taxonomic note confirming the name *K. aerogenes* for the former organism *E. aerogenes*. In 1971, it was proposed that *E. aerogenes* be placed in the *Klebsiella* genus based on biochemical and physical properties of the bacteria. At that time, however, the name *K. aerogenes* was thought to already be validly published and therefore a new name, *Klebsiella mobilis*, was proposed. But the proposed name *K. mobilis* was determined (and continues) to be illegitimate according to the Rules of the International Code of Nomenclature of Prokaryotes. Upon further investigation it was found that the name *K. aerogenes* was actually never validly published, and could therefore be used, hence the recent name change to *K. aerogenes*.³

In 2016 IJSEM published a comprehensive comparative genomic analysis of the order *Enterobacteriales* using data from 1548 core proteins, 53 ribosomal proteins and 4 multilocus sequence analysis proteins.⁴ Based on phylogenetic analyses and conserved molecular characteristics, a proposal was made to rename the order *Enterobacteriales* to order *Enterobacterales* ord. nov. which consists of seven families: *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. see Figure (below).

Figure. Schematic of the Phylogeny and Taxonomy of the New Order *Enterobacterales*



What does this mean for antimicrobial susceptibility testing (AST)?

In 2018 *Klebsiella* (formerly *Enterobacter*) *aerogenes* replaced *E. aerogenes* in the CLSI document M100 28th ed.⁵ The same characteristics such as intrinsic resistance patterns and higher tendency to develop resistance to third-generation cephalosporins as applied to *E. aerogenes* likewise apply to *K. aerogenes*. But rules for extended-spectrum β -lactamase (ESBL) testing and use of cefazolin as a surrogate for oral cephalosporins for urinary isolates do not apply to *K. aerogenes*, as these have not applied to *E. aerogenes* in the past.

Oh No, *Enterobacterales*?? More Nomenclature Changes! (Continued)

Currently in CLSI document M100 29th ed., breakpoints are listed for *Enterobacteriaceae* (Table 2A) and Non-*Enterobacteriaceae* (Table 2B-5).⁶ Organisms such as *Escherichia coli*, *Proteus mirabilis*, and *Serratia marcescens* all belong to the former *Enterobacteriaceae* family and therefore all have the same breakpoint listed in Table 2A. However, with the creation of six additional families within the order of *Enterobacterales*, some of the organisms that once belonged to the family *Enterobacteriaceae* now belong to new families; ie, *P. mirabilis* belongs to the *Morganellaceae* family and *S. marcescens* belongs to the *Yersiniaceae* family. Since these organisms no longer belong to the family *Enterobacteriaceae* but remain appropriate for inclusion in Table 2A with other members of the *Enterobacteriaceae* family, the name of Table 2A had to change to *Enterobacterales*. Referring to this Table as the order *Enterobacterales* captures all the organisms for which these breakpoints apply. This change will be included in the upcoming CLSI document M100 30th ed. with the replacement of “*Enterobacteriaceae*” with “*Enterobacterales*” throughout the document.

Tips for the bench:

- When you identify *Klebsiella aerogenes*, think “*Enterobacter aerogenes*” and apply the same AST rules that you applied to *Enterobacter* spp.
- When you see *Enterobacterales*, think of the old family “*Enterobacteriaceae*” which included all of the members of the *Enterobacterales* order.

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