

Meeting Title:	Subcommittee on Antimicrobial Susceptibility Testing (AST)	Contact:	egomez@clsi.org
Meeting Location:	Dallas, Texas, USA		
Meeting Dates and Times: All times are Central Standard (US) time.	Plenary 1: Monday, 2 June 2025, 7:30 AM - 12:00 PM Plenary 2: Monday, 2 June 2025, 1:00 - 5:30 PM Plenary 3: Tuesday, 3 June 2025, 7:30 AM - 12:00 PM		
Meeting Purpose:	The purpose of this meeting is to review and discuss AST Working Group and Subcommittee business in preparation for publication of the next edition of M100 (36th).		
Requested Attendee(s):	SC Chairholder, Vice-Chairholder, Secretary, Members, Advisors, and Reviewers; Expert Panel on Microbiology Chairholder and Vice-Chairholder; Other Interested Parties; CLSI Staff		
Attendee(s):			
Amy J. Mathers, MD, D(ABMM) AST Subcommittee Chairholder		University of Virginia Medical Center	
James S. Lewis, PharmD, FIDSA AST Subcommittee Vice-Chairholder		Oregon Health and Science University	
Alexandra L. Bryson, PhD, D(ABMM) AST Subcommittee Secretary		Virginia Commonwealth University Health	
Members Present:			
Kevin Alby, PhD, D(ABMM)		University of North Carolina Hospital	
April M. Bobenchik, PhD, D(ABMM)		Penn State Health, Milton S. Hershey Medical Center	
Shelley Campeau, PhD, D(ABMM)		Scientific and Medical Affairs Consulting, LLC	
Tanis Dingle, PhD, D(ABMM), FCCM		Alberta Precision Laboratories	
German Esparza, MSc		Proasecal SAS Columbia	
Mark Fisher, PhD, D(ABMM)		ARUP	
Stephanie Mitchell, PhD, D(ABMM)		Cepheid	
Navaneeth Narayanan, PharmD, MPH		Rutgers University, Ernest Mario School of Pharmacy	
Elizabeth Palavecino, MD		Wake Forest Baptist Medical Center	
Virginia M. Pierce, MD, FIDSA		University of Michigan Medical School	
Audrey N. Schuetz, MD, MPH, D(ABMM)		Mayo Clinic (Rochester, MN)	
Patricia J. Simner, PhD, D(ABMM)		Johns Hopkins University School of Medicine, Department of Pathology	
Pranita D. Tamma, MD, MHS		John Hopkins University School of Medicine, Department of Pediatrics	
Members Absent:			
Joseph D. Lutgring, MD		Centers for Disease Control and Prevention	
Advisors Present:			
Mariana Castanheira, PhD		Element/JMI Laboratories	
Sharon K. Cullen, BS, RAC		Beckman Coulter, Inc. Microbiology Business (retired contractor)	
Lindsay Donohue, PharmD, BCIDP		University of Virginia Medical Center	
Rebekah Dumm, PhD, D(ABMM)		Washington University School of Medicine	
Andrea L. Ferrell, MLS(ASCP)		BD	
Elizabeth Hirsch, PharmD		University of Minnesota	
Andre Hsiung, M(ASCP), MS, MBA		Hardy Diagnostics	
Antonieta Jimenez Pearson, MQC, PhD		INCIENSA	
Kristie Johnson, PhD, D(ABMM)		University of Maryland	

William Miller, MD	Methodist Hospital
Samia Naccache, PhD, M(ASCP), D(ABMM)	LabCorp
Mike Satlin, MD	Weill Cornell Medicine
Jolyn Tenllado	bioMérieux
Katsunori Yanagihara, MD, PhD	Japanese Society for Clinical Microbiology
Barbara L. Zimmer, PhD	Beckman Coulter
Advisors Absent:	
Amelia S. Bhatnagar, MPH	Centers for Disease Control and Prevention
Marcelo Galas, BSc	Pan American Health Organization
Soren Gattermann, PhD	EUCAST
Romney M. Humphries, PhD, D(ABMM), FIDSA, FAAM	Vanderbilt University Medical Center
Dmitri Iarikov, MD, PhD	FDA Center for Drug Evaluation and Research
Thomas J. Kirn, MD, PhD	Rutgers Robert Wood Johnson Medical School
Joe Kuti, PharmD, FIDP, FCCP	Consultant
Maria Machado	Centers for Disease Control and Prevention
Brigit Quinn, MS	SeLux
Ribhi Shawar, PhD, D(ABMM), F(AAM)	FDA Center for Devices and Radiological Health
Melvin P. Weinstein, MD	Robert Wood Johnson University Hospital
Reviewers and Guests (Non-SC-roster attendees): see Plenary Attendee List below	
Staff:	
Kristine D. Bell, MBA, MLS(ASCP)DLM	CLSI
Emily Gomez, MS, MLS(ASCP)MB	CLSI
Barb Jones, PhD	CLSI
Christine Lam, MT(ASCP)	CLSI

Plenary Agendas

PLENARY AGENDA: SESSION 1 - IN PERSON Monday, 2 June 2025 7:30 AM - 12:00 PM Central Standard Time (US)			
Time	Item	Presenter	Page
7:30 AM - 7:40 AM (10 min)	Opening Remarks	A. Mathers	7
7:40 AM - 7:45 AM (5 min)	Approval of Meeting Agenda	A. Mathers	7
7:45 AM - 7:55 AM (10 min)	CLSI Welcome and Update	B. Jones	7
7:55 AM - 8:05 AM (10 min)	AST Subcommittee Update	E. Gomez	8
8:05 AM - 8:15 AM (10 min)	Expert Panel on Microbiology Update	A. Schuetz	11
8:15 AM - 8:25 AM (10 min)	Veterinary AST Subcommittee Update	R. Bowden	13
8:25 AM - 8:55 AM (30 min)	Investigational Only Breakpoints Update	A. Mathers	19
8:55 AM - 9:30 AM (30 min)	Joint CLSI-EUCAST WG	J. Hindler E. Matuschek	23
9:30 AM - 10:00 AM (30 min)	Break		
10:00 AM - 10:30 AM (30 min)	Anaerobe WG	D. Carpenter S. Copsey-Mawer	28
10:30 AM - 12:00 PM (1 hr 30 min)	Quality Control WG	S. Cullen C. Pillar	31
PLENARY AGENDA: SESSION 2 - IN PERSON Monday, 2 June 2025 1:00 PM - 5:30 PM Central Standard Time (US)			
Time	Item	Presenter	Page
1:00 PM - 3:00 PM (2 hr)	Breakpoints WG: Part 1	N. Narayanan M. Satlin	55

3:00 PM - 3:20 PM (20 min)	Break		
3:20 PM - 5:30 PM (2 hr)	Breakpoints WG: Part 2	N. Narayanan M. Satlin	55
PLENARY AGENDA: SESSION 3 - IN PERSON Tuesday, 3 June 2025 7:30 AM - 12:00 PM Central Standard Time (US)			
Time	Item	Presenter	Page
7:30 AM - 9:30 AM (2 hr)	Methods WG: Part 1	T. Dingle K. Johnson	115
9:30 AM - 9:50 AM (20 min)	Break		
9:50 AM - 10:50 AM (1 hr)	Methods WG: Part 2	T. Dingle K. Johnson	115
10:50 AM - 11:20 AM (30 min)	Text and Tables WG	A. Bobenchik S. Campeau	151
11:20 AM - 11:50 AM (30 min)	Outreach WG	J. Hindler A. Schuetz	157
12:00 PM	Closing Remarks	A. Mathers	161

Summary of Voting Decisions and Action Items

Summary of Passing Votes			
#	Motion Made and Seconded	Results ^a	Page ^b
1.	To approve the June 2025 meeting agenda.	12-0-0-2	7
2.	To revise the CLSI M100 investigational breakpoint definition to read, “Antimicrobial agents that are investigational for the organism group designated by “Inv.” in Tables 2 have not yet been approved any regulatory agency.” and to form an hoc working group to review investigational (Inv.) antimicrobial agents to align with the CLSI M23 investigational breakpoint definition.	13-0-0-1	22
3.	To approve the objective for the Joint CLSI EUCAST Working Group as “Respond to differences between CLSI and EUCAST AST methods to determine if harmonization can be achieved as determined by the AST Subcommittee.”	12-0-0-2	23
4.	To approve the updated anaerobe antibiogram with an introductory paragraph and discussed footnotes and to align with the CLSI M45 species.	13-0-0-1	30
5.	To accept the GDC-0829 MIC QC range for <i>E. coli</i> ATCC 25922 (0.12-0.5 µg/mL).	13-0-0-1	34
6.	To accept the GDC-0829 MIC QC for <i>P. aeruginosa</i> ATCC 27853 (0.25-2 µg/mL) with the comment, “ <i>P. aeruginosa</i> ATCC 28753 range is 0.25-2 µg/mL with mode 0.5-1 µg/mL. Results at 0.25 µg/mL and 2 µg/mL were seen less frequently in Tier 2 studies and if observed frequently in routine testing, consider troubleshooting.”.	13-0-0-1	35
7.	To accept the aztreonam-nacubactam disk (10/20 µg) diffusion QC for <i>P. aeruginosa</i> ATCC 27853 (17-23 mm), <i>K. pneumoniae</i> ATCC 700603 (20-26 mm), and <i>K. pneumoniae</i> BAA-2814 (18-26 mm) with comment about reading the colonies within the inner zone. Identify <i>K. pneumoniae</i> ATCC 700603 and <i>K. pneumoniae</i> BAA-2814 as QC strains for routine QC with green shading.	13-0-0-1	37
8.	To amend the previously approved motion (#7) and not add a comment about reading the colonies within the inner zone for aztreonam-nacubactam disk diffusion QC.	13-0-0-1	37
9.	To accept the cefepime-nacubactam disk (10/20 µg) diffusion QC for <i>P. aeruginosa</i> ATCC 27853 (21-29 mm), <i>K. pneumoniae</i> ATCC 700603 (24-29 mm), and <i>K. pneumoniae</i> BAA-2814 (20-27 mm) and for the QC Working Group to revisit the <i>E. coli</i> ATCC 25922 data for January 2026. Identify <i>K. pneumoniae</i> BAA-2814 as QC strain for routine QC with green shading.	13-0-0-1	39
10.	To accept the spectinomycin disk (100 µg) diffusion QC for <i>Neisseria gonorrhoeae</i> ATCC 49226 (24-30 mm).	12-0-0-2	43
11.	To add the footnote for Table 5A-1 cefiderocol MIC QC with mode 0.12-0.25 µg/mL as “ <i>P. aeruginosa</i> ATCC 27853 is recommended for routine QC (0.06-0.5 µg/mL, mode 0.12-0.25 µg/mL). Additional investigation may be required if frequent MIC results at 0.06 µg/mL or 0.5 µg/mL are observed (for troubleshooting, see Table 5G).”	9-4-0-1	49
12.	To amend the previously approved motion #11 and accept the Table 5A-1 cefiderocol QC footnote as “ <i>P. aeruginosa</i> ATCC 27853 is recommended for routine QC (0.06-0.5 µg/mL, mode 0.12-0.5 µg/mL). Frequent MICs at 0.5 µg/mL may be observed with some media manufacturers, which may result in falsely elevated MICs when testing clinical isolates (eg, false resistant results).”	13-0-0-1	49
13.	To add reference to CLSI M02 and M07 Quick Guides in Tables 4D and 5G, respectively.	12-0-0-2	51
14.	To accept the aztreonam-avibactam MIC breakpoints ($S \leq 4/4$, $I 8/4$, $R \geq 16/4$ µg/mL) for Enterobacterales.	11-2-0-1	62

Summary of Passing Votes

15.	To accept the aztreonam-avibactam disk diffusion breakpoints ($S \geq 25$ mm, I 22-24 mm, $R \leq 21$ mm) for Enterobacterales with the comment, "Disk diffusion may overcall resistance. Confirmatory testing with an MIC method can be performed on isolates that test intermediate by disk diffusion." and wordsmithing of comment to be similar to ceftazidime-avibactam.	13-0-0-1	66
16.	To place aztreonam-avibactam in Table 1 Tier 3 with other novel β -lactamase/ β -lactamase inhibitors.	12-0-0-2	66
17.	To accept the trimethoprim-sulfamethoxazole MIC breakpoints ($S \leq 0.5$, I 1, $R \geq 2$ $\mu\text{g/mL}$) for β -hemolytic streptococci with the first comment with only second sentence, the second comment without "uncomplicated" and reword as "not routinely reported other than..." to match other comments in M100, the Table 1 comment, and placement in Table 1 Tier 4.	10-3-0-1	67
18.	To form an ad hoc working group to review oral cephalosporin breakpoints.	13-0-0-1	81
19.	To accept the ceftriaxone MIC breakpoints for <i>Neisseria gonorrhoeae</i> ($S \leq 0.12$, I 0.25, $R \geq 0.5$ $\mu\text{g/mL}$) based on a dosage of 500 mg and removal of the disk diffusion breakpoints with a comment about pending disk diffusion breakpoint review.	13-0-0-1	94
20.	To accept the cefixime MIC breakpoints for <i>Neisseria gonorrhoeae</i> ($S \leq 0.06$, I 0.12, $R \geq 0.25$ $\mu\text{g/mL}$) based on a dosage of 800 mg and removal of the disk diffusion breakpoints with a comment about pending disk diffusion breakpoint review.	13-0-0-1	94
21.	To remove the tetracycline urine breakpoints for <i>Acinetobacter</i> spp.	13-0-0-1	97
22.	To add the minocycline breakpoints comment for <i>Acinetobacter</i> spp.	13-0-0-1	98
23.	To accept the amikacin MIC breakpoints ($S \leq 8$, I 16, $R \geq 32$ $\mu\text{g/mL}$) for <i>Acinetobacter</i> spp. based on a dosage of 20 mg/kg/day.	13-0-0-1	104
24.	To accept the amikacin disk diffusion breakpoints ($S \geq 20$, I 17-19, $R \leq 16$ mm) for <i>Acinetobacter</i> spp.	13-0-0-1	106
25.	To accept the gentamicin and tobramycin MIC breakpoints ($S \leq 2$, I 4, $R \geq 8$ $\mu\text{g/mL}$) for <i>Acinetobacter</i> spp. based on a dosage of 7 mg/kg/mL.	13-0-0-1	110
26.	To accept the gentamicin disk diffusion breakpoints ($S \geq 19$, I 14-18, $R \leq 13$ mm) for <i>Acinetobacter</i> spp.	13-0-0-1	111
27.	To accept the tobramycin disk diffusion breakpoints ($S \geq 17$, I 13-16, $R \leq 12$ mm) for <i>Acinetobacter</i> spp.	13-0-0-1	113
28.	To approve the early growth AST methods phase 1 study as proposed, to consider additional time points for QC, and to return to the Subcommittee for approval for phase 2.	12-1-0-1	117
29.	To discontinue the high cefazolin inoculum effect method study and disband the ad hoc working group until more data is available.	10-3-0-1	123
30.	To accept the expected resistance definition.	10-3-0-1	131
31.	To include the resistance (R) for cephalosporins, cephamycin, and aminoglycosides for <i>Salmonella/Shigella</i> with a footnote warning in the intrinsic resistance table and Table 1A.	13-0-0-1	122
32.	To accept the piperacillin-tazobactam 16-18 hour direct blood culture disk diffusion breakpoint ($R \leq 17$ mm) for Enterobacterales with <i>Proteus</i> spp. included.	13-0-0-1	142
33.	To remove the <i>Burkholderia cepacia</i> complex ECVs.	13-0-0-1	148

^a Key for voting: X-X-X-X = For-against-abstention-absent

^b Page links can be used to go directly to the related topic presentation and voting discussions.



NOTE 1: The information contained in these minutes represents a summary of the discussions from a CLSI committee meeting, and do not represent approved current or future CLSI document content. These summary minutes and their content are considered property of and proprietary to CLSI, and as such, are not to be quoted, reproduced, or referenced without the expressed permission of CLSI. Thank you for your cooperation.

NOTE 2: Discussions recorded in this summary may be paraphrased.

2025 JUNE AST MEETING SUMMARY MINUTES PLENARY 1: Monday, 2 June 2025 7:30 AM - 12:00 PM Central Standard Time (US)	
#	Description
1.	<u>OPENING REMARKS (A. MATHERS)</u> Dr. Mathers opened the meeting at 7:30 AM Central Standard (US) time by welcoming the participants to the hybrid CLSI meeting in Dallas, Texas.
2.	<u>APPROVAL OF MEETING AGENDA</u> A motion to approve the June 2025 meeting agenda was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)
3.	<u>CLSI WELCOME AND UPDATE (B. JONES)</u> Dr. Jones provided an update on CLSI activities. The main points included: • Thank you volunteers for their hard work and dedication to CLSI • Starting in January 2026, all Committees Week meetings will be in-person only. There will be no virtual streaming.

4. **AST SUBCOMMITTEE UPDATE (E. GOMEZ)**

Ms. Gomez provided an update on the AST Subcommittee. The main points included:

- AST Subcommittee Chairholders and Secretary
 - Chairholder: Amy J. Mathers, MD, D(ABMM)
 - Vice-Chairholder: James S. Lewis II, PharmD, FIDSA
 - Secretary: Alexandra L. Bryson, PhD, D(ABMM)
- AST Subcommittee Members
 - Kevin Alby
 - April Bobenchik
 - Shelley Campeau
 - Tanis Dingle
 - German Esparza
 - Mark Fisher
 - Joseph Lutgring
 - Stephanie Mitchell
 - Navaneeth Narayanan
 - Elizabeth Palavecino
 - Virginia Pierce
 - Audrey Schuetz
 - Patricia Simner
 - Pranita Tamma
- AST Subcommittee Roster Changes
 - Holly Huse has rotated off as an advisor.
- New Ad Hoc Working Groups
 - Breakpoints Working Group
 - Aztreonam-Avibactam AHWG
 - Joint CLSI EUCAST Working Group
 - New Drug Alternative Methods AHWG
- Subcommittee Voting Rules
 - 2/3 majority of members to approve
 - 1 vote to approve from each constituency (professions, industry, government) is no longer needed

**Subcommittee on Antimicrobial Susceptibility Testing
Chairholder's Rules on Voting**

**June 2025 AST Subcommittee Roster
14 voting members (excludes Chairholder and Vice-chairholder)**

<u>Committee Status</u>	<u>"Pass" Vote</u>
All members present and voting	14-0; 13-1; 12-2; 11-3; 10-4; 9-5
One member not present or abstaining	13-0; 12-1; 11-2; 10-3; 9-4
Two members not present or abstaining	12-0; 11-1; 10-2; 9-3
Three members not present or abstaining	11-0; 10-1; 9-2
If more than three members not present	Chairholder's discretion to conduct vote or table until sufficient members are present, or an electronic vote is taken.

Guidance on Considerations of Conflicts of Interest by Subcommittee Members Voting on an Issue

On any subcommittee business for which a subcommittee vote is required, all subcommittee members are expected to cast a vote, from the following voting options:

- Accept
- Accept with comments, and/or qualifications
- Reject with specified supporting reason(s)
- Abstain due to conflict of interests*

*Any personal gain within 3 years or imminently expected as a result of working with a specific drug (occasionally might apply if did such work with direct competitor[s]).

Note: "Personal gains" do not include payments only to your institution or research funds. These need to be declared but do not require a declared abstention.

- M100 36th Edition is publishing in January 2026.
- M100 Subcommittee Reminders

- January and June 2025 meeting decisions are incorporated into M100 36th edition
- No additional decisions after the June 2025 meeting
- Any pending M100 comments or revisions need to be provided to the Text and Table Working Group Chairholders (April B. and Shelley) by end of day on Tuesday
- Meet set review deadlines
- Important Upcoming Dates
 - Second Text and Tables Working Group Review: 18 June to 3 July
 - AST Subcommittee Supplemental Review: 13 August -26 August
- Supplemental Review
 - Vote and Comment: AST Subcommittee Members
 - Need 2/3 approval votes
 - Comment Only: AST Subcommittee Chairholders, Secretary, Advisors, and Reviewers
 - Working group members and advisors are AST Subcommittee Reviewers
 - Completed using the Edaptive Platform
 - Focus on tracked changes (new revisions to 36th edition)
 - Comments regarding content outside the tracked changes, email Text and Tables Working Group Chairholders or the applicable working group chairholders
- 2026 Meeting Dates
 - January 2026
 - 25 - 27 January 2026 in Tempe, Arizona
 - Virtual Only Working Group Meetings in weeks of 5 January and 12 January 2026
 - Meeting materials due 8 December 2025
 - June 2026
 - 31 May - 2 June 2026 in Chicago, Illinois
 - Virtual Only Working Group Meetings in weeks of 4 May and 11 May 2026
 - Meeting materials due date TBD
- A celebration of dedication was held for Lauri D. Thrupp, MD.

5. **EXPERT PANEL ON MICROBIOLOGY UPDATE (A. SCHUETZ)**

Dr. Schuetz provided an update on the activities of the CLSI Expert Panel on Microbiology. The main points included:

- CLSI Governance Structure
 - Expert panels are constituted for various technical subject areas. Currently, there are 10—one for each of CLSI’s technical areas. Each panel is made up of subject-matter experts who provide advice to document development committees and the consensus council, as needed.
 - The expert panels report to the Consensus Council and may take directives from CLSI’s Board of Directors.
 - Document development committees and subcommittees report to the Expert Panels and Consensus Council.
- Expert Panel Responsibilities
 - Identifying documents and products for development
 - Proposing those projects to the Consensus Council
 - Reviewing proposals from other sources and advises the Consensus Council on suitability
 - Advising document development committees and Consensus Council at all stages of document development
 - Reviewing, commenting, and voting on Proposed Drafts of consensus documents within the panel’s area of expertise
 - Reviewing documents within their area of expertise to recommend reaffirmation, revision, consolidation or division, or withdrawal, or archiving
- Active Microbiology Document Development Committees

Document Number	Document Name	Publication Year of Prior Version
M29-ED5	Protection of Laboratory Workers From Occupationally Acquired Infections	2014
M35-ED3	Abbreviated Identification of Bacteria and Yeasts	2008
M52-ED2	Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems	2015
M56-ED2	Principles and Procedures for Detection of Anaerobes in Clinical Specimens	2014
M58-ED2	Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry	2017
M63-ED1	Principles and Procedures for the Gram Stain	N/A
M66-ED1	Methods for Active Surveillance of Multidrug-Resistant Organisms	N/A
M67-ED1	Verification of Laboratory Automation in Microbiology	N/A
M68-ED1	Validation of Commercial Antimicrobial Susceptibility Test (AST) Breakpoints	N/A
M69-ED1	Antibody and Antigen Testing for Infectious Diseases	N/A
M70-ED1	Laboratory Testing for Lyme Borreliosis	N/A

- Upcoming Revisions to Microbiology Documents

Document Number	Document Name	Publication Year of Prior Version
M40-ED2	Quality Control of Microbiological Transport Systems	2014
M48-ED2	Laboratory Detection and Identification of Mycobacteria	2008

- Recent Publications
 - M64-ED1 Implementation of Taxonomy Nomenclature Changes
 - Published December 2024
- Additional Microbiology Documents

Document Number	Document Name	Publication Year of Prior Version
M47-ED3	Principles and Procedures in Blood Cultures	2022
M54-ED2	Principles and Procedures in Detection and Culture of Fungi in Clinical Specimens	2021

- How can you help?
 - Reach out to Microbiology Expert Panel members or advisors with ideas for documents
 - Respond to requests for volunteers for document development committees (DDCs)

6. **VETERINARY AST SUBCOMMITTEE UPDATE (R. BOWDEN)**

Mr. Bowden provided an update on the activities of VAST Subcommittee. The main points included:

- Working Group on VAST Breakpoints/Editorial Tables (VET01S)

Subgroup 1: Breakpoint Comment Review and Harmonization

- Standardize verbiage of comments throughout document – *many updates approved in Jan. 2025*

Subgroup 2: Human Breakpoint Addition and Removal (inclusion/exclusion)

- Develop criteria for evaluating if an M100 breakpoint should or should not be included in VET01S
tentative criteria presented in Jan. 2025

Subgroup 3: Appendix A updates – *many updates approved in Jan. 2025*

Subgroup 4: *Staphylococcus* subgroup

- Oxacillin BP work continues for *S. pseudintermedius*-group, *S. coagulans*, and *S. Schleiferi*
new oxacillin and penicillin data anticipated for presentation at Jan. 2026 meeting

Subgroup 5: Table 1 Reorganization

- Will function as a standalone AHWG – *work will begin in late fall 2025*
- Subgroup 2: Human Breakpoint Addition and Removal (Inclusion/Exclusion)
 - An example of VET01S Tables 2:
 - Originally only human breakpoints
 - Now >200 vet-specific breakpoints
 - Vet-specific breakpoints = white boxes
 - Human breakpoints = grey boxes
 - Longterm goal has been to "make the grey go away!"

Table 2D. *Enterococcus* spp. (Continued)

Table 2b: Enterococcus spp. (continued)											
Test/ Report Group	Body Site	Antimicrobial Agent	Antimicrobial Agent Class or Subclass	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
					S	I	R	S	I	R	
Cats											
A	Ur	Amoxicillin-clavulanate	β-lactam combination agents	-	-	-	-	≤ 8/4	-	≥ 16/8	(10) These breakpoints were derived from published literature in which amoxicillin-clavulanate was administered to nonazotemic cats.
A	Ur	Ampicillin	Penicillinase-labile penicillins	10 µg	≥ 17	-	≤ 16	≤ 8	-	≥ 16	See comment (9).
Humans											
(11) The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. Ampicillin results may be used to predict susceptibility to amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam among non-β-lactamase-producing enterococci. Ampicillin susceptibility can be used to predict imipenem susceptibility, providing the species is confirmed to be <i>E. faecalis</i> . ^c											
(12) Rx: High-dose IV ampicillin is the recommended therapy for serious enterococcal infections from nonurinary sites . Chloramphenicol may be considered as an alternative. Additionally, combination therapy with ampicillin, penicillin, or vancomycin (for susceptible strains only), plus an aminoglycoside, has been used for serious enterococcal infections, such as endocarditis, unless high-level resistance to both gentamicin and streptomycin is documented; such combinations are predicted to result in synergistic killing of the <i>Enterococcus</i> . See additional testing and reporting information in Table 7H. ^{4,c}											
(13) Penicillin or ampicillin resistance among enterococci due to β-lactamase production has been reported very rarely. Penicillin or ampicillin resistance due to β-lactamase production is not reliably detected with routine disk or dilution methods but is detected using a direct, nitrocefin-based β-lactamase test. Because of the rarity of β-lactamase-positive enterococci, this test does not need to be performed routinely but can be used in selected cases. A positive β-lactamase test predicts resistance to penicillin, as well as amino- and ureidopenicillins (see Glossary I). ^c											
		Ampicillin	Penicillinase-labile penicillins	10 µg	≥ 17	-	≤ 16	≤ 8	-	≥ 16	See comments (11), (12), and (13).
		Chloramphenicol	Phenolics	30 µg	≥ 18	13-17	≤ 12	≤ 8	16	≥ 32	(14) Not routinely reported on isolates from the urinary tract. ^c

working criteria, not a set of new categories for publication

Category 1

Human BPs included for use to detect important mechanisms of resistance that impact clinical efficacy
(ex. PEN for beta-lactamase and OX for methicillin-resistance detection among *Staphylococcus* spp.)

Category 2

Human BPs included because there are no or few vet-specific BPs *and* there is some evidence that the BPs have applicability in predicting clinical outcomes in veterinary species

- (ex. trimethoprim-sulfamethoxazole)

Category 3

Human BP entries included without BPs

- (ex. chloramphenicol BPs, which canine and equine PK data suggests are inapplicable to dogs and horses)

Category 4

Human BP that should be fully removed from VET01S, either because there are a sufficient number of vet-specific BPs, or because there is no clinical applicability to veterinary medicine

- (ex. Enterobacterales BPs for amikacin and streptomycin)

- Subgroup 3: Appendix A update

- In VET01S-Ed8, categories will be redefined to closely align with descriptions and action steps found in M100 Appendix A
- *Staphylococcus* spp. chloramphenicol removed. Linezolid-R added to Category I.
- *E. faecalis* and *E. faecium* linezolid-R added to Category I
- *Streptococcus suis* penicillin-R, ampicillin-R, ceftiofur-R, florfenicol-R added to Category II
- *B. bronchiseptica* aminoglycosides and fluoroquinolones removed (no breakpoints). Florfenicol-R added to Category II.
- *P. multocida* florfenicol-R added to Category II and tidipirosin-I/R added to Category II.

- Working Group on Generic Drugs

- Multiple fluoroquinolone breakpoint revisions and additions approved in January 2025

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments	
					S	SDD	I	R	S	SDD	I	R		
Cats														
A		Enrofloxacin	Enterobacterales	5 —	≥23 —	—	16-22 —	≤16 —	≤0.5 ≤ 0.12	—	1-2 0.25	≥4 ≥ 0.5		
A		Enrofloxacin	<i>Staphylococcus spp.</i> <i>pseuintermedius</i>	5 —	≥23 —	—	16-22 —	≤16 —	≤0.5 ≤ 0.12	—	1-2 0.25	≥4 ≥ 0.5		
A		Enrofloxacin	<i>P. multocida</i>	—	—	—	—	—	≤ 0.12	—	0.25	≥ 0.5	(comment to be added that enrofloxacin should not be reported for <i>P. multocida</i> if testing is performed in CAMHB-LHB as no QC exists)	
A		Marbofloxacin	Enterobacterales	5 —	≥23 —	—	16-22 —	≤16 —	≤1 ≤ 0.12	0.25	2 —	≥4 ≥ 0.5		
A		Marbofloxacin	<i>P. aeruginosa</i>	5 —	≥23 —	—	16-22 —	≤16 —	≤1 —	≤ 0.5	2 —	≥4 ≥ 1		
A		Marbofloxacin	<i>Staphylococcus spp.</i>	5 —	≥23 —	—	16-22 —	≤16 —	≤1 —	≤ 0.5	2 —	≥4 ≥ 1		
A		Marbofloxacin	<i>P. multocida</i>	—	—	—	—	—	≤ 0.12	0.25	—	≥ 0.5	(comment to be added that enrofloxacin should not be reported for <i>P. multocida</i> if testing is performed in CAMHB-LHB as no QC exists)	

- Ad Hoc Working Group on Disk Diffusion Breakpoints
 - 2024 project to establish ampicillin, penicillin, and tetracycline disk breakpoints for *M. haemolytica* and *P. multocida*
 - 100 isolates tested per microorganism-antimicrobial agent combination (5 laboratories, 20 isolates each)
 - Analyzed manually and by dBETs
 - VET02 criteria was followed. Dry-form BMD MICs were used.

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL			
					S	SDD	I	R	S	SDD	I	R
Cats												
A	SST, Ur	Ampicillin	<i>P. multocida</i>	10	≥ 25	—	19-24	≤ 18	≤ 0.25	—	0.5	≥ 1
Cattle												
A	Resp	Ampicillin	<i>M. haemolytica</i>	—	—	—	—	—	≤ 0.03	—	0.06- 0.12	≥ 0.25
A	Resp	Penicillin	<i>M. haemolytica</i>	—	—	—	—	—	≤ 0.25	—	0.5	≥ 1
A	Resp	Tetracycline	<i>M. haemolytica</i>	30	≥ 23	—	20-22	≤ 19	≤ 2	—	4	≥ 8
A	Resp	Ampicillin	<i>P. multocida</i>	—	—	—	—	—	≤ 0.03	—	0.06- 0.12	≥ 0.25
A	Resp	Penicillin	<i>P. multocida</i>	10	≥ 25	—	19-24	≤ 18	≤ 0.25	—	0.5	≥ 1
A	Resp	Tetracycline	<i>P. multocida</i>	30	≥ 22	—	18-21	≤ 17	≤ 2	—	4	≥ 8
Swine												
A	Resp	Ampicillin	<i>P. multocida</i>	10	≥ 25	—	19-24	≤ 18	≤ 0.5	—	1	≥ 2
A	Resp	Penicillin	<i>P. multocida</i>	10	≥ 25	—	19-24	≤ 18	≤ 0.25	—	0.5	≥ 1
A	Resp	Tetracycline	<i>P. multocida</i>	30	≥ 23	—	19-22	≤ 18	≤ 0.5	—	1	≥ 2

- Working Group on Aquatic Animals (AWG)
 - New MIC ECVs approved in September 2024 and April 2025 for the next edition of VET04
 - Individual analyses for *Aeromonas* but < 100 per species. ECVs aligned, VAST approved setting "group" ECVs.

Organism	MIC ECVs
<i>Aeromonas</i> group (<i>A. hydrophila</i> , <i>A. sobria</i> , <i>A. veronii</i>)	Enrofloxacin, florfenicol, gentamicin, oxolinic acid, oxytetracycline, ormetoprim- sulfadimethoxine, trimethoprim-sulfamethoxazole
<i>Edwardsiella ictaluri</i>	Ampicillin, enrofloxacin, florfenicol, gentamicin, oxolinic acid, oxytetracycline, ormetoprim-sulfadimethoxine, trimethoprim-sulfamethoxazole
<i>E. piscicida</i>	Ampicillin, enrofloxacin, florfenicol, gentamicin, oxolinic acid, oxytetracycline, ormetoprim-sulfadimethoxine, trimethoprim-sulfamethoxazole
<i>Vibrio parahaemolyticus</i> (28°C and 35°C)	Ceftazidime (35°C only), enrofloxacin florfenicol, gentamicin, oxolinic acid, oxytetracycline, trimethoprim-sulfamethoxazole
<i>Yersinia ruckeri</i>	Ampicillin, enrofloxacin, florfenicol, gentamicin, oxolinic acid, oxytetracycline, ormetoprim-sulfadimethoxine, trimethoprim-sulfamethoxazole

- Working Group on Education
 - Two invited speaker educational plenary presentations were given at the January 2025 meeting:
 - "Dealing with Breakpoints in the Human World" by Dr. Romney Humphries. Covered the landscape of human breakpoint setting in the US (CLSI and FDA) and the impact of MIC variability on breakpoints and device clearance.
 - "A Look at Published Data on the Relationship of AST and Clinical Outcomes in Bovine Respiratory Disease" by Dr. Mike Apley. Provided insights on gaps in understanding and the difficulties polymicrobial disease poses for accurate data interpretation
 - Videos - series of ~3-minute shorts focused on education of veterinarians/laboratory clients
 - "Susceptible Dose-Dependent" -- what it means and how to apply it
 - "Why can't I get AST on Mycoplasmas?"
 - How breakpoints and interpretive criteria are established
 - Topical therapy and AST
- VET06 Methods for AST of Infrequently Isolated or Fastidious Bacteria Isolated From Animals
 - Retaining use of susceptible/intermediate/resistant. Every table will carry a warning box to remind users that breakpoints do not meet the same level of evidence as is required for breakpoints found in VET01S.
 - Revised and new breakpoints for multitude of species and organism groups.
 - Anaerobic bacteria: removed chloramphenicol, pradofloxacin, and tetracycline from recommended primary testing. Removed tetracycline breakpoints due to concerns about clinical inapplicability among veterinary species.
 - *Campylobacter jejuni/coli*: methods and QC updated to match M45. Ciprofloxacin and azithromycin breakpoints were removed due to concerns about clinical inapplicability among veterinary species.
 - *H. pylori*: removed as most vet infections are due to *Helicobacter* species other than *H. pylori* and there is no data on whether the methods or breakpoints are applicable.
 - Actinomycetes and rapidly growing mycobacteria: removed, WG and subcommittee felt these are better housed in existing M documents

7. **INVESTIGATIONAL ONLY BREAKPOINTS UPDATE (A. MATHERS)**

Dr. Mathers provided information and led a discussion on investigational only breakpoints. The main points included:

- Revisiting the Investigational (Inv.) Definition
 - There is a conflict in the current M100 and CLSI M23 Inv. Breakpoint definitions
 - It may be important to resolve this conflict for incoming international agents that want recognition in CLSI and testing by laboratories outside the US
 - A recent concept that the investigational breakpoints would all be posted on the CLSI website rather than in a document in the next version
 - Need resolution on the definition before work to remove investigational breakpoints and place on website as well as guidance on approach to companies not planning to file with FDA
- Current Investigational Definition (M100 34th Edition)

Antimicrobial Agent Test and Report Designations and Additional Considerations for Agents Not Listed in Tables 1

Designation	Definition	Test	Report*	Additional Testing and Reporting Considerations
Other	Antimicrobial agents with established clinical breakpoints designated by an * in Tables 2 that are generally not candidates for testing and reporting in the United States	By request	By request	• Test and report only by clinician request and only following consultation with the antimicrobial stewardship team and other relevant institutional stakeholders to ensure appropriateness of the request. • Agents with an "Other" designation may not reflect current consensus recommendations for first-choice and alternative drugs for the specific organism or organism group.
Inv.	Antimicrobial agents that are investigational for the organism group designated by "Inv." in Tables 2 have not yet been approved by the FDA for use in the United States.	By request	By request	Test and report only by clinician request and only following consultation with the antimicrobial stewardship team and other relevant institutional stakeholders to ensure appropriateness of the request. These agents would likely be clinically available for compassionate use only.

- M23 6th Edition with highlights of changes from 5th Ed.
 - 4.3 Investigational Breakpoints (5th Ed 4.3 was called "Provisional Breakpoints)
 - **For development:** For antimicrobial agents that are in development (ie, for which registration has not yet occurred for any indication), information on zone diameter and MIC relationships, distributions of MICs for organisms relevant to the intended clinical uses, and PK/PD indices may be submitted to the relevant CLSI subcommittee and to any relevant CLSI working group (eg, the CLSI Working Group on AST Breakpoints) co-chairholders at any time*. The relevant CLSI subcommittee can then assist in the selection of "investigational" (previously provisional) breakpoints to be used by clinical investigators during clinical trials that assess efficacy.
 - **Publication guidance:** Investigational (Provisional) breakpoints that are not yet approved by a regulatory agency are not published in CLSI documents. If an antimicrobial agent has regulatory agency approval for some organisms but the approval does not include a specific organism, it may be published in CLSI documents as investigational for that specific organism.
 - 5th Ed was submitted to the Chairholders of the AST Subcommittee and Breakpoints Working Group
- Cefepime-zidebactam requested recognition for laboratories in India to test in clinical setting of ongoing trial
 - CLSI Investigational Use-Only Breakpoints
 - Although investigational breakpoints are published in the current edition of CLSI M100, a revision to CLSI M23 required that investigational breakpoints not be included in future editions of CLSI M100.

- The following susceptible-only breakpoints were established using *in vitro* susceptibility testing on contemporary global resistant isolates, *in vivo* mouse model data, and pharmacokinetic and pharmacodynamic data paired with models to achieve efficacy against carbapenem-resistant isolates and are considered **investigational use only**. Antimicrobial susceptibility testing by disk diffusion or agar dilution was not reviewed. Therefore, breakpoints are provided only for cation-adjusted Mueller-Hinton broth (CAMHB).
- Fine for now as trial is ongoing but will need to understand how to post after sufficient clinical trial data available as company not filing with FDA

Organism	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
CEFEPIME-ZIDEBACTAM										
(1) The breakpoints were based on in vitro susceptibility testing on contemporary isolates, in vivo mouse model data, as well as pharmacokinetic and pharmacodynamic data paired with models to achieve efficacy .										
Acinetobacter baumannii	—	—	—	—	—	≤ 64/64	—	—	—	See comment (1).
Pseudomonas aeruginosa	—	—	—	—	—	≤ 64/64	—	—	—	See comment (1).
Enterobacterales	—	—	—	—	—	≤ 64/64	—	—	—	See comment (1).

• Current Investigational Breakpoints in M100

Cefetamet (Inv.)	Table 2A-1	Enterobacterales	Not approved by FDA for human use but approved elsewhere in Asia
Ceftibuten (U, Inv.) ^b	Table 2A-1	Enterobacterales	Approved by FDA for other organisms. Use for Enterobacterales is off-label (but it does mention that this drug may be active)
Fleroxacin (Inv.)	Table 2A-1	Enterobacterales	Not FDA approved. Do believe approved elsewhere
Pefloxacin (Inv.) (surrogate test for ciprofloxacin)	Table 2A-2	Salmonella/Shigella spp.	Not FDA approved but approved for use elsewhere but only surrogate use for this species in this table
Teicoplanin (Inv.)	Table 2C	Staphylococcus spp.	Not FDA approved. Approved in 1 st Europe in 1988. Also approved elsewhere now
Fleroxacin (Inv.)	Table 2C	Staphylococcus spp.	Not FDA approved. Do believe approved elsewhere
Teicoplanin (Inv.)	Table 2D	Enterococcus spp	Not FDA approved. Approved in 1 st Europe in 1988. Also approved elsewhere now
Cefetamet (Inv.)	Table 2E	Haemophilus influenzae/ parainfluenzae	Not approved by FDA for human use but approved elsewhere in Asia
Fleroxacin (Inv.)	Table 2E	Haemophilus influenzae/ parainfluenzae	Not FDA approved. Do believe approved elsewhere

- Are there agents listed in M100 which are not approved by FDA?
 - Some agents are listed as “other” because not reviewed by FDA or was reviewed by FDA but no longer available in the US (either withdrawn for toxicity, no longer manufactured)
 - Pivmecillinam (tested as mecillinam) was in the document as other prior to 2024 although it had not been reviewed by FDA but approved in Europe (1974)
 - Not clear if there are other examples of agents reviewed and approved by other agencies
- How should we move forward?
 - Investigational breakpoint - a breakpoint for an antimicrobial agent in development and for which there is no regulatory approval. This breakpoint is based on relevant microbiological and pharmacokinetic/pharmacodynamic data and is not published in CLSI documents.
 - Not 100% clear that all non-FDA approved agents are identified in our documents
 - Change from provisional in M23 in 6th Edition does not specify FDA
 - Should the FDA be the only regulatory agency for CLSI documents which are international in distribution?
 - Considerations for moving forward reviewing data outside FDA collaborations
 - How to understand labeling and review of other accreditation agencies?
 - How to advise companies outside the US whom seek breakpoints?
 - Do we need a working group to review all investigational breakpoints in the M100?
- Proposed change in M100 language to align with M23
 - Antimicrobial agents that are investigational for the organism group designated by “Inv.” in Tables 2 have not yet been approved by ~~the FDA for use in the United States~~ **any regulatory agency**
 - This breakpoint is based on relevant microbiological and pharmacokinetic/pharmacodynamic data and is not published in future CLSI documents (may need an ad hoc working group to review all Inv.)

SC DISCUSSION (MAIN POINTS)

- CLSI should be cautious about reviewing drugs that do not have a complete data set, particularly new drugs without clinical outcomes data yet. To be an international organization, CLSI should consider drugs approved by a regulatory organization.
- CLSI M23 made the change in language to say a regulatory organization and they thought the M100 would follow in language. Companies like coming for feedback for the investigational drugs. Table 1 does have a requirement for FDA approval, so perhaps CLSI needs to look at the table.
- Should an “Inv.” be next investigational breakpoints?
- There are investigational drugs that do not have data vs drugs that have approval from a different regulatory organization.
- Why do investigational breakpoints need to be removed from M100 and put on the website?
- This was to try and clean up M100; however, when CLSI went to clean up the document it turns out that some of the drugs currently classified as investigational have approval in other countries.
- Please be cautious with wording as the FDA uses the term “provisional” and that is for sponsors going into phase III clinical trials. Laboratories want to have guidance that it is OK to participate in a clinical trial. Please check all CLSI documents for consistency on wording, as an example remember to check the language in the CLSI M24-S or CLSI M23.
 - CLSI is looking to see how the bacteriology group defines terms and will then apply them to the mycobacterial and antifungal documents.
- CLSI could use the * symbol to designate drugs approved outside of the US.
- CLSI could reach out to the WHO to ask their expertise on which drugs are being used internationally.
- Did the CLSI M23 Working Group consider the specific wording about not including the investigational breakpoints in the CLSI documents?

- The M23 Working Group did not want laboratories to be confused about drugs that were not commercially available.
- Would cefazolin and *Klebsiella pneumoniae* be considered investigational in this definition?
- Concerns about removing breakpoints for drugs that are technically off label use, but commonly used.
- CLSI intentionally chose the term “provisional” implied there is some data to support them, whereas “investigational” is based on PK/PD and is the best estimate of the breakpoint.
- Should not be publishing investigational breakpoints.
- If a drug has been approved by a regulatory organization outside of the US and CLSI has reviewed a sufficient data package, then that is a breakpoint and it should be in the M100.
- If a sponsor does not qualify for Tiers 1,2,3, or 4, but have a full data package then get an * for other. If CLSI does not have a full data package, then an investigational breakpoint is appropriate.
- Teicoplanin is an example where need to remove the “Inv”. It is approved outside of the US and is used widely. Need to look at and possibly remove screening tests.
- Need an ad hoc working group or a defined set of people to document why each drug was removed or changed. CLSI should have a record.
- CLSI may want to change the wording to clarify the off label wording
- Suggestion to add a note that the Inv definition is under review.
- Support to update the definition now.
- Is the definition of a regulatory body organization to mean the regulatory organization for a country? What if the standards between organizations are not equivalent?
 - CLSI has not defined this.
 - The European agencies all have different standards so there is no way to rank or classify agencies.
 - Some regulatory organizations have reviewed which is beyond the CLSI scope. CLSI would just look at the data package to determine if there is enough data to support.

A motion to revise the CLSI M100 investigational breakpoint definition to read, “Antimicrobial agents that are investigational for the organism group designated by “Inv.” in Tables 2 have not yet been approved any regulatory agency.” and to form an hoc working group to review investigational (Inv) antimicrobial agents to align with the CLSI M23 investigational breakpoint definition was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

NOTE: A comment will be added that the Inv. breakpoints in M100 are pending review and may not accurately reflect the new definition.

8. **JOINT CLSI- EUCAST WORKING GROUP (J. HINDLER)**

JOINT WORKING GROUP GOALS

- Goal #1: Describe a method for disk content determination which can be used early in the drug development process to avoid having different disk contents in the CLSI and EUCAST standards.
- Goal #2: Discuss differences between CLSI and EUCAST QC criteria, methods for establishing QC criteria and the possibility of harmonizing CLSI and EUCAST QC criteria.
- Expand goals beyond disk content and QC criteria?
- Projects / Discussions Beyond Disk Content or QC Criteria
 - New Alternative Methods Ad Hoc Working Group
 - Reading disk diffusion and MIC endpoints
 - Colony counts
 - Differences between CLSI and EUCAST antifungal AST
- New Proposed Working Group Goal
 - Goal #3: Explore other differences between CLSI and EUCAST AST recommendations to determine if harmonization can be achieved.
 - Approved by the Working Group.

SC DISCUSSION (MAIN POINTS)

- There are differences in interpretations but what about methods?
 - There were some method differences between CLSI and EUCAST.
- Countries struggle to determine what to do with reading CLSI vs EUCAST for reading the inner colonies or not.
 - For Fosfomycin, EUCAST and CLSI decided to disagree on whether to read inner colonies.
- This is a vague Joint CLSI-EUCAST Working Group goal, so it would be better to have more specific goals.
- Suggestion to change the wording to include methods for reading disk diffusion and MIC methods.
- The main reason CLSI started this working group was to review new alternative methods.
- The goal is to be definitive of what is being asked of the working group. CLSI does not want a goal that is infinite.
- The working group should not proactively look for work but if there is an issue be ready to access.

A motion to approve the objective for the Joint CLSI EUCAST Working Group as “Respond to differences between CLSI and EUCAST AST methods to determine if harmonization can be achieved as determined by the AST Subcommittee.” was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)

DISK CONTENT SELECTION STUDIES

WG Assigned Study #	Agent	Sponsor	Notes
JWG-2023-2	Piperacillin-tazobactam (reassessment Phase 1)	JMI/EDL	Evaluating 9 disk masses (5/0.5 - 30/6 µg) (currently CLSI disk is 100/10 µg; EUCAST 30/6 µg)
JWG-2025-2	Cefiderocol (reassessment Phase 1)	JMI/EDL	Evaluating 10 disk masses (1-30 µg) (currently 30 µg)
JWG-2024-1	GDC0829	Genentech	Phase 1 complete Proceed to Phase 2 with 5, 7.5, 10, and 20 µg disks

PREQC MHA AGAR STUDIES

WG Assigned Study #	Agent	Sponsor	Notes
JWG-2022-6	Debio 1452	Debiopharm	Final discussions at QCWG
JWG-2025-1	Zosurabalpin	Roche	Little variation between media manufacturers. Noted some differences in one manufacturer's disk performance; asked manufacturer to reassess disk

M23S: COMMERCIAL DISK PRODUCTION FOR PHASE 2 TESTING

- CRO/Pharma company can either use home-made disks (in duplicate) or commercial disks (singly) for the shortlisted disk masses in Phase 2.
- How are disks commercially provided manufactured / quality controlled?
- Joint Working Group is surveying disk manufacturers to determine their processes for disk preparation for disk content selection studies.

NEW DRUG ALTERNATIVE METHODS AD HOC WORKING GROUP REPORT

- Objective: Develop a standard that drug developers can use to identify when the reference method for AST as described in CLSI M07 or ISO 20776-1, does not provide reliable results for an antimicrobial agent, such that an alternative method is needed. This standard will include:

- A list of criteria indicating a need for identifying an alternative reference AST method
- A list of situations that do not indicate the need for an alternative method
- Hypothetical examples to illustrate the criteria
- A list of desired characteristics for an alternative reference method
- Issues discussed to date
 - Reference method should be simple enough for a large number of laboratories to perform when commercial methods are unlikely to develop testing. Reference methods should not require specialized equipment. Try not to modify the method if just one org group isn't working. If the pathogen is important, then alternatives can be considered.
 - Acceptable methods should be those already recognized by CLSI or EUCAST. This includes methods for other orgs like anaerobes.
 - Need a list of drug-specific deviations that are already recognized.
 - When can an alternative medium be used?
 - Define the observations that drive a change and demonstrate that the alternative method proposal addresses that gap. Include examples to illustrate the criteria.
 - Use acceptable methods even if other methods were used for early development
- Proposed Document Outline
 - 1. Background
 - A. Definitions: Reference method, standard methods, modified methods, alternative methods, surrogate methods
 - B. How is a reference method used during drug development (eg, develop an MIC method and then validate a disk diffusion method)
 - C. How is a reference method used after drug development (eg, who needs to perform a reference method)
 - D. Benefits and challenges with one method for all bugs and drugs
 - II. Recognized modifications of the reference method
 - A. Changes to promote growth of fastidious bacteria
 - B. Changes to enable drug activity *in vitro* (eg, cefiderocol, daptomycin, oxacillin)
 - C. Changes to enable more accurate testing (eg, agar dilution for mecillinam)

SC DISCUSSION (MAIN POINTS)

- The original scope was to focus on what data is needed to show that the reference method is not sufficient and that is captured here. The intent was not to give advice on how to develop the second method, but to give guidance on when it is acceptable to deviate from the standard method. The problem is that there are companies seeking alternative methods, who do not actually need to be moving to alternative methods.
- When companies believe they need to change the method then they could come to this group for guidance on if they truly need to change the method or not.
- The AHWG needs guidance on different inflection points: Do I need a different method? Is the method I picked acceptable?
- This should be a CLSI M23 supplement to explain when a method change is necessary.
- Concern that companies cannot wait 6 months to have a discussion about changing the method.
- If CLSI is going to put up roadblocks on new methods, CLSI needs to let companies know early enough.
- The methods development happens way ahead of the QC process.
- CLSI needs a clear procedure on how companies can submit information to CLSI for approval for or not for different methods.
- It is important for CLSI and EUCAST to agree on when methods can be changed so companies do not get different information.
- Is the new document a best practice guideline or a strictly enforced rule?

- It will be guidance in CLSI M23 or a supplement to CLSI M23
- These companies need to have a clinical microbiologist, CLSI cannot fill that role.
- Need a checklist of criteria for companies that includes data requirements for approval for a new method.
- CLSI needs to provide clear guidance but not be consultants.
- Each drug is different. There needs to be one checkpoint.
- This is a huge time commitment.
- Narrow the scope to a best practices document for now, then CLSI needs to evaluate what resources CLSI wants to dedicate.
- The AHWG will focus on a list of criteria for identifying an alternative reference AST method.
- Continuity is important, it would be good to have a subgroup under the Methods Working Group and include EUCAST and industry members. It needs to start and stay in the Methods Working Group.
- Need the document to have minimum thresholds for data to be shown.

COLONY COUNTS (NOT PRESENTED BUT INCLUDED IN BACKGROUND MATERIALS)

- Presented in January 2025
 - Colony counts from McFarland 0.5 suspensions may vary by species
 - CLSI only addresses colony counts for *E. coli* ATCC 25922
 - EUCAST defers to ISO 20776-1
 - Some adjust inoculum suspensions to achieve recommended CFU/mL. No guidance from CLSI or EUCAST for this.
- Questions
 - What colony counts are obtained during QC studies?
 - Are inoculum suspensions adjusted to obtain recommended CFU/mL? How?
 - Should we provide further guidance for inoculum preparation to enhance consistency in CFU/mL ...for reference methods.
 - Collecting data from previous QC studies and other sources
- Colony count “ranges” listed in various standards

Reference	Acceptable CFU/mL	Comments
CLSI M02 M07 McFarland 0.5	1-2 10^8	<i>E. coli</i> ATCC 25922
ISO 20776-1 McFarland 0.5	1-2 10^8	
CLSI M07 BMD	2-10 $\times 10^5$ Most 3-4 $\times 10^5$	<i>E. coli</i> ATCC 25922
ISO 20776-1 BMD	Target = 5 $\times 10^5$ Range = 2-8 $\times 10^5$	States: "Different dilutions of the McFarland 0.5 suspension may be necessary as determined by colony counts in preliminary tests." Depends on growth phase of culture... mostly for fastidious organisms such as <i>S. pneumoniae</i> where old cultures can have fewer viable cells
FDA Guidance for Industry BMD	Approximately 5 $\times 10^5$	
Fuchs 1996 BMD	Proposed 1-9 $\times 10^5$	

9. **ANAEROBE WORKING GROUP (D. CARPENTER AND S. COPSEY-MAWER)**

WORKING GROUP UPDATES

- CLSI M11: Reaffirmed and will need to be reviewed again in 2030
- Anaerobe “Hot Topic” article will be in Fall 2025 newsletter
- Disk testing study is ongoing

M100 ANAEROBE ANTIBIOGRAM

- Anaerobe Cumulative Antibigram: Data Analysis
 - Inclusion Criteria
 - Clinical isolates submitted Jan 2017 - Dec 2024
 - All isolates underwent routine antimicrobial susceptibility testing
 - Isolates tested using CLSI reference method (agar dilution)
 - Current CLSI breakpoints applied
 - Organisms with >30 isolates
 - Isolates from at least 2 of 3 sites or >500 isolates if single site (with notation)
 - Mayo
 - Total isolates submitted: 43,534
 - Total isolates included: 42,063
 - IHMA
 - Total isolates submitted: 7,737
 - Total isolates included: 7,717
 - UK Anaerobe Reference Unit (ARU), Public Health Wales
 - Total isolates submitted: 8,238 (2017-2023)
 - Total isolates included: 8,095
- Anaerobe Cumulative Antibigram: Comparison
 - As compared to prior (2013-2016) CLSI anaerobe antibiogram:
 - 4-year vs 8-year time frame
 - Data are more robust
 - More species level identification (due to improved identification systems/increasing use of MALDI)
 - Many more isolates and genera
 - Worldwide representation
 - Additional antibiotics include amox-clav, ceftriaxone, and penicillin.
 - Antibiotics not included in this updated antibiogram include cefoxitin, imipenem, and ampicillin-sulbactam
 - Overall Conclusions
 - The % S difference between ertapenem (82-84% S) and meropenem (≥93% S) noted for *Bacteroides/Parabacteroides* species in prior CLSI anaerobe antibiogram was not seen with these data
 - Marked decrease in penicillin susceptibility for *Prevotella* species

- %S to clindamycin much higher for *Cutibacterium acnes* now (89% S) (6503 isolate) than in past (53% S) but current study had 6503 isolates compared to the prior study which only had 34 isolates
- %S to clindamycin much higher for *Fusobacterium necrophorum-nucleatum* now (98% S) than in past (77% S) but current study had 1274 isolates compared to the prior study which had only 44 isolates
- Anaerobe Cumulative Antibigram: Updated footnotes
 - ¹Isolates from a single site, only species with > 500 isolates included
 - ²*B. fragilis* cefoxitin susceptibility was 87.3%; isolates were from a single site (n=858)
 - ³Using FDA-recognized interpretive criteria, *B. fragilis* tigecycline susceptibility was 93.2% (MIC ≤ 4); isolates were from a single site (n=858).
 - ⁴44.9% of *P. bivia* isolates were intermediate to moxifloxacin (MIC=4); isolates were from a single site
 - ⁵*F. nucleatum* ertapenem susceptibility was 99.2%; isolates were from a single site (n=500)
 - ⁶Vancomycin MICs for 3033 *C. difficile* isolates demonstrated 97.6% wild type (MIC ≤ 2 µg/mL) and 2.4% non-wild-type (MIC ≥ 4 µg/mL); the majority of isolates (75.9%) were from intestinal sources
 - ⁷Thirty-six isolates of *C. acnes* from a single site generated MIC values for rifampin ≤ 0.03 µg/mL using the agar dilution method. These are no interpretive breakpoints for this organism/antimicrobial agent combination.
 - ⁸Vancomycin MICs for 1903 *C. acnes* isolates demonstrated 100% wild type (MIC ≤ 2 µg/mL).
 - ⁹A dash(-) indicates that data were insufficient.
 - ¹⁰Data were generated from unique isolates from patient specimens submitted to: Mayo Clinic College of Medicine and Science, Rochester, Minnesota; UK Anaerobe Reference Unit, Public Health Wales, Cardiff, UK; and International Health Management Associates Inc., Schaumburg, Illinois. All testing was performed by the agar dilution method. Information and analysis of previous versions of this table have been published.
- The proposed anaerobe antibiogram tables can be found in the Anaerobe Working Group meeting materials.
- Future steps
 - Anaerobe Working Group will work with Text and Tables Working Group to format anaerobe antibiogram for M100.
 - Publication in progress.
- Anaerobe Working Group Discussion and Recommendation
 - Motion to accept the update anaerobe antibiogram. WG Vote: 11-0-0-0.

SC DISCUSSION (MAIN POINTS)

- Ampicillin-sulbactam was not tested in the three reference laboratories so that is why that drug is not included in the antibiogram.
- The antibiogram should include a footnote or a way to designate all the organism in the “Other category”.
- How is this handled by Text and Tables Working Group?
 - Planning to do something similar to the QC appendix, so the Text and Tables Working Group does not have to track and make changes in Edaptive.
- Consider removing the decimal points in the data to make it cleaner and follow CLSI M39.
- It would be good to highlight all the isolates are world-wide, perhaps in the title of the antibiogram.
- The CLSI M45 reference method hasn’t been established for some of the organisms.
 - Dr. Simmer will send wording/organism list to the Anaerobe Working Group.
- What is the timeframe for isolates? 2017-2024
- Is it possible to get a US specific cut?
 - The data is international and there isn’t a way to separate the US data.

- *Veillonella* had a big difference between amoxicillin/clavulanate and piperacillin/tazobactam. Is it correct?
 - The data is correct. The European data seems to be driving the difference between the drugs.
 - Amoxicillin/clavulanate is tested at ratio whereas piperacillin/tazobactam is tested at the fixed drug. At low levels the MICs might be artificially low.
- Will the antibiogram bias the readers since not all active drugs are included?
 - The solution is to add in a footnote that says not all active drugs are included in the antibiogram.
- Depending on what MALDI database is use, the species level data may not be accurate.
- Is there an option to add a genus level?
 - The Anaerobe Working Group did consider that and decided not to go that route. They see susceptibility differences by the species, so they wanted to publish that.
- The Mayo data tests at the breakpoint, so they do not have data on the spread of MICs.
- There could be bias on which isolates are tested because it is possible more resistant isolates get tested for second line drugs.

A motion to approve the updated anaerobe antibiogram with an introductory paragraph and discussed footnotes and to align with the CLSI M45 species was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

10. QUALITY CONTROL WORKING GROUP (S. CULLEN AND C. PILLAR)

TIER 2 QC

DEBIO-1452

• Background

Drug: Debio-1452 (FAB 0.1)		Abbreviation (Glossary II & III): FAB	Previous ID: ANF-1252
Solvent (Table 6A): DMSO		Diluent (Table 6A): DMSO	Preparation (Table 6C combination agents): See Table 8B preparing dilutions of water-insoluble antimicrobial agents
Route of administration (Glossary II): Oral, IV		Class (Glossary I & II): FabI inhibitor	Subclass (Glossary I & II): NA
Study Report by: IHMA		Pharma Co: Debiopharm	Control Drugs: Rifampin
Additional Information (M23 requirements)	• Tier 1 Impact Assessment (stability, inoculum, reading, incubation time, etc): NA • ISO/TS 16782 assessment of Tier 2 study materials: Confirmed		
Footnotes:	• Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC): NA		
Discussion	Previously reviewed January 2024. 2 mm difference in zone diameters between disk manufacturers with lots in the Tier 2 Study. Approved a narrower range 20-27 for <i>S. aureus</i> ATCC 25923 requesting additional information from sponsor to reassess at future meeting. Vote (11/0/1/2) was to keep the range "as-is" and monitor in Tier 3. Also suggested pursuing a range for <i>S. aureus</i> ATCC 29213 as potential supplemental QC strain that is easier to read (fuzzy zones observed with <i>S. aureus</i> ATCC 25923) to potentially help with troubleshooting. Data on next slide is a new Tier 2 Study after Mast and Oxoid collaborated to harmonize disk potency. New disks were evaluated by IHMA and EDL laboratories.		

IHMA to present *S. aureus* ATCC 29213 data for Debio-1452 (0.1 µg) disks in January

Drug Name:		Debio 1452 (0.1 µg disks)				Votes:		11/0/1/2 Keep the same (20-27), monitor in Tier 3			
QC Strain	Range	% In	Median	Mm	Disk	Media	Labs	Gavan	Range Finder	Comments	
<i>S. aureus</i> ATCC 25923 Jan 2024, page 6	20-28 20-27	98.1 97.1	24	9 8	25 23	24 (2), 23 (1)	22 (1), 23 (2), 24 (2), 25 (3), 26 (1)	20-28, 98.1%, 9 mm	20-28, 98.1%, 9 mm	Variability: Disk 2mm, Media 1mm, Lab 5mm Media mfg lot 2 mode 23mm	
<i>S. aureus</i> ATCC 25923 Re-test, new disk lot page 7	18-26 19-26	100% 100%	22 22	9 8	21 22	21 (2), 22 (1), 24 (2)	21 (1), 22 (2), 23 (2)	18-26, 100%, 9mm	19-26, 100%, 8mm	Variability: Disk 1mm, Media 4mm, Lab 3mm Only 5 labs & 256 results included.	
<i>S. aureus</i> ATCC 25923 Combined, page 8&9	19-27	98.0%	23	9	NA	22 (2), 23 (1), 24 (2)	NA	19-27, 98.0%, 9 mm	NA		
<i>S. aureus</i> ATCC 25923 June 2024 exc Lab 8&9, Pg 26	20-28	98.1%	24	9	23, 25	23 (1), 24 (2)	23 (2), 24 (2), 25 (3)	20-28, 98.1%, 9 mm	20-28, 98.1%, 9 mm	Lab 8&9 not outliers but data repeated due to rifampin QC out Variability: Disk 2mm, Media 2mm, Lab 3mm	

CLSI range 20-27 approved January 2024 with action to address concerns about 2mm disk manufacturer variance (across all media).

Mast and Oxoid collaborated and made new disk lots that were evaluated by IHMA and EDL laboratories.

Re-test: Initially each QC strain was tested in triplicate using a single inoculum suspension at IHMA by a single analyst. Study expanded to include IHMA and EDL, 2 readers per lab, 10 replicates, generating 256 new data points. Each lab performed testing media sourced from 3 local manufactures, overall, 5 manufactures tested with new disk lots

Pre-Tier 2 MH agar comparison: Study design: 2 disk mfg, 4 MHA mfg, triplicate with 72 total results.. Conclusions: Media types varied +/- 1mm, Disk mfg varied 2 mm. Fuzzy zones for *S. aureus* ATCC 25923.

Leave range as 20-27 (still >95% in range). With no change made to disk manufacturing median ranged from 23, 25, 21, 22). Add to Tier 3 to monitor. Pursue *S. aureus* ATCC 29213 as option for supplemental QC for troubleshooting (tighter range, data is available) .

GDC-0829

- Background

Drug: GDC-0829		Abbreviation (Glossary II & III): TBD	Previous ID: NA
Solvent (Table 6A): Water		Diluent (Table 6A): Water	Preparation (Table 6C combination agents): No special instructions
Route of administration (Glossary II): IV		Class (Glossary I & II): arylomycin (new class small molecule)	Subclass (Glossary I & II): TBD
Study Report by: IHMA		Pharma Co: Roche_Genentech	Control Drugs: Meropenem
Additional Information (M23 requirements)	• Tier 1 Impact Assessment (stability, inoculum, reading, incubation time, etc): • 31 parameters evaluated with triplicate testing for 6 strains (QC & clinical isolates) • Minimally impacted by parameter variations (less sensitive than control drug meropenem and CZA). No impact with E. coli ATCC and P. aeruginosa ATCC 27853. • A. baumannii strains MIC varied by two to four doubling dilutions with acidic pH, supplementation with calcium, prolonged incubation of 48h, the addition of 50% human serum (see appendix) • Assessment of MICs in 20 biological replicates against each of the three reference strains tested showed high reproducibility and narrow ranges of two to three doubling dilutions • Agar dilution comparable to BMD • ISO/TS 16782 assessment of Tier 2 study materials: Yes		
Footnotes:	• Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC): None		
Discussion	GDC-0829 belongs to a novel class of antibiotics derived from the arylomycin natural chemical structure. GDC-0829 inhibits LepB, an inner membrane-bound type I signal peptidase essential in Gram negative species. Inhibition of LepB activity results in the toxic accumulation of pre-proteins. GDC-0829 is active against Enterobacteriaceae, Acinetobacter baumannii, and Pseudomonas aeruginosa both in vitro and in vivo and shows a low frequency of resistance against all the target species.		

- Proposed MIC QC Ranges

Drug Name:		GDC-0829				Votes:		12/0/0/2 for <i>E. coli</i> ATCC 25922 for 0.12-0.5 µg/mL 10/2/0/2 for <i>P. aeruginosa</i> ATCC 27853 for 0.25-2 µg/mL		
QC Strain	Range	% In	Mode	Dil	Shoulder	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
<i>Escherichia coli</i> ATCC 25922	0.125-0.5	100%	0.25	3	45% @ 0.125	0.13 (1), 0.25 (2)	0.13 (2), 0.25 (7)	0.125-0.5, 3, 100%	0.125-0.5, 3, 100%	Some media variability.
<i>P. aeruginosa</i> ATCC 27853	0.25-1 0.5-2 0.25-2	100% 99.6% 100%	0.5	3	54% @ 1	0.5 (2), 1 (1)	0.5 (8), 1 (1)	0.25-1, 3, 100%	0.25-1, 3, 100%	Media variability (Lot 1 commonly used, mode at top of proposed range). No significant impact with parameters evaluated in Tier 1 study. Option 5 approved. Include 0.5-2 with footnote to address early Tier 3 data

P. aeruginosa ATCC 27853: **Mode in additional studies (early Tier 3) was 1 µg/mL with some results at 2 µg/mL. See next slide.**

EDL proposes to include 2 µg/mL within the QC range. EDL does not support a four-dilution range 0.25-2 µg/mL (potentially allowing too much deviations) but would accept a three-dilution range 0.5-2 µg/mL based on

- 1) infrequent occurrences at 0.25 µg/mL.
- 2) high likelihood of Lot 1 medium use in future laboratories and the non-negligible frequency of the occurrence at 2 µg/mL with this medium
- 3) bimodal distribution; significant shoulder at 1 µg/mL in the Tier 2 study and in the global distribution.

Options

- 1) *P. aeruginosa* ATCC 27853 range 0.25-2 (4 dil): not acceptable to EUCAST, not ideal to have different EUCAST & CLSI ranges.
- 2) *P. aeruginosa* ATCC 27853 range 0.5-2 (3 dil) 99.6% in range: 0.25 rare – only 1 occurrence, acceptable to EUCAST
- 3) Only use *E. coli* ATCC 25922 range 0.125-0.5: acceptable to EUCAST, requires additional dilution for on-scale QC
- 4) *E. coli* ATCC 0.125-0.5 and *P. aeruginosa* ATCC 27853 0.25-1 (monitor QC): risk 10% of QC could be invalid
- 5) ***P. aeruginosa* ATCC 27853 range is 0.25-2 µg/mL with mode 0.5-1 µg/mL. Results at 0.25 and 2 were seen less frequently in Tier 2 studies and if observed frequently in routine testing, consider troubleshooting.**

QCWG 2023 Jan

9

SC DISCUSSION (MAIN POINTS)

- For *P. aeruginosa* ATCC 27853, the sponsor wanted an MIC of 2 µg/mL included since the mode is 1 µg/mL.

A motion to accept the GDC-0829 MIC QC range for *E. coli* ATCC 25922 (0.12-0.5 µg/mL) was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

A motion to accept the GDC-0829 MIC QC for *P. aeruginosa* ATCC 27853 (0.25-2 µg/mL) with the comment, “*P. aeruginosa* ATCC 28753 range is 0.25-2 µg/mL with mode 0.5-1 µg/mL. Results at 0.25 µg/mL and 2 µg/mL were seen less frequently in Tier 2 studies and if observed frequently in routine testing, consider troubleshooting.” was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

AZTREONAM-NACUBACTAM

Background

Drug: Aztreonam/nacubactam (10/20 µg)	Abbreviation (Glossary II & III): ANC	Previous ID: ??
Solvent (Table 6A): ??	Diluent (Table 6A): ??	Preparation (Table 6C combination agents): ??
Route of administration (Glossary II): ??	Class (Glossary I & II): β-lactam combination agents	Subclass (Glossary I & II): NA
Study Report by: IHMA	Pharma Co: Meiji Seika Pharma Co., Ltd.	Control Drugs: Cefepime 30 µg, aztreonam 30 µg, and meropenem/vaborbactam 20/10 µg

Additional Information (M23 requirements)	- Tier 1 Impact Assessment (stability, inoculum, reading, incubation time, etc): ?? - ISO/TS 16782 assessment of Tier 2 study materials: ??
Footnotes:	Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC): NA
Discussion	Nacubactam is a novel diazabicyclooctane beta-lactamase inhibitor with activity against serine β-lactamases (classes A and C and some class D). Nacubactam also has direct activity against penicillin binding protein 2 in Enterobacterales. Testing also included 4 clinical strains resistant to single drug (2 wild-type, 2 non-wild-type)



Red circle = breakthrough colony

QCWG 2023 Jan

11

Proposed Disk Diffusion QC Ranges

Drug Name:		Aztreonam/nacubatam (1:1, 10/20 µg) ANC				Votes:		12/0/0/2		
QC Strain	Range	% In	Median	Mm	Media	Disk	Labs	Gavan	Range Finder	Comments
<i>E.coli</i> ATCC 25922 (page 28)	27-37	99.3%	32	11	32 (2), 33 (1)	32 (1), 33 (1)	28 (1), 30 (1), 32 (3), 34 (2)	29-35, 7mm, 86.9%	27-37, 8mm, 99.3%	Aztreonam CLSI range 28-36 Variability: media 1mm, disk 1mm, labs 7mm. Range not needed.
<i>P. aeruginosa</i> ATCC 27853 (page 33)	17-23	95.6%	20	7	19 (1), 20 (2)	19 (1), 20 (1)	18 (1), 19 (3), 20 (4), 21 (1)	17-23, 7 mm, 95.6%	16-23, 8 mm, 97.8%	Aztreonam CLSI range 23-29 Variability: media 1mm, disk 1mm, labs 4mm
<i>K. pneumoniae</i> ATCC 700603 (page 38)	20-26	99.6%	23	7	23 (3)	22 (1), 24 (1)	22 (1), 23 (5), 24 (2), 25 (1)	20-26, 7mm, 99.6%	20-26, 7mm, 99.6%	Aztreonam CLSI range 10-16 Variability: media none, disk 2mm, labs 4mm Routine QC (shade green)
<i>K. pneumoniae</i> ATCC BAA-2814 (page 41)	18-26	95.8%	22	9	22 (2), 23 (1)	21 (1), 23 (1)	21 (2), 22 (2), 23 (2), 24 (1), 25 (1)	18-26, 9mm, 95.8%	18-27, 10mm, 98.1%	No CLSI range, study results ≤6 Routine QC (shade green) Variability: media 1mm, disk 2mm, labs 5mm. Excluded lab 9 (expired)

- Breakthrough colonies seen with QC strains except *K. pneumoniae* ATCC 700603. No footnote needed since ranges based on reading inner zone per normal instructions. Used Gavan range since it was same for inner and outer zones and included ≥95% of results. Range finder range only differed by 1mm for some bug/drugs.
- Refer to Tables 3, 6, 10 in report for proposed ranges and performance when including and excluding breakthrough colonies
- A fifth lot of MH agar tested with *P. aeruginosa* ATCC 27853 for repeat test due to expiry of medium 4 plates.
- Breakthrough colonies were also observed with *P. aeruginosa* ATCC 27853 with MEV disk. 96.4% were in range using inner zones but 100% if using outer zones. Excluding out of range MEV data had no impact of proposed ranges. Added to Tier 3 monitoring.

SC DISCUSSION (MAIN POINTS)

- E. coli* ATCC 25922 is commonly used in laboratories, so consider if it should be included.
 - This organism does not truly QC the combination. There was additional variability in this organism, so the working group thought it should be excluded.
- Suggestion to add a footnote that colonies for the inner zone should be read.
- Ranges for additional strains can be helpful for laboratories to have an already characterized isolate for validation.
- For *E. coli*, 7 mm range by Gavin statistic only captured 87% of the data, which was below the threshold. Using RangeFinder it was an 11 mm range to get above 95% and then the zone sizes overlap with aztreonam alone.
- For *E. coli*, it also had laboratory to laboratory variability.

A motion to accept the aztreonam-nacubactam disk (10/20 µg) diffusion QC for *P. aeruginosa* ATCC 27853 (17-23 mm), *K. pneumoniae* ATCC 700603 (20-26 mm), and *K. pneumoniae* BAA-2814 (18-26 mm) with comment about reading the colonies within the inner zone was made and seconded. Identify *K. pneumoniae* ATCC 700603 and *K. pneumoniae* BAA-2814 as QC strains for routine QC with green shading. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

A motion to amend the previously approved motion and not add a comment about reading the colonies within the inner zone for aztreonam-nacubactam disk diffusion QC was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

CEFEPIME-NACUBACTAM

- Background

Drug: cefepime/nacubactam (10/20 µg)	Abbreviation (Glossary II & III): FNC	Previous ID:
Solvent (Table 6A): ??	Diluent (Table 6A): ??	Preparation (Table 6C combination agents): ??
Route of administration (Glossary II): ??	Class (Glossary I & II): β-lactam combination agents	Subclass (Glossary I & II): NA
Study Report by: IHMA	Pharma Co: Meiji Seika Pharma Co., Ltd.	Control Drugs: Cefepime 30 µg, aztreonam 30 µg , and meropenem/vaborbactam 20/10 µg

Additional Information (M23 requirements)	- Tier 1 Impact Assessment (stability, inoculum, reading, incubation time, etc): ?? - ISO/TS 16782 assessment of Tier 2 study materials: ??
Footnotes:	Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC): NA
Discussion	Nacubactam is a novel diazabicyclooctane beta-lactamase inhibitor with activity against serine β-lactamases (classes A and C and some class D). Nacubactam also has direct activity against penicillin binding protein 2 in Enterobacterales. Testing also included 4 clinical strains resistant to single drug (2 wild-type, 2 non-wild-type

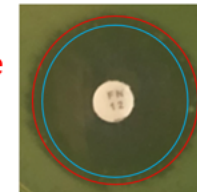
- Proposed Disk Diffusion QC Ranges

Drug Name:	cefepime/nacubactam (10/20 µg) FNC					Votes:		12/0/0/2		
QC Strain	Range	% In	Median	Mm	Media	Disk	Labs	Gavan	Range Finder	Comments
<i>E.coli</i> ATCC 25922 (page 45)	30-37	99.6	34	8	33 (1), 34 (2)	33 (2)	29 (1), 31 (1), 32 (1), 33 (1), 34 (3), 35 (2)	31-37 7mm, 97.5%	30-37, 8mm, 99.6%	Cefepime CLSI range 31-37 Variability: media 1mm, disk none, labs 6mm
<i>P. aeruginosa</i> ATCC 27853 (page 50)	21-29	97.6%	25	9	25 (2), 26 (1)	25 (2)	22 (2), 24 (1), 25 (3), 26 (2), 27 (1),	22-28, 7mm, 94.3%	21-29, 9mm, 97.6%	Cefepime CLSI range 25-31 Variability: media 1mm, disk none, labs 6mm
<i>K. pneumoniae</i> ATCC 700603 (page 55)	24-29	100%	27	6	27 (3)	27 (2)	26 (4), 27 (4), 28 (1)	25-29, 5mm, 98.1%	24-29, 6mm, 100%	Cefepime CLSI range 23-29 Variability: media none, disk none, labs 3mm
<i>K. pneumoniae</i> ATCC BAA-2814 (page 57)	20-27	97.9%	23	8	23 (2), 24 (1)	23 (1), 24 (1)	22 (1), 23 (3), 24 (2), 25 (2)	20-26, 7mm, 93.8%	20-27, 8mm, 97.9%	No CLSI range, results from this study 6-10 Routine QC (shade green) Variability: media 1mm, disk 1mm, labs 4mm

Breakthrough colonies seen with QC strains except *K. pneumoniae* ATCC 700603.

No footnote needed since ranges based on reading inner zone per normal instructions. Only slight difference between range using inner or outer zone. Range finder used to include ≥95% in range.

A fifth lot of MH agar tested with *P. aeruginosa* ATCC 27853 for repeat test due to expiry of medium 4 plates
Refer to Tables 13, 16, 20 in report for proposed ranges and performance when including and excluding breakthrough colonies. See page 62 in report.



Blue line: inner colony diameter
Red line: outer zone diameter

SC DISCUSSION (MAIN POINTS)

- E. coli* ATCC 25922 was excluded to be consistent with aztreonam/nacubactam, not because of variability.
- E. coli* ATCC 25922 should never be used as the only QC. If CLSI wants this organism, then it needs to be clear to not use this one QC strain to test the drug.
- Is a footnote needed on reading the inner colonies?
 - Agreement that footnote is not needed for ceftazidime-nacubactam and aztreonam/nacubactam.

A motion to accept the cefepime-nacubactam disk (10/20 µg) diffusion QC for *P. aeruginosa* ATCC 27853 (21-29 mm), *K. pneumoniae* ATCC 700603 (24-29 mm), and *K. pneumoniae* BAA-2814 (20-27 mm) and for the QC Working Group to revisit the *E. coli* ATCC 25922 data for January 2026 was made and seconded. Identify *K. pneumoniae* ATCC BAA-2814 as QC strain for routine QC with green shading. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

CLINDAMYCIN

Background

Drug Name:	Clindamycin (2µg) Tier 3 Anaerobe Disk/FAA media	Votes:	x/x/x/x (Defer vote until January)
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QC Strain	Range	% In	Median	Mm	Media	Disk	Labs	Gavan	Range Finder	Comments
<i>Bacteroides fragilis</i> ATCC 25285	23-29	99.1%	26	7	24 (2), 25 (2), 26 (5), 27 (4), 28 (1)	24 (1), 26 (1), 27(1)	24 (2), 25 (1), 26 (1), 27 (2)	23-29, 7mm, 99.1%	NA (dataset too large to analyze)	EUCAST range: 23-29, 7mm, target 26 Variability: Lab 4 mm, Disk lot 1 mm, Media 2mm

No current ranges for disk diffusion for anaerobes.

Only one disk manufacturer was used (4 lots).

- QC ranges were established using data from only one disk manufacturer. Disk from other manufacturers were not available at the time of testing.
- 6 labs, 14 media lots (FAA) from 2 media manufacturers.
- Replicates: 1 to 442 per site.
- Total data points: 1733
- EUCAST recently revised range to 22-28 based on Tier 3 feedback with signal at bottom of range with potential for out of range low results (data not shown but will be provided for Jan 2026 meeting).
- Feedback provided on tables: include separate tables for the different studies (e.g., media, disk lots) and one slide with totals excluding redundant data.
- Discussion: Defer vote until Jan 2026 to consider data used to support recent EUCAST range change. Could any differences between anaerobe chamber results and anaerobe boxes contribute to lab variability?

SC DISCUSSION (MAIN POINTS)

- CLSI M23 outlines a tier 2 study but also allows for an alternative study design.

TIER 3 DISK DIFFUSION QC

- Antimicrobial/organism combinations monitoring/compiling data to re-evaluate the current QC range or have no QC ranges.
- CLSI M23 Tier 3 requirements: 3 laboratories, 2 media lots, 10 reps/lab and 50 reps per media, 2 disk lots for a total of 500 results.
- Refer to separate files for additional details and raw data.
- Discussion: Add meropenem-vaborbactam to Tier 3 list for *P. aeruginosa* ATCC 27853 (control drug for aztreonam-nacubactam)

QC Strain (ATCC)	Antimicrobial	Current Range	Action Recmd	Concern	Update	Date Reported
<i>N. gonorrhoeae</i> ATCC 49226	Spectinomycin 100 µg	23-29	Continue to monitor until June 2025. Request additional data. Approved range change to 24-30 11/0/2/1)	QC study out high	June 2025: No additional data. June 2022: Observations in gentamicin QC study, especially with one lab and media	June-22
<i>E. coli</i> NCTC 13353	Ceftibuten 30 µg		Continue to monitor until January 2027. Request additional data.	Zone diameters in the lower part of range and out of range	June 2025: No additional data.	Jan-24

SPECTINOMYCIN

- Background

June 2022 QCWG Presentation

Spectinomycin Control Disk from Manufacturer A on 3 Lots of Media

Table 4: *Neisseria gonorrhoeae* ATCC 49226

Quality Control Results with Spectinomycin

Spectinomycin 100 µg Disks															QC Range 23 mm - 29 mm																
	Lab 1			Lab 2			Lab 3			Lab 4			Lab 5			Lab 6			Lab 7			Lab 8			Lab 9			All Labs			
Media Lot	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	
ZOI (mm)																															
22																												0	0	0	
23																												0	0	0	
24				1	3																				1			1	4	0	
25		2		1	1								3				1	1	3						5	6		12	10	1	
26	4	1		3	3	3		6	1			1	5	2			2		7	2					5	3	8	24	20	12	
27	5	5	1	4	3					3	6			7			2	4	2		8						1	14	33	4	
28	1	2	2	1		3	4	3	3	3	2		1	1	1	3		6			1		3				1	13	11	17	
29			6				5	1	3	4	1	2	1		6	3	2	1		2								13	4	20	
30			1			4	1		3			6			1	2	1			3		4					3	5	18		
31												2			1					3	3	2	2				3	2	8		
32																				1		1	1				0	1	2		
33															1						2		6				2	0	7		
34																					5		1				5	0	1		
35																												0	0	0	
36																												0	0	0	
Total	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	90	90	90	
Combined	30			30			30			30			30			30			30			30			30			270			

Abnormal Central Tendency Statistics

- Lab 8: Mean & Mode
- Lab 5: Mode

Almost all of Lab 8's Spec. data is out-of-range

Most out-of-range values from Lot 3

June 2022 QCWG Presentation

Spectinomycin Control Disk from Manufacturer A on 3 Lots of Media- Excluding Lab 8

Table 6: *Neisseria gonorrhoeae* ATCC 49226

Quality Control Results with Spectinomycin

Spectinomycin 100 µg Disks													QC Range 23 mm - 29 mm															
	Lab 1			Lab 2			Lab 3			Lab 4			Lab 5			Lab 6			Lab 7			Lab 9			All Labs			
Media Lot	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	
ZOI (mm)																												
22																									0	0	0	
23																									0	0	0	
24				1	3																1				1	4	0	
25		2		1	1								3				1	1	3			5	6		12	10	1	
26	4	1		3	3	3		6	1		1		5	2			2		7	2		5	3	8	24	20	12	
27	5	5	1	4	3					3	6			7		2	4	2		8				1	14	33	4	
28	1	2	2	1		3	4	3	3	3	2		1	1	1	3		6			1			1	13	8	17	
29			6				5	1	3	4	1	2	1		6	3	2	1		2					13	4	20	
30			1			4	1		3			6			1	2	1			3				3	1	18		
31												2			1					3				0	0	6		
32																				1				0	0	1		
33															1									0	0	1		
34																								0	0	0		
35																								0	0	0		
36																								0	0	0		
Total	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	80	80	80	
Combined	30			30			30			30			30			30			30			30			240			

High variability with
Spec. on Lot 3

Spectinomycin *N. gonorrhoeae* ATCC 49226 Additional Analysis

Compare Media Lots (Excluding Lab 8)

Media Lot	Lot 1	Lot 2	Lot 3
ZOI (mm)			
22			
23			
24	1	4	
25	12	10	1
26	24	20	12
27	14	33	4
28	13	11	17
29	13	4	20
30	3	5	18
31	3	2	8
32		1	2
33	2		7
34	5		1
35			
36			
Mean	27.62	26.94	29.09
SD	2.38	1.59	2.01
+2 SD	32.4	30.1	33.1
-2 SD	22.9	23.8	25.1

RangeFinder

All Labs		
Calculated QC Range	Gavan QC Range	Mean
23 to 33	24 to 31	27.9
Range	Range	StDev
11	7	2.2
% Obs. Captured	% Captured	+2 SD
97.8%	93.3%	32.3
Prob'ty Outside Range		-2 SD
0.023		23.5

Exclude Lab 8		
Calculated QC Range	Gavan QC Range	Mean
24 to 31	25 to 29	27.4
Range	Range	StDev
8	4	1.7
% Obs. Captured	% Captured	+2 SD
99.2%	85.4%	30.8
Prob'ty Outside Range		-2 SD
0.041		24.0

- Quality Control Working Group Discussion and Recommendation
 - Discuss the potential to change the QC range from 23-29 mm to 24-30 mm (88.5% including Lab 8, 96.7% excluding Lab 8)
 - No results 23 mm or below
 - 9.6% (30/270) results at 30 mm across multiple laboratories

A motion to accept the spectinomycin disk (100 µg) diffusion QC for *Neisseria gonorrhoeae* ATCC 49226 (24-30 mm) was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)

TIER 3 MIC QC

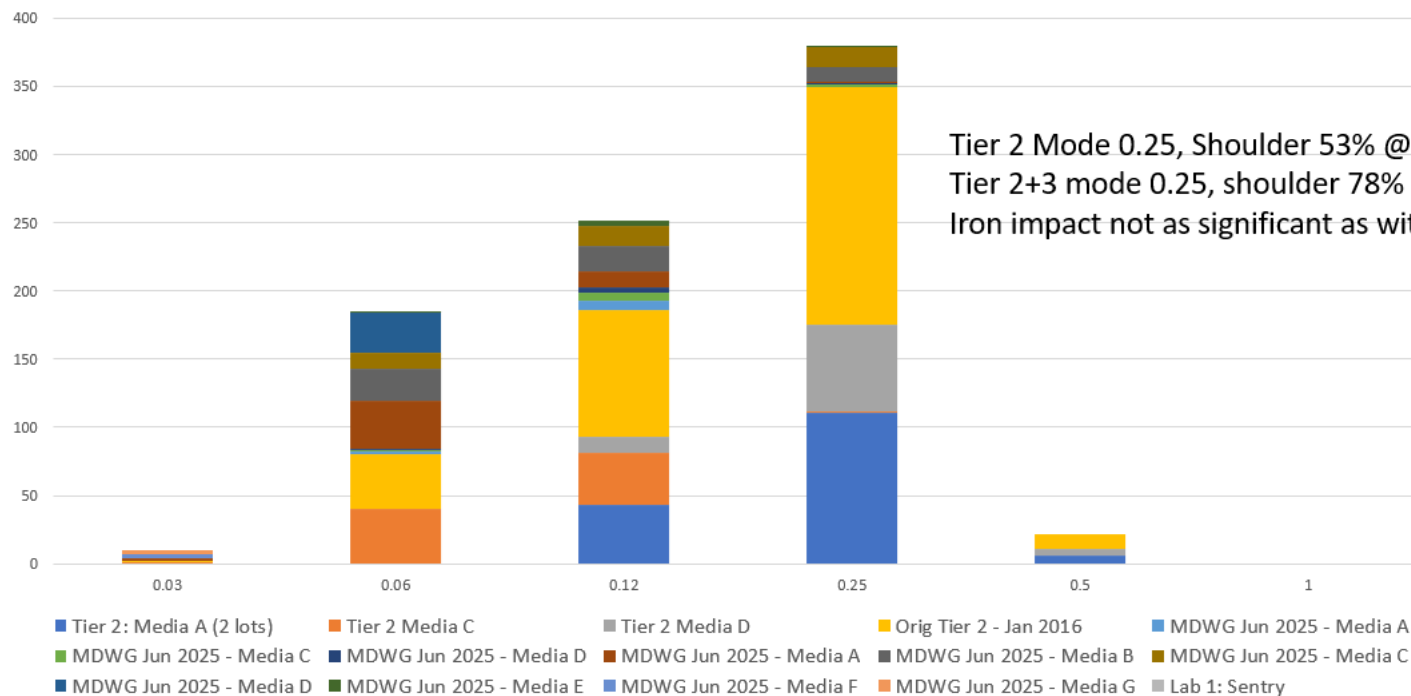
QC Strain (ATCC)	Antimicrobial	Current Range	Action Recommended	Concern/Analysis	Reported
<i>E. coli</i> ATCC 25922	Aztreonam/ avibactam	0.03/4-0.12/4	QCWG Jan 2025 supported including 0.25/4 in the QC range but after addition of new data and discussion with EUCAST, shoulder is now <50% so no change proposed. Continue to monitor	Report for shoulder/bimodal distribution with large amount of data at high end of current range. Dec 2023: Additional data added from 3 labs, resulting in 5 total labs with Tier 3 data (n=2158) + Tier 2 (n=237). Tier 3 data has 56% shoulder at 0.12/4, with 3 of 5 labs demonstrating bimodal distributions or a mode at the high end of the range; <1% out of QC high. NOTE: Aztreonam alone was changed from 0.06-0.25 to 0.06-0.5 for the same reason. June 2024: Added supplemental data provided by one lab in Jan 2024 bringing Tier 3 data to n=2198, did not impact prior analysis. Jan 2025: Added supplemental data provided by one lab bringing Tier 3 data to n=2270; that new set was from three sites, one with a mode at 0.12/4 and two bimodal at 0.06/4 and 0.12/4; shoulder at 0.12/4 is 59% of the mode. Jun 2025: Added 11 results from one lab and >450+ results from an additional lab bringing Tier 3 data to n=3064; new data more consistent with current range dropping shoulder at 0.12/4 to 48% of the Tier 3 overall mode.	21-Jun

QC Strain (ATCC)	Antimicrobial	Current Range	Action Recommended	Concern/Analysis	Reported
<i>K. pneumoniae</i> ATCC BAA-1705	Meropenem	8-64	New signal, request more data	This is for QC integrity check. Signal from Thermo study with three sites had 23/80 (28%) of results out of QC low with MIC values (≤ 4). One lab provided 32 additional datapoints from 3 years with 30 results at 32 and 2 results at 16. Two additional sites provided data for 32 and 143 total instances where >99% were >4.	25-Jun
<i>E. coli</i> NCTC 13353	Ceftibuten	16-64	New signal, request more data	Signal from Microbiologics study where this organism was tested 20 times over 4 days across three media lots and had 100% out of QC high (≥ 128) while the same panel tested mid-range for two other QC organisms.	25-Jun
<i>S. pneumoniae</i> ATCC 49619	Doxycycline	0.016-0.12	No new data provided for review.	Signal from EDL 5 lab dried panel study where nearly 70% of results tested at 0.12, the high end of the range; requesting frozen reference method data to see if further monitoring or adjustment is warranted Jun 2024: no reference data submitted	23-Jun
<i>P. aeruginosa</i> ATCC 27853	Cefiderocol	0.06-0.5	Add footnotes to <i>P. aeruginosa</i> ATCC 27853 (with mode) and SR27001 (for media QC). Add to Troubleshooting guide	Elevated MICs observed in MDWG evaluation of multiple media manufacturers and chelation process may correlate with elevated clinical MICs (false resistant). Identified QC <i>P. aeruginosa</i> SR27001 to qualify media. <i>P. aeruginosa</i> 27853 not adequate alone to evaluate media but frequent MICs at edge or outside QC range may be indicate poor correlation with clinical isolates.	25-Jan

CEFIDEROCOL

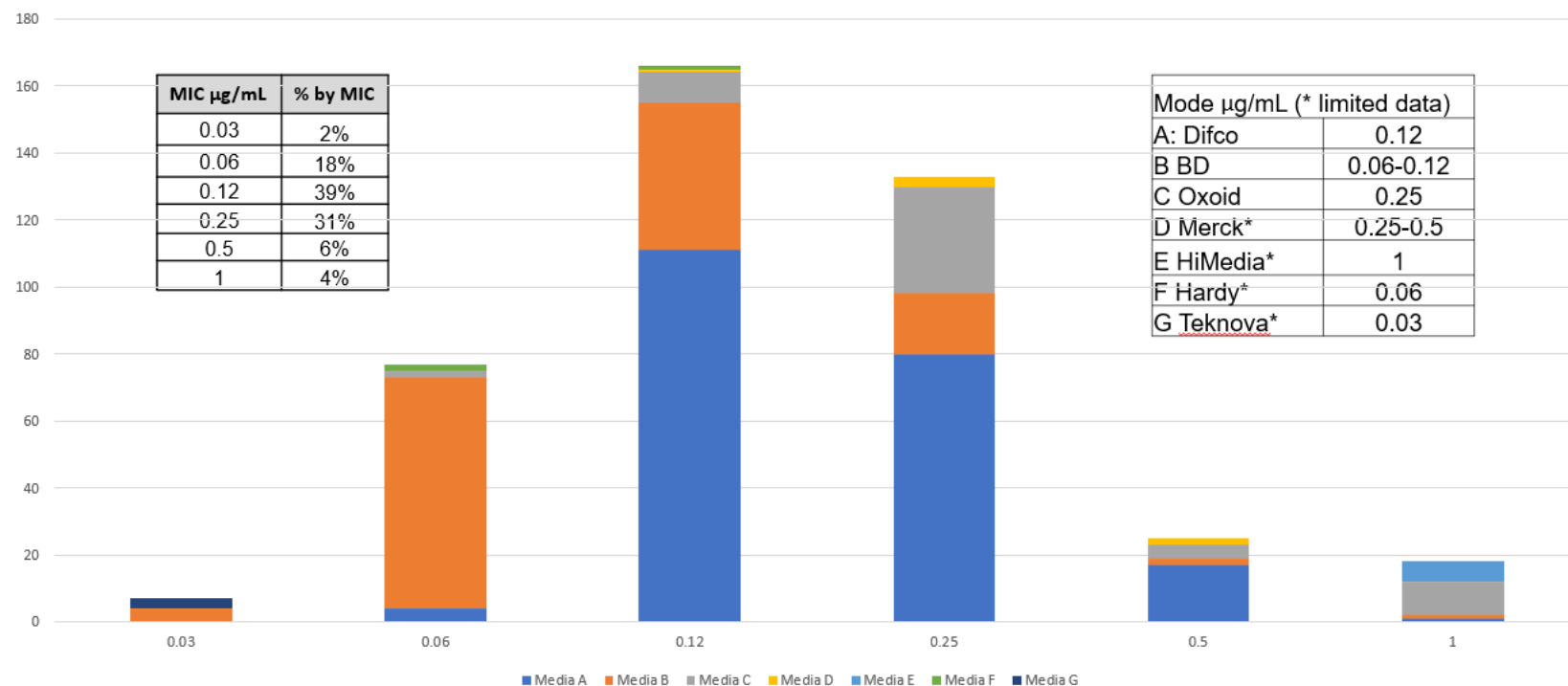
- Background

Cefiderocol Tier 2 & 3 Data: *E. coli* ATCC 25922



Tier 2 Mode 0.25, Shoulder 53% @ 0.12
Tier 2+3 mode 0.25, shoulder 78% @ 0.12
Iron impact not as significant as with other organisms

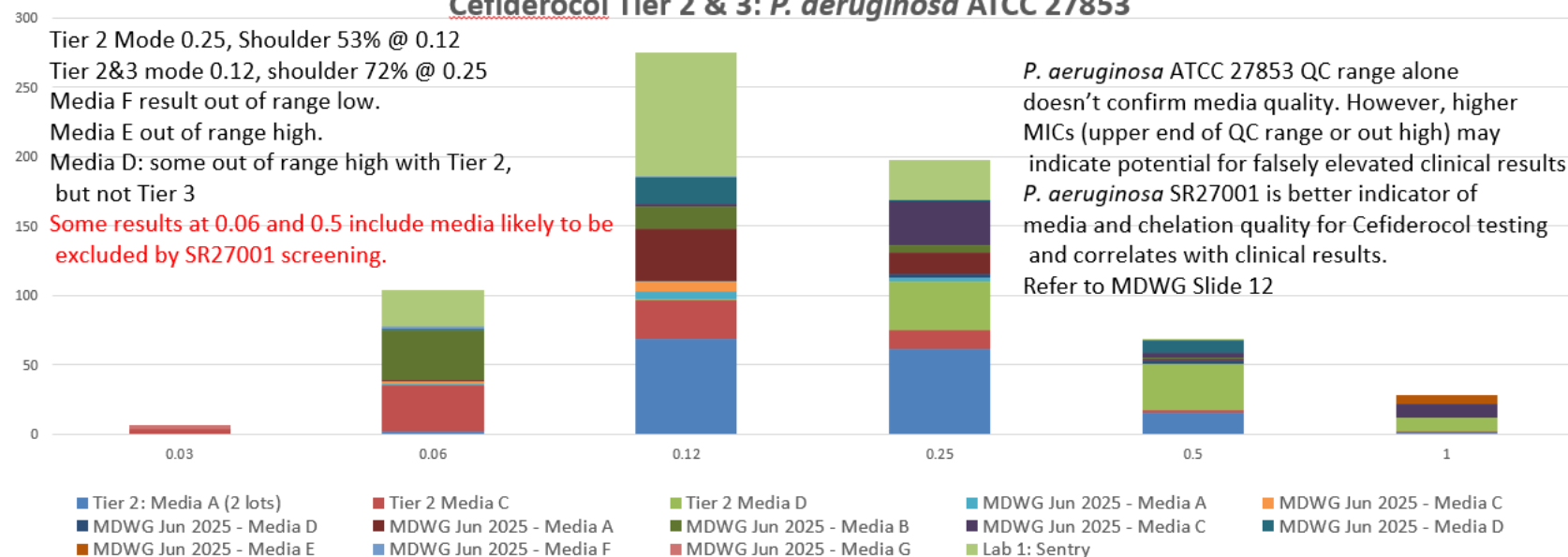
Cefiderocol *P. aeruginosa* ATCC 27853 by Media (Tier 2&3 exc Sentry)



Significant difference in media manufacturers with *P. aeruginosa* ATCC 27853

Overall mode 0.12 with 80% shoulder at 0.25 µg/mL

Cefiderocol Tier 2 & 3: *P. aeruginosa* ATCC 27853



Quality Control Working Group Discussion and Recommendation

Proposed Footnotes to QC Table 5A-1:

- To determine the suitability of the media for cefiderocol, test lot and/or media manufacturer using *P. aeruginosa* SR27001. If the MIC is $\leq 2 \mu\text{g/mL}$ and 2 dilutions or more lower in ID-CAMHB compared to CAMHB, the medium is acceptable. Elevated MICs ($\geq 4 \mu\text{g/mL}$) were observed with some media manufacturers and/or inadequate chelation which may result in falsely elevated MICs (false resistant) with clinical isolates. Note: Guidelines were established based on investigation of media performance with Cefiderocol and did not follow M23 Tier 2 guidelines. (could add reference if study gets published).
- P. aeruginosa* ATCC 27853 is recommended for routine QC (0.06-0.5 $\mu\text{g/mL}$, mode 0.12-0.25 $\mu\text{g/mL}$). Additional investigation may be required if frequent MIC results at 0.06 $\mu\text{g/mL}$ or 0.5 $\mu\text{g/mL}$ are observed (for troubleshooting, see Table 5G).

- Motion to accept proposed footnotes. WG Vote: 10-0-2-2.

SC DISCUSSION (MAIN POINTS)

- When the QC range is high or low, then see clinical isolate MICs shifting high or low.
- The original proposal did not have a cutoff and thought that was acceptable when the Cefiderocol AHWG reviewed this.
 - Does this MIC cutoff help guide people?
 - The original intent was to indicate that the two dilution drop was to indicate adequate chelation. The strain can be used to QC media.
 - Oxoid data gives higher MICs for all QC organisms. There is concern that there isn't data to say the higher Oxoid QC MICs are not accurate.
- Since the strain has not been deposited yet, does that prevent this from going into the upcoming M100?
 - The intended user is for anyone making the media themselves. This is not going to be a routine QC organism.

- There are lots of values at 0.06 in the cefiderocol data.
- The mode should be 0.12 - 0.25 (there is an error on the slide saying it is to 0.5, but that is too high).
- It look like the BD media will be out at 0.06.
- What counts as “frequently”?

A motion to add the footnote for Table 5A-1 cefiderocol MIC QC with mode 0.12-0.25 µg/mL as “*P. aeruginosa* ATCC 27853 is recommended for routine QC (0.06-0.5 µg/mL, mode 0.12-0.25 µg/mL). Additional investigation may be required if frequent MIC results at 0.06 µg/mL or 0.5 µg/mL are observed (for troubleshooting, see Table 5G).” was made and seconded. Vote: 9 for, 4 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:

- Cefiderocol AHWG should have time to review this data.
- Concern that media at 0.06 needs to be evaluated because that is the BD media, which is one of the main medias used.

CEFIDEROCOL CONTINUED

Additional discussions occurred during the Methods Working Group presentation.

SC DISCUSSION (MAIN POINTS)

- Do not want to exclude 0.06 because that value does occur between 0 and 18% of the time based on media used.
- Revise the approved motion footnote.
- Why use 10% as a cutoff? It seems like a normal bell-shaped curve might end up with a 10% at these MICs.
- Perhaps saying if an MIC 0.5 µg/mL is the most common result, then that is an issue.
- Other places in CLSI use different percent cutoffs for frequency, so it can be confusing to have a percent.

A motion to amend the previously approved motion and accept the Table 5A-1 cefiderocol QC footnote as “*P. aeruginosa* ATCC 27853 is recommended for routine QC (0.06-0.5 µg/mL, mode 0.12-0.5 µg/mL). Frequent MICs at 0.5 µg/mL may be observed with some media manufacturers, which may result in falsely elevated MICs when testing clinical isolates (eg, false resistant results).” was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

CEFIDEROCOL TROUBLESHOOTING GUIDE TABLE

- Proposed additions to Table 5G MIC and 4D Disks

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
Cefiderocol	P. aeruginosa ATCC 27853 E. coli ATCC 25922	MIC too high	Incomplete removal of iron during chelation. Media manufacturer or lot gives elevated MICs compared to media used to establish CLSI reference method.	Repeat chelation process. Measure iron concentration in media. Use another lot or manufacturer of media. Confirm media quality with QC P. aeruginosa SR27001
Various	Any	Many MICs too high or too low	Possible reading/ transcription error	Recheck readings. Refer to M07-Ed12-QG Use alternative lot.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
Various	Any	Zone too small (resistant) or too large (susceptible)	Possible reading/ transcription error	Recheck readings. Refer to M02-Ed12-QG Use alternative lot.

80% READ FOOTNOTES

- Contezolid
 - Jan 2025 QCWG recommendations
 - Add footnote regarding trailing and read at 80% reduction in growth (use same footnote as other oxazolidinones)
 - Did not include recommendations for text
 - M100 35th Edition has similar comments for other oxazolidinones but not directly applicable to contezolid
- New proposed footnote to add to contezolid in Table 5A-1
 - n. For *S. aureus* ATCC® 29213 and *E. faecalis* ATCC® 29212 when testing contezolid by broth microdilution MIC, trailing growth can make end point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see CLSI M07), Read the end point at the concentration in which there is ≥ 80% reduction in growth compared with the control (see CLSI M07).
- Sulfamethoxazole, Trimethoprim and Trimethoprim/Sulfamethoxazole (1/19)
 - No footnote currently included to indicate reading is different
 - Proposed comment to add to Table 5A-1 for these antimicrobial agents.
 - Read the end point at the concentration in which there is 80% reduction in growth compared to the control.
 - Trailing growth can be seen; therefore, read the end point at the concentration in which there is 80% reduction in growth compared to the control.

READING GUIDES AND FOOTNOTES

- M07-Ed12-QG
 - Page 2, Figure 4: For reading certain classes of antimicrobials or antimicrobial agents (eg, lincosamides, tetracyclines, macrolides, phenicols, oxazolidinones, streptogramins, fusidic acid), trailing growth can occur. Read the MIC as the first well where trailing in growth begins.
 - Page 3, Figure 6: Trimethoprim-sulfamethoxazole or sulfisoxazole MIC end points should be read at 80% inhibition.
- M100 35th Edition Table 5A-1
 - Linezolid: r, Tedizolid: x
 - r. QC range for *S. aureus* ATCC 25923 with linezolid is 1-4 µg/mL; this strain exhibits less trailing, and MIC endpoints are easier to interpret. *S. aureus* ATCC 25923 is considered a supplemental QC strain and is not required for routine QC of linezolid MIC tests.
 - x. QC range for *S. aureus* ATCC 25923 with tedizolid is 0.12-0.5; this strain exhibits less trailing, and MIC endpoints are easier to interpret. *S. aureus* ATCC 25923 is considered a supplemental QC strain and is not required for routine QC of tedizolid MIC tests.
 - Trimethoprim and Trimethoprim-sulfamethoxazole: v, Sulfisoxazole: v and w
 - v. Very medium-dependent, especially with enterococci.
 - w. Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
 - CLSI 100 Table 5G Troubleshooting: Proposed addition in red font (same for Table 4D for disks).

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
Various	Any	Many MICs too high or too low	Possible reading/transcription error	Recheck readings. Refer to M07-Ed12-QG Use alternative lot.

A motion to add reference to CLSI M02 and M07 Quick Guides in Tables 4D and 5G, respectively was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)

UPDATE AND FEEDBACK ON ROUTINE QC CHANGES

- Document Status
 - Appendix I: Selection of QC Strains and QC Testing Frequency published with CLSI M100 35th Edition
 - Rationale Document: complete, final edits from CAP before publishing
 - Quick Guide: complete, final edits from CAP before publishing
 - IQCP MIC Tool: complete, final edits from CAP before publishing
 - IQCP Example: final ASM edits before publishing
- Educational sessions: 23 February 2025
- General Feedback: minimal so far, Outreach Working Group is requesting feedback
- CAP Feedback: checklist revisions in process
- Future actions/improvements: to be determined

FUTURE MEETING ITEMS

- B-Lactamase/B-Lactamase Inhibitors Combination QC: Potential Harmonization and Improvements
 - CLSI will continue to recommend best QC strains for routine testing to minimize number of QC strains for users to test
 - EUCAST currently recommends a susceptible and resistant QC strain. They will consider options to indicate when a susceptible strain is recommended (eg, manufacturer vs user)
 - Best practice to include 3 lots of media when doing MIC Tier 3 for control drug (if room on panel) to help monitor and reevaluate if needed for Tier 3.
 - Continue to use Tier 3 process to monitor/reassess when control drug from a Tier 2 study has high % out of range or results at extremes of QC range.
 - Consider adding information on *E. coli* ATCC 25922 (just says B-lactamase negative).
 - Consider adding information on B-lactamase inhibitors if they have activity against QC strain (to assist with setting range and confirm alignment of range for B-lactamase and B-lactamase inhibitors)
 - Compile list of differences between CLSI and EUCAST QC ranges. Review and triage in June (eg, no action, assess with Tier 3)
- QC Strain Descriptions: Are changes needed?
 - *E. coli* ATCC 25922: Should we change description of strain?
 - *E. coli* ATCC 25922 produces low levels of ESC-5, a chromosomally-encoded non-inducible Ambler class C enzyme with limited cephalosporinase activity (<https://www.ncbi.nlm.nih.gov/nuccore/AY899338?from=110&to=1243>) (Tracz et al. 2005 [<https://academic.oup.com/jac/article/55/5/768/691267>]).
 - The MICs of certain B-lactam agents that are sensitive to hydrolysis by this enzyme may therefore be slightly reduced (eg, by one to three doubling dilutions) against *E. coli* ATCC 25922 in the presence of a B-lactamase inhibitor with a spectrum of inhibition that includes *AmpC*.
 - Questions if the enzyme described above is expressed enough to warrant additional comments.
 - Should we add statement in Tables 4A-2, 5A-2, Appendix C, or Q&A about potential differences in QC ranges for single agent vs combination (may be the same or B-lactamase inhibitor may be ≥ 1 dilution different than B-lactamase)? Reassess after comparing CLSI and EUCAST ranges?
- Proposal to Add Mode/Median or Targets to QC Tables
 - EUCAST
 - In EUCAST QC tables, both ranges and targets are listed. Repeat testing of EUCAST quality control strains should yield individual MIC and zone diameter values randomly distributed within the recommended ranges. If the number of tests is ≥ 10 , the mode MIC should be the target value and the mean zone diameter should be close to the target value (optimally ± 1 mm from the target).
 - CLSI
 - Previously included mode/median in tables but this information was removed several years ago.
 - Mode/Mean included in QC Working Group summaries for Tier 3 assessments and potential use in Tables.
 - Recently added mode to selected QC strains/antimicrobials in CLSI M100 to provide additional information to better evaluate quality eg,
 - Colistin mode for multiple QC strains,
 - Imipenem need further action if *P. aeruginosa* MIC is repeatably 4, range 1-4
 - Could/should CLSI add mode/median or target to QC Tables?
 - Would additional information improve evaluation of quality?
 - Options/references to compile and add to CLSI tables?
 - EUCAST targets: either refine (target too tight), accept as is, reject or additional action
 - QC Working Group summaries: compile from previously approved reports

	• Resurrect modes/means from older CLSI M100 versions • Tier 3 data: accept as is, reject, additional action or collect more/contemporary data • Define what to publish for disks and how to establish (eg, median or mean, +/- x mm) ▪ Are regulatory impacts different in US and EU (eg, user inspections, AST device clearance/labeling)? ▪ Options to add to CLSI M100 tables (eg, in phases or wait until complete, separate or same table). • Guidance for study design/data requirements to develop QC ranges for ○ Qualitative (eg, screening, specialty tests) ○ Supplemental use only ○ When changing solvents
11.	<u>ADJOURNMENT</u> Dr. Mathers thanked the participants for their attention. The meeting was adjourned at 12:00 PM Central Standard (US) time.

2025 JUNE AST MEETING
SUMMARY MINUTES
PLENARY 2: Monday, 2 June 2025
1:00 PM - 5:30 PM
Central Standard Time (US)

#	Description
1.	<u>OPENING</u> Dr. Mathers opened the meeting at 1:00 PM Central Standard (US) time.

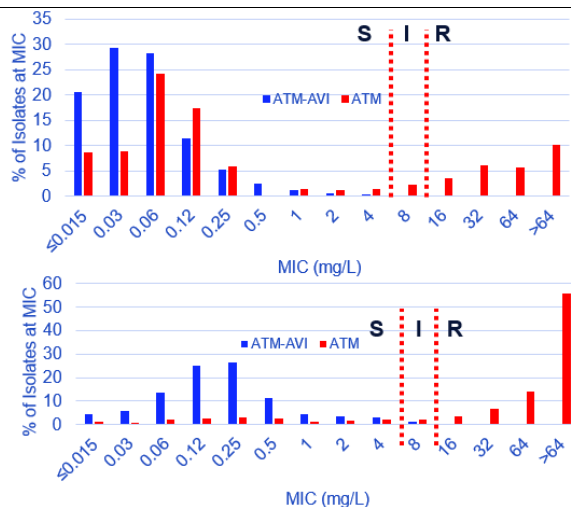
2. BREAKPOINTS WORKING GROUP (N. NARAYANAN AND M. SATLIN)

AZTREONAM-AVIBACTAM (ATM-AVI) MIC BREAKPOINTS FOR ENTEROBACTERALES

- FDA Indication (7 February 2025)
 - ATM-AVI in combination with metronidazole, is indicated in patients 18 years and older who have limited or no alternative treatment options for the treatment of cIAI, including those caused by the following susceptible Gram-negative microorganisms: *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae* complex, *Citrobacter freundii* complex, and *Serratia marcescens*. Approval of this indication is based on limited clinical safety and efficacy data for ATM-AVI
 - Dose = 1.5 g aztreonam/0.5 g avibactam q6h by 3-h iv infusion
- Mode of Action
 - Aztreonam not hydrolyzed by metallo- β -lactamases (MBL)
 - Avibactam prevents hydrolysis of aztreonam by serine- β -lactamases
 - ATM-AVI effective against MBL-producing isolates
 - Non-monobactam-avibactam combinations ineffective due to hydrolysis of β -lactam by MBLs
- *In vitro* Activity and Epidemiological Cutoff Value Data
 - Comparative activity of ATM and ATM-AVI for key MBL-producing and non-MBL-producing Enterobacterales species
 - Similar to overall Enterobacterales and non-MBL-containing Enterobacterales, AVI potentiated the activity of ATM against MBL-positive Enterobacterales; MIC₉₀s were at least 8-fold lower for ATM-AVI relative to ATM among the MBL-positive Enterobacterales.
 - Comparative activity of ATM and ATM-AVI for 2,530 MBL-producing Enterobacterales with or without additional β -lactamase enzymes
 - Production of additional β -lactamase enzymes may result in important differences in susceptibilities
 - *In vitro* activity of ATM and ATM-AVI against carbapenemase-producing Enterobacterales (INFORM/ATLAS 2017-2021)
 - ATM-AVI shows potent activity against carbapenemase-producing Enterobacterales, with MIC₉₀ values between 0.5 - 1 mg/L
 - MIC distribution of ATM-AVI for MBL-producing Enterobacterales
 - Rosolini GM, et al. J Glob Antimicrob Resist. 2024:123-131

Enzyme	n	MIC ≤4 µg/mL	MIC ₉₀ µg/mL
NDM	1421	98%	0.5
VIM	242	100%	1
IMP	49	100%	1
All MBL	1707	98%	1

- ATM-AVI and ATM MIC distributions for all Enterobacterales and MBL-containing Enterobacterales from surveillance studies (2017-2021)
 - Avibactam restores activity of ATM in Enterobacterales, including MBL-containing isolates
 - Dotted red lines represent proposed breakpoints: Susceptible (S)/Intermediate (I)/Resistant (R) MIC of ≤4 mg/L/8 mg/L/≥16 mg/L



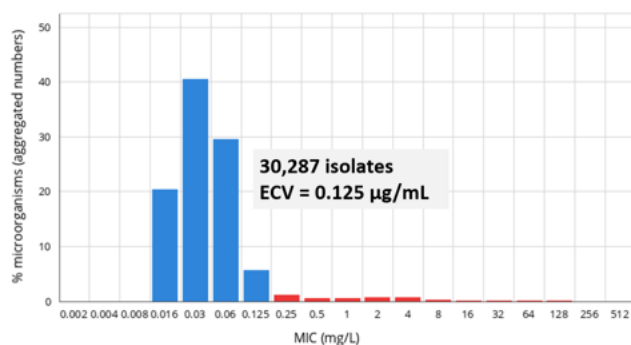
- ATM-AVI Epidemiological Cutoff Values (ECVs) for Enterobacteriales by species
 - ATM-AVI ECVs for Enterobacteriales range from 0.125 mg/L (*E. coli*) to 0.5 mg/ml (*K. pneumoniae*)

	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	Distributions	Observations	(T)ECOFF	Confidence interval
<i>Escherichia coli</i>	0	0	0	6153	12,260	8,951	1,719	348	150	167	201	195	83	36	10	8	6	0	0	5	30,287	0.125	0.06 - 0.25
<i>Klebsiella pneumoniae</i>	0	0	0	2,051	7,615	7,938	4,514	2,651	938	285	127	54	4	6	3	7	6	0	0	5	26,199	0.5	0.25 - 1
<i>Klebsiella oxytoca</i>	0	0	0	717	1,668	1,161	348	100	26	9	8	12	1	0	0	0	0	0	0	5	4,050	0.125	0.125 - 0.25
<i>Klebsiella aerogenes</i>	0	0	0	122	610	1,215	479	371	211	64	26	5	1	1	2	1	0	0	0	5	3,108	0.25	0.06 - 1
<i>Enterobacter cloacae</i>	0	0	0	254	1,565	2,495	970	629	627	354	129	38	2	3	0	2	1	0	0	5	7,069	0.25	0.125 - 0.25
<i>Citrobacter freundii</i>	0	0	0	244	783	712	505	264	102	22	23	8	0	0	1	1	0	0	0	5	2,665	0.25	0.25 - 1
<i>Citrobacter koseri</i>	0	0	0	166	692	281	58	17	8	0	3	3	0	0	0	1	0	0	0	3	1,229	0.125	0.03 - 0.125
<i>Serratia marcescens</i>	0	0	0	46	284	2,028	1,331	365	116	38	22	13	1	0	1	1	0	0	0	4	4,246	0.25	0.125 - 0.5

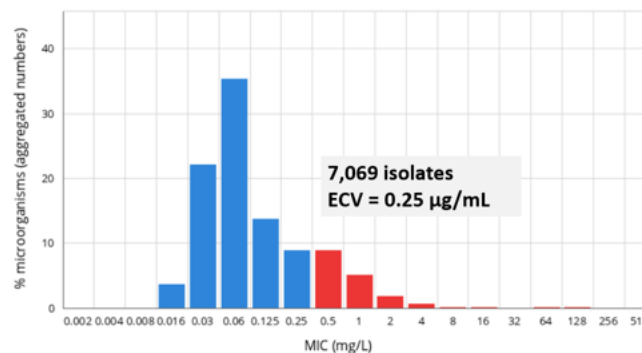
Epidemiologic cutoff values were calculated using the epidemiologic cutoff values finder statistical tool. Available at https://www.eucast.org/mic_distributions_and_ecoffs/

Turnidge J, et al. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. Clin Microbiol Infect. 2006;12(5):418-25

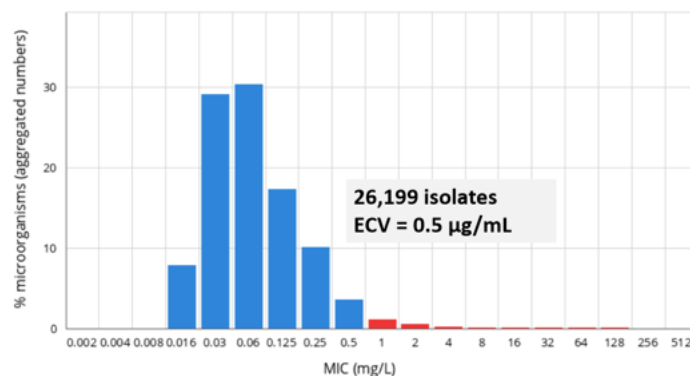
ATM-AVI & *Escherichia coli*



ATM-AVI & *Enterobacter cloacae*



ATM-AVI & *Klebsiella pneumoniae*



- MIC distribution for *E. coli* isolates with PBP3 insertions
 - Periasamy H, et al. J Antimicrob Chemother. 2020; 75:1650-51. Livermore DM, et al. Int J Antimicrob Agents. 2023; 61:106776
 - ATM binds primarily to PBP3
 - 4 amino acid PBP3 insertions (ie, YRIN or YRIK) increase ATM-AVI MIC, particularly when present with CMY enzymes (ie, AmpC)
 - In Asia and Middle East, these mutants represent >20% of NDM-producing *E. coli*. Present in all regions of the world.
 - Committee did not ultimately factor this into the breakpoint recommendation: No data indicating that *E. coli* with PBP3 insertion and ATM-AVI MICs $\leq 4/4$ µg/mL are more likely to fail treatment than wild-type isolates
- Inferring breakpoints from ECV data alone
 - To avoid cutting into the wild-type distribution, based on ECV alone susceptibility breakpoint should be no lower than 0.5 µg/mL
- Pharmacokinetic-Pharmacodynamic Data

- PK/PD targets were derived from hollow fiber infection models and mouse models (thigh and lung infection) testing both *E. coli* and *K. pneumoniae*

Drug (Target)	Hollow Fiber Target (to achieve 1-log reduction)	Murine Target (to achieve 80% max killing ^a)	Target Used for Monte Carlo Simulations
Aztreonam (fT>MIC)	50-55%	Not tested	60%
Avibactam (fT>C _T of 2.5 mg/L)	41-58%	14-43%	50%
			Joint Target

^a80% max killing correspond to different log reductions depending on the *species* and *model*

-*E. coli*: ~1.5-log (thigh) or ~2-log (pneumonia)

-*K. pneumoniae*: ~0.5-log (thigh) or ~2-log (pneumonia) reductions

- Probability of target attainment analysis performed for 5,000 simulated patients with cIAI and HAP/VAP using steady state plasma exposures
- Joint PTA: 60% fT>MIC for ATM + 50% fT>C_T of 2.5 mg/L for AVI
- >90% joint PTA for MICs ≤ 8 mg/L = PK/PD cutoff
- Key Discussion Point: Limited data with isolates near the proposed breakpoint and less robust data in non-*E. coli* Enterobacterales
 - Data in *E. coli* appear strong and were key to guiding PK/PD breakpoint selection
 - In discussing with EUCAST, they also largely relied on *E. coli* data
 - *In vitro* time-kill data found that ATM-AVI caused >3-log killing at 24 h in most isolates
 - Exceptions: AmpC-positive isolates of *C. freundii* or *S. marcescens*
 - Preclinical *in vivo* data in species other than *E. coli* are less robust
 - *K. pneumoniae* failed to achieve 1-log₁₀ kill for 2 of 3 isolates in the murine thigh infection model, thus, a target of near maximal activity (EC80) was chosen instead of 1-log₁₀ kill
 - No *K. pneumoniae* isolates with ATM-AVI MICs >0.5 mg/L were evaluated
 - ATM-AVI murine thigh infection model data show 1.5-2.3 log reduction against *E. coli* but <1 log reduction in 2/3 *K. pneumoniae* strains
 - Human simulated regimens (HSRs) for ATM 2g q6h (1hr infusion) alone or in combination with AVI 375mg or 600mg in neutropenic mouse thigh infection model. Approved dose: ATM 1.5g/AVI 0.5g q6h (3h infusion).
 - Active against *E. coli* with ATM-AVI MICs to 8-16 mg/L
 - Less active against *K. pneumoniae* and only tested against isolates with ATM-AVI MICs ≤ 0.5 mg/L
 - ATM-AVI murine pneumonia model data show similar bacterial killing for *E. coli* and *K. pneumoniae*
- Inferring breakpoints from PK-PD data alone
 - Based on PK-PD alone susceptibility breakpoint should be no higher than 8 µg/mL
 - Applicability for non-*E. coli* Enterobacterales species is less clear
 - All *K. pneumoniae* isolates tested had ATM-AVI MICs ≤ 0.5 mg/L and maximum killing varied between the thigh infection model (0.3-1 log₁₀ CFU reduction) and murine pneumonia model (2-3 log₁₀ CFU reduction)

- Clinical Outcome Data

- Two Phase 3 Studies for ATM-AVI
 - Summary of Indication: ATM-AVI, in combination with metronidazole, in adults with cIAI caused by susceptible Gram-negative microorganisms for which there are limited or no treatment options
 - REVISIT study: Randomized, open-label clinical trial comparing ATM-AVI (± metronidazole) versus meropenem (± colistin) for 422 patients with intra-abdominal infections and pneumonia. Included 7 NDM-producing Enterobacterales.

Favorable Microbiological Response at Test of Cure Visit

Organism	ATM-AVI Group (n, %)	Meropenem Group (n, %)
Enterobacterales	130 (75%)	71 (72%)
<i>E. coli</i>	91 (72%)	44 (76%)
<i>K. pneumoniae</i>	14 (52%)	15 (65%)

Clinical cure at Test of Cure Visit

Organism*	ATM-AVI Group (n, %)	Meropenem Group (n, %)
<i>E. coli</i>	89 (78%)	43 (74%)
<i>K. pneumoniae</i>	15 (56%)	16 (70%)

*Data missing for other Enterobacterales species

MIC (µg/mL)												
		0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8
Enterobacterales	Number of isolates	7	4	39	69	30	12	9	0	2	0	2
	Clinical cure	86%	100%	72%	81%	77%	58%	33%	NA	50%	NA	0%
	Microbiological cure	86%	100%	74%	83%	77%	58%	33%	NA	50%	NA	0%
<i>E. coli</i>	Number of isolates	3	1	31	59	15	3	0	0	0	0	2
	Clinical cure	100%	100%	71%	83%	73%	100%	NA	NA	NA	NA	0%
	Microbiological cure	100%	100%	74%	85%	73%	100%	NA	NA	NA	NA	0%
<i>K. pneumoniae</i>	Number of isolates	0	0	4	4	5	6	7	0	1	--	--
	Clinical cure	NA	NA	75%	50%	100%	33%	43%	NA	0%	--	--
	Microbiological cure	NA	NA	75%	50%	100%	33%	29%	NA	0%	--	--

- ASSEMBLE study: Randomized, open-label study of 12 patients with NDM-producing Enterobacteriales intra-abdominal infections, pneumonia, urinary tract infections, and bloodstream infections A prospective, randomized, open-label, comparative study

Organism	ATM-AVI MIC range	Clinical cure percent
<i>E. coli</i> (3)	0.5-1 µg/mL	0%
<i>K. pneumoniae</i> (8)	0.12-0.5 µg/mL	63%

- Inferring breakpoints from clinical outcomes data alone
 - Based on clinical outcomes data alone susceptibility breakpoint should be no higher than 0.25 µg/m
- ATM-AVI Enterobacteriales MIC Breakpoints
 - ECV data alone: ≥0.5 µg/mL
 - PK-PD data alone: ≤8 µg/mL
 - Clinical data alone: ≤0.25 µg/mL

Organization	Minimum Inhibitory Concentration (µg/mL)		
	Susceptible	Intermediate	Resistant
Sponsor proposed	≤4/4	8/4	≥16/4
FDA	≤4/4	8/4	≥16/4
EUCAST	≤4/4	--	>4/4

- ATM-AVI AHWG Discussion and Recommendation
 - Vote 1: Harmonize with FDA and EUCAST for all Enterobacteriales
 - AHWG Vote: 0-7-0-0
 - Vote 2: Harmonize with FDA and EUCAST for *E. coli* only
 - “Not having a breakpoint has never discouraged the expert from using drugs off label and without established breakpoints.”
 - If we set a separate breakpoint for *K. pneumoniae* it would likely cut into the wild-type distribution as it would be no higher than 0.25 µg/mL
 - AHWG Vote: 7-0-0-0
 - Vote 3: Harmonize with FDA and EUCAST for all Enterobacteriales include proposed comment
 - Proposed Comment: “Breakpoints are based largely on data for *E. coli*. For Enterobacteriales other than *E. coli*, data may not support these breakpoints.”
 - Preference to re-review non-*E. coli* data when more becomes available in coming years rather than not include ATM-AVI as an option for *K. pneumoniae* given the rising prevalence of NDM-producing *K. pneumoniae* globally

- Many countries will not have access to cefiderocol, at least for several years
- AHWG Vote: 7-0-0-0
- Breakpoints Working Group Discussion and Recommendation
 - Lack of 1-log kill in the thigh infection model with NDM-producing *K. pneumoniae*
 - Clinical cutoff and lack of isolates with higher MICs within the breakpoint for 'susceptible' (FDA and EUCAST deliberations)
 - Current CLSI breakpoint for aztreonam alone is ≤ 4 mcg/mL (S)
 - PK/PD data driven by *E. coli*
 - Should have a high bar to deviate from FDA STIC
 - Motion to accept ATM-AVI MIC breakpoints ($S \leq 4/4$, $I 8/4$, $R \geq 16/4$ $\mu\text{g/mL}$) for Enterobacterales with the comment "Breakpoints are based largely on data for *E. coli*. For Enterobacterales other than *E. coli*, data may not support these breakpoints." WG Vote: 8-0-1-4.

SC DISCUSSION (MAIN POINTS)

- Is it common to see differences between *E. coli* and *Klebsiella* in PK/PD models?
 - *Klebsiella* is always harder to get a 1-log kill. This is not unique to aztreonam-avibactam. This happens with many drugs.
- In the past FDA has focused on highest MIC clinically demonstrated to work, so it is surprising the FDA went for a higher breakpoint of 4 $\mu\text{g/mL}$.
 - The sponsor believes the totality of the data is likely what FDA used to make their decision. The sponsor does not think the comment that the "data does not support" is not accurate and that it is more fitting that there is a lack of data.
- Why did EUCAST chose a breakpoint of 8 $\mu\text{g/mL}$?
 - EUCAST does not have the intermediate category. If the susceptible breakpoint is 4 $\mu\text{g/mL}$, then the resistant breakpoint is 8 $\mu\text{g/mL}$.
- For isolates that re-grew in the models, there is no follow-up data to know what the MICs were after the isolates re-grew.
- There is no clinical data at MICs above 0.5 $\mu\text{g/mL}$. There is concern about the lack of data at the higher MICs.
- For the hollow fiber data with *E. coli*, the PK/PD data looks good. There is a concern about taking this drug off the table as an option.
- There was concern about deviating from the aztreonam breakpoint of 4 $\mu\text{g/mL}$.
- CLSI has never held new β -lactam/ β -lactamase inhibitor drugs to the standard of having clinical isolates near the breakpoint. Rely on the parent breakpoint (aztreonam in this case) to help set the breakpoint for the new inhibitors.
- If the breakpoint is set at anything other than 4 $\mu\text{g/mL}$, CLSI needs to re-evaluate the aztreonam breakpoint of 4 $\mu\text{g/mL}$.
- One study is all intraabdominal infections.
- The extra footnote might not be needed. In the second study, this isn't an MIC vs exposure issue. They did dose ranging (a high dose of the inhibitor). Adding more inhibitor doesn't improve the killing. This is probably a bug-drug model. Not sure why the lung model behaves differently. In ceftibuten, *Klebsiella* did not have as good of a kill. Is one log kill truly meaningful vs a $\frac{3}{4}$ log kill?
- Is the study a neutropenic pneumonia model? Yes.
- Adding in a comment sets a precedent that CLSI might not want to because there are lots of studies that do not cover all the representative organisms.
- *Klebsiella* is the main organism where this drug is going to be used, which is why it is being looked at so closely.
- The comment is an extra challenge for laboratories to implement or decide what to do with. Consider removing the comment.
- The FDA package insert specifically includes *E. coli*, *Klebsiella*, *Citrobacter*, and *Serratia*.
- The Aztreonam-Avibactam AHWG felt a little uncomfortable with the *Klebsiella* data.
- Every gram-negative drug that has come through CLSI is largely based on *E. coli* data, so there is no reason to specifically call this out for this drug. Need to be careful about over editorializing this drug. This information could go in a publication or rationale document.

A motion to accept the aztreonam-avibactam MIC breakpoints ($S \leq 4/4$, $I 8/4$, $R \geq 16/4$ µg/mL) for Enterobacterales was made and seconded. Vote: 8 for, 5 against, 0 abstain, 1 absent (Fail)

Against Vote Reasoning:

- Want a comment that states not a lot of data exists for species outside of *E. coli*.
- The breakpoint should be lower and the aztreonam breakpoint needs to be re-evaluated.
- Concern that the aztreonam breakpoint is not accurate

SC DISCUSSION (MAIN POINTS)

- The aztreonam breakpoint was recently re-evaluated and it checked every box that CLSI holds as a standard.
- The EUCAST resistant breakpoint is 4 µg/mL, so think of that, not the susceptible breakpoint 1 µg/mL.
- CLSI should not discourage people from bringing forward *Klebsiella* data because it is known that it does not perform as well in animal models as *E. coli*.
- Can see a two log kill for *Klebsiella* against some drugs like cefiderocol. There are no MICs of 1 or 2 in the pre-clinical data for *Klebsiella*.
- In the pneumonia model, it was well beyond a 1 log kill.
- The breakpoint for aztreonam was changed to avoid false susceptibility for KPCs and it wasn't based on PK/PD data.
- CLSI M23 states that at least 4 isolates for each species should be tested.
- If this comment is for information, then the M100 is probably not the place for this. Maybe it should be communicated somewhere else.
- Just because CLSI has not added a comment before doesn't mean a comment should not be added now. It is hard for laboratories to find primary references.

A motion to accept the aztreonam-avibactam MIC breakpoints ($S \leq 4/4$, $I 8/4$, $R \geq 16/4$ µg/mL) for Enterobacterales with the comment, "Breakpoints are based largely on data for *E. coli*." was made and seconded. Vote: 5 for, 8 against, 0 abstain, 1 absent (Fail)

Against Vote Reasoning:

- The breakpoint is too high.
- Do not want the comment added.

A motion to accept the aztreonam-avibactam MIC breakpoints ($S \leq 4/4$, $I 8/4$, $R \geq 16/4$ µg/mL) for Enterobacterales was made and seconded. Vote: 11 for, 2 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:

- The breakpoint is too high.

AZTREONAM-AVIBACTAM (ATM-AVI) DISK DIFFUSION BREAKPOINTS FOR ENTEROBACTEREALES

- ATM-AVI Enterobacterales Disk Diffusion Breakpoints

Organization	Disk Diffusion Zone Diameters (mm)		
	Susceptible	Intermediate	Resistant
Sponsor proposed	≥25	22-24	≤21
FDA	≥21	18-20	≤17
EUCAST	≥25	--*	<25

* area of technical uncertainty: 22-24 mm

- Datasets
 - LabCorp/ JMI study
 - Phase III clinical trial isolates (405 Enterobacterales including 224 *E. coli*, 106 *K. pneumoniae*)
 - 31 MBL producers
 - 1 site: BMD and disk diffusion performed at LabCorp, data analysis by JMI
 - 1 replicate per isolate for both BMD and disk diffusion
 - 1 disk brand: MAST
 - 1 brand of Mueller Hinton broth and agar (unknown brand, info not provided)
 - EUCAST study
 - 201 Enterobacterales (unknown origin) including 76 *E. coli*, 47 *K. pneumoniae*, 14 *E. cloacae*
 - 4 MBL producers
 - 1 site: EUCAST development laboratory
 - 2 disk brands: MAST, Oxoid
 - 2 Mueller Hinton agar brands: BD BBL, Oxoid
 - Unknown: BMD replicates and MH broth brand (info not provided)
- Why are FDA and EUCAST disk breakpoints different?
 - FDA only used the LabCorp/ JMI study with clinical trial isolates. Limited isolates at high ATM-AVI MICs.
 - EUCAST did their own study. More representative isolates with higher MICs.
 - Combined both datasets = 35 total MBL-producers

FDA breakpoints

	S	I	R	ATU
FDA	≥ 21	18-20	≤ 17	-
EUCAST	≥ 25	-	< 25	22-24

Very major error and Minor error rate too high

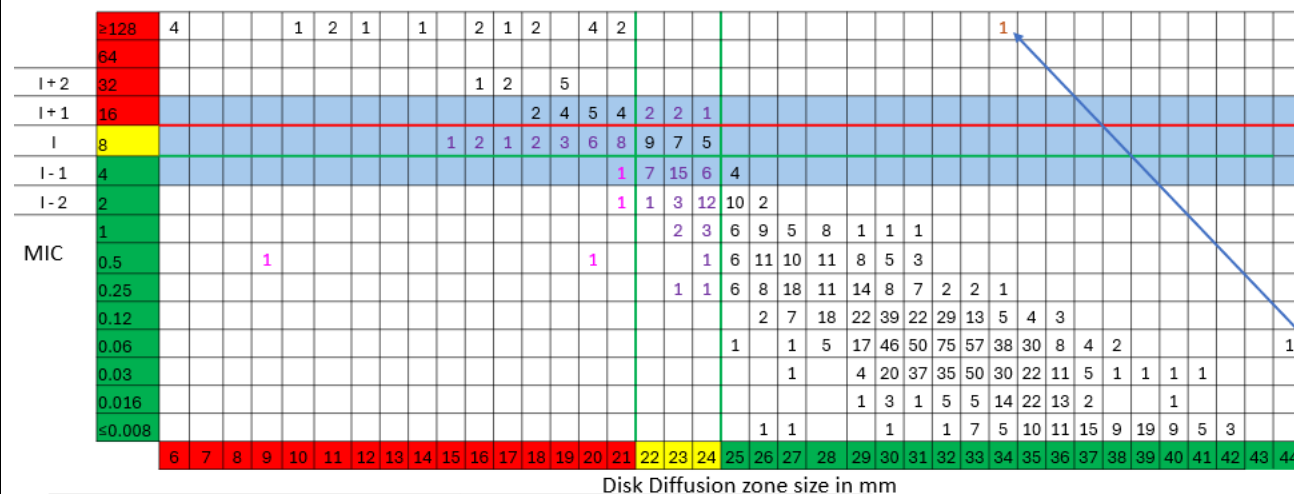
[illegible]

Disk Diffusion zone size in mm

MIC range	Number	Very Major Errors		Major Errors		Minor Errors	
		n	%	n	%	n	%
≥ I+2	29	3	10.3%	N/A	N/A	11	38%
I+1 to I-1	97	9	9.3%	0	0%	44	45%
≤ I-2	1083	N/A	N/A	1	0.1%	1	0.1%
Total	1209	12	1.0%	1	0.1%	56	5%

ATM-AVI Disk Proposal (similar to EUCAST)

	S	I	R	ATU
EUCAST	> 25	-	< 25	22-24
Proposal to CLSI	≥ 25	22-24	≤ 21	



1 VME (technically too high but it is one *Morganella* isolate and it was not re-tested)

MIC range	Number	Very Major Errors		Major Errors		Minor Errors	
		n	%	n	%	n	%
≥ I+2	29	1	3.4%	N/A	N/A	0	0%
I+1 to I-1	97	0	0%	1	1%	56	58%
≤ I-2	1083	N/A	N/A	3	0.3%	24	2%
Total	1209	1	0.1%	4	0.3%	80	7%

Minor error too high but can be addressed with a comment

- ATM-AVI AHWG Discussion and Recommendation

	S	I	R	ATU
FDA	≥ 21	18-20	≤ 17	-
EUCAST	≥ 25	-	< 25	22-24
Proposal to CLSI	≥ 25	22-24	< 21	

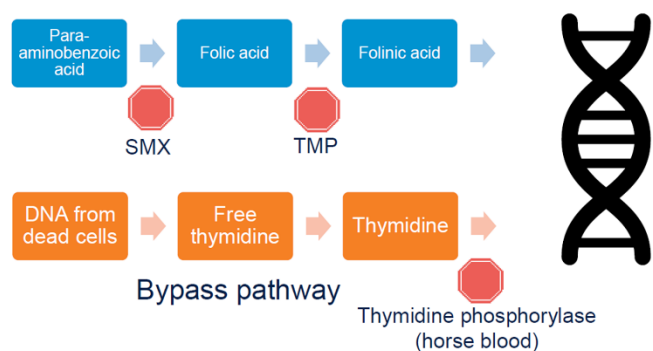
- Proposed comment: “Disk diffusion may overcall resistance. If clinically necessary, confirmatory testing with an MIC method can be performed on isolates that test intermediate by disk diffusion.”

	○ Motion to accept the ATM-AVI disk diffusion breakpoints ($S \geq 25$ mm, I 22-24 mm, $R \leq 21$ mm) for Enterobacterales with the proposed comment. AHWG deferred to clinical microbiologists who agreed with motion. ● Breakpoints Working Group Discussion and Recommendation ○ Proposal for disk diffusion breakpoints deviates from breakpoints approved by FDA. ○ Dataset for FDA decision was based on more limited data. FDA could revisit with updated dataset. ○ Motion to accept the ATM-AVI disk diffusion breakpoints ($S \geq 25$ mm, I 22-24 mm, $R \leq 21$ mm) for Enterobacterales with the proposed comment “Disk diffusion may overcall resistance. Confirmatory testing with an MIC method can be performed on isolates that test intermediate by disk diffusion.” WG Vote: 8-0-1-4. SC DISCUSSION (MAIN POINTS) ● During the Breakpoints Working Group meeting, the FDA stated they would be willing to look at the new data and update their breakpoints.
	A motion to accept the aztreonam-avibactam disk diffusion breakpoints ($S \geq 25$ mm, I 22-24 mm, $R \leq 21$ mm) for Enterobacterales with the comment, “Disk diffusion may overcall resistance. Confirmatory testing with an MIC method can be performed on isolates that test intermediate by disk diffusion.” and wordsmithing of comment to be similar to ceftazidime-avibactam was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)
	AZTREONAM-AVIBACTAM (ATM-AVI) TABLE 1 PLACEMENT ● Breakpoints Working Group Discussion and Recommendation ○ Motion to place ATM-AVI in Table 1 in Tier 3 with other novel β-lactamase/β-lactamase inhibitors. WG Vote: 8-0-1-4.
	A motion to place aztreonam-avibactam in Table 1 Tier 3 with other novel β-lactamase/β-lactamase inhibitors was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)
	TRIMETHOPRIM-SULFAMETHOXAZOLE (SXT) MIC BREAKPOINTS FOR β-HEMOLYTIC STREPTOCOCCI ● Background ○ Large colony forming pyogenic strains ○ Species from the pyogenic or beta-hemolytic group are further characterized by the presence of Lancefield antigens, which do not always correlate with the proper streptococcal species designations

Lancefield Antigen Group	Organisms
Group A	<i>S. pyogenes</i>
Group B	<i>S. agalactiae</i>
Group C	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> <i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i> (alpha-hemolytic) <i>S. equi</i> subsp. <i>equi</i> <i>S. equi</i> subsp. <i>zooepidemicus</i>
Group G	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> <i>S. canis</i>

- Resistance

Resistance to TMP/SMX



Goldstein EJC and Proctor RA. Clin Infect Dis 2008; 46(4): 584-593. <https://doi.org/10.1093/cid/cin5536>

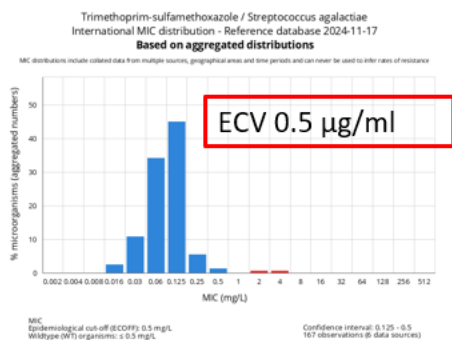
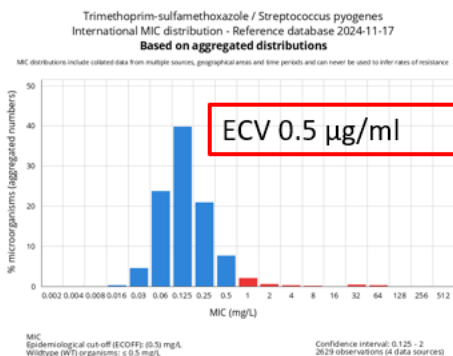
UCSF

- Antimicrobial Susceptibility Testing Methods

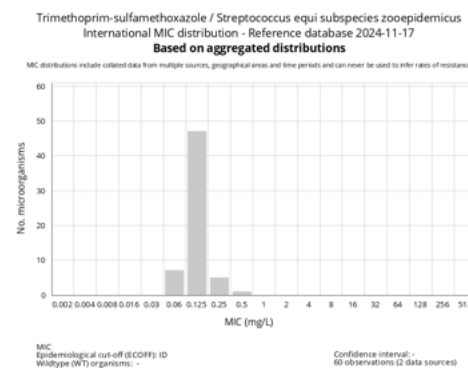
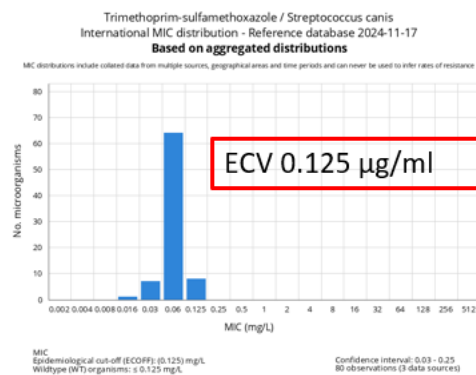
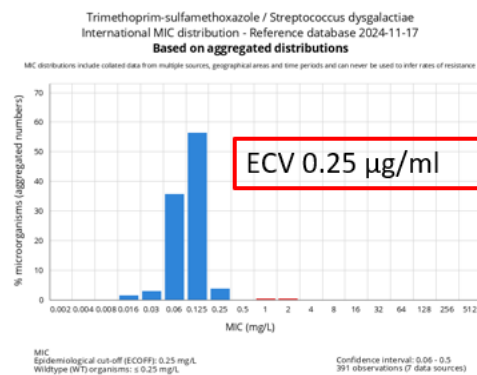
- SXT disk content the same for CLSI and EUCAST breakpoints: 1.25/23.75 µg

	Disk Diffusion	Broth Microdilution	Agar Dilution	SXT Breakpoints?
CLSI	MHA with 5% sheep blood 35°C ± 2°C 5% CO ₂ ; 20–24 hours	CAMHB with lysed horse blood (2.5% to 5% v/v) 35°C ± 2°C ambient air; 20–24 hours	MHA with sheep blood (5% v/v) 35°C ± 2°C ambient air; 20–24 hours (CO ₂ if necessary, for growth with agar dilution)	No
EUCAST	Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) Sealed panels, air, 35±1°C, 18±2h (for glycopeptides 24h)	Cation-adjusted Mueller-Hinton broth + 5% lysed horse blood and 20 mg/L β-NAD (MH-F broth) 5% CO ₂ , 35±1°C, 18±2h	Guidance not available	Yes

- β-hemolytic Streptococci and SXT
 - Early studies demonstrated beta-hemolytic streptococci had variable rates of resistance to SXT (method dependent)
 - Perpetuated the thought that beta-hemolytic streptococci had high levels of resistance to SXT (or even thought to be intrinsically resistant)
- What is the difference between sheep and horse blood?
 - Lysed horse blood contains thymidine phosphorylase, which neutralizes thymidine
 - Thymidine phosphorylase converts thymidine to thymine and hence overcomes the inhibition of folate metabolism that occurs in the presence of thymidine
 - Can no longer serve as an exogenous source of thymidine
 - No other mammalian blood carries thymidine phosphorylase
 - Thymidine content regulated in MHA (>0.03 mg/L)
 - *Enterococcus faecalis* 29212/33186 used to QC thymidine content in media
- Epidemiologic Cutoff Values (ECVs)

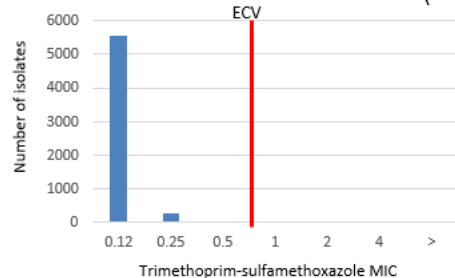


ECV of 0.125-0.5 µg/ml
species dependent



- Contemporary Data Sets

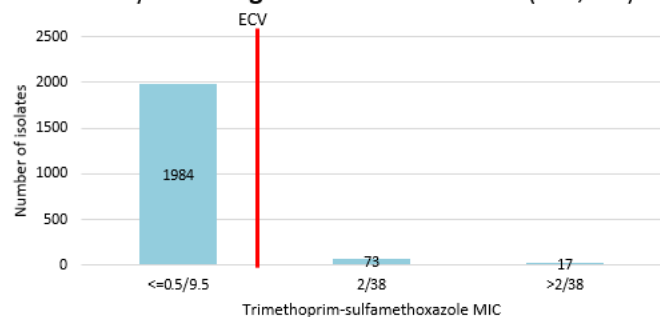
SENTRY BMD Data 2018-2023 (n: 5929)



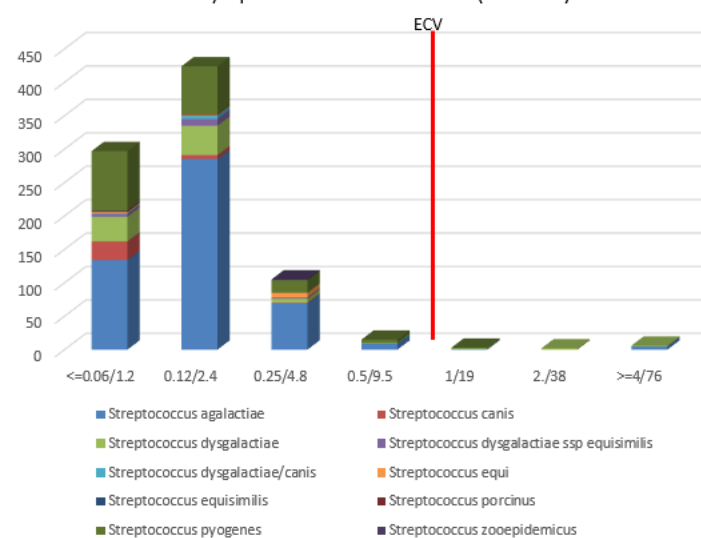
- Organisms included:
- *S. pyogenes* (2,604)
 - *Streptococcus agalactiae* (2,241)
 - *S. dysgalactiae* (1,040)
 - *S. canis* (36)
 - *S. equi* (8)

MIC₅₀: ≤0.12
MIC₉₀: ≤0.12

Mayo Clinic Agar Dilution MHA & LHB (n: 2,074)



ARUP Lyophilized BMD Data (n: 852)



- Streptococcus agalactiae
- Streptococcus dysgalactiae
- Streptococcus dysgalactiae/canis
- Streptococcus equisimilis
- Streptococcus pyogenes
- Streptococcus canis
- Streptococcus dysgalactiae ssp equisimilis
- Streptococcus equi
- Streptococcus porcinus
- Streptococcus zooepidemicus

Little data on performance of commercial AST devices.
Available data suggests poor performance.

- Current EUCAST SXT Breakpoints (v15, 2025)

Table 1: Current trimethoprim-sulfamethoxazole breakpoints and ECOFFs (Breakpoint table v15, 2025)

Species	(T)ECOFF	Current breakpoints S ≤ / R >	fAUC/MIC for ECOFF	fAUC/MIC for current S- bp
Enterobacterales ¹	0.5	2/4	=30/0.5=60	=30/2=15
<i>E. coli</i>	0.5	2/4	=30/0.5=60	=30/2=15
<i>K. pneumoniae</i>	0.5	2/4	=30/0.5=60	=30/2=15
<i>Acinetobacter</i> spp. ²	0.5	2/4	=30/0.5=60	=30/2=15
<i>Staphylococcus</i> spp. ³	(0.25)	2/4	=30/0.25=120	=30/2=15
<i>S. aureus</i>	(0.25)	2/4	=30/0.25=120	=30/2=15
<i>Streptococcus</i> A/C/G	0.5 ⁴	1/2	=30/0.5=60	=30/1=30
<i>S. pneumoniae</i>	1	1/2	=30/1=30	=30/1=30
<i>H. influenzae</i>	0.5	0.5/1	=30/0.5=60	=30/0.5=60
<i>M. catarrhalis</i>	1	0.5/1	=30/0.5=60	=30/1=30
<i>Aeromonas</i> spp. ⁵	(1)	2/4	=30/1=30	=30/2=15

⁴ The ECOFF stated is the highest ECOFF of the different *Streptococcus* ABCG; ECOFFs for the different species range from 0.125 to 0.5.

- EUCAST Public Consultation

Table 2: Proposed breakpoints for trimethoprim-sulfamethoxazole (Breakpoint table v16, 2026):

Species	Proposed clinical breakpoints (based on ECOFFs) ¹	Wild type SIR placement
Enterobacterales except <i>Serratia</i> and <i>Salmonella</i>	0.5/0.5	S
<i>Serratia</i> spp. and <i>Salmonella</i> spp.	0.001/2	I
<i>Acinetobacter</i> spp.	0.5/0.5	S
<i>Staphylococcus</i> spp. ²	0.5/0.5	S
<i>Streptococcus</i> A/C/G	0.5/0.5	S
<i>S. pneumoniae</i>	1/1	S
<i>H. influenzae</i>	0.5/0.5	S
<i>M. catarrhalis</i>	1/1	S
<i>Aeromonas</i> spp.	1/1	S

¹ There are some PK/PD data suggesting that the clinical breakpoint for lower UTI could be higher than the ECOFF, but there are few strains with MICs between 0.5 and 2 mg/L, and it would be challenging to have a breakpoint here, as some strains with trimethoprim R would then become trimethoprim-sulfamethoxazole S (due to sulfamethoxazole susceptibility). Thus, the EUCAST

https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/2025/Consultation_trimethoprim-sulfa_20250507.pdf

- Thoughts From the Microbiologists
 - If we approve setting SXT breakpoints for β -hemolytic streptococci, recommend (reference) BMD MIC testing if performed and add a comment
 - The only approved MIC method for testing is (reference) broth microdilution. Agar based methods using MHA with 5% Sheep Blood (eg, disk diffusion, gradient diffusion, agar dilution) should not be performed due to increased thymidine content leading to false resistance.
 - Potential Next Steps: Perform a method evaluation study
 - MHA + 5% sheep and MHF for agar based methods compared to reference BMD
 - Disk and gradient diffusion
 - Assess disk correlates
 - Evaluate multiple agents (not limited to SXT)
- SXT PK/PD Summary
 - PK/PD Targets for β -hemolytic streptococci remain undefined
 - Probably reasonable to assume acceptable PTA for stasis based on arbitrary fAUC_{0-24} /MIC target of 25 up to an MIC of 0.5 mg/L for 5 mg/kg/day or 1 DS Tab twice daily
 - Purely based on extrapolation from very limited data
- Clinical data pertaining to SXT vs β -hemolytic Streptococci

- Is SXT effective against infections caused by B-hemolytic streptococci?
- Most published studies (predominantly uSSSI and cSSSI) do not answer this question (wrong patient population, wrong comparators)
- Very limited data from RCTs comparing SXT to standard of care for infections likely to be caused by B-hemolytic streptococci, but rarely including microbiologic or serologic evidence of B-hemolytic streptococci infection
- With those caveats the preponderance of available data suggest efficacy with proper dosing
- Clinical Data Summary
 - Clinical data are incomplete and lack microbiology confirmation of B-hemolytic streptococci infection in SSSI
 - Available data for uSSSI and cSSSI suggest efficacy
 - Available data for group A streptococci pharyngitis raise a question regarding efficacy vs. comparator (PCN G), but
 - Dosing of SXT in that study may not be adequate
 - Given the small sample size, 95% CI crosses 1
 - Lack of data for efficacy:
 - UTI due to group B streptococci
 - Step-down for more severe B-hemolytic streptococci skin infections
 - Oral option for bloodstream/other deep seated B-hemolytic streptococci infections
- SXT B-hemolytic Streptococci AHWG Discussion and Recommendation
 - Reasonable to recommend SXT breakpoints for B-hemolytic streptococci at least for uncomplicated SSSI
 - Data for other clinical indications and B-hemolytic streptococci species is very limited
 - Most non-purulent cellulitis is caused by B-hemolytic streptococci and treated without cultures, so would include all B-hemolytic streptococci for any B-hemolytic streptococci breakpoint if possible
 - Options discussed
 - Option 1: ECV for B-hemolytic streptococci
 - Option 2: Breakpoint for B-hemolytic streptococci
 - Limit testing to Tier 4 as mostly used empirically and the data only support uSSTI and maybe cSSTI/uUTI
 - Testing issues - Limited to BMD
 - Option 3: Breakpoint for B-hemolytic streptococci
 - Add as Tier 2 agent
 - Add a comment that the clinical data is limited to SSTI/UTI - thus each institution needs to evaluate how to test and report
 - Group A Streptococci vs all B-hemolytic Streptococci
 - Distributions and ECVs are similar among all B-hemolytic streptococci
 - No clinical data to indicate that we should separate them
 - Often do not have culture results for non-purulent cellulitis
 - Table 2H-1 encompasses all B-hemolytic streptococci; rare exceptions of Q-D (group A streptococci only)/dalbavancin (group A streptococci/group B streptococci/*S. dysgalactiae*)/telavancin (group A streptococci/group B streptococci only)
 - AHWG Vote: Unanimously to include all B-hemolytic streptococci and not limiting it to group A streptococci
 - ECV vs Breakpoint
 - Lack of awareness/use of ECVs by clinical laboratories
 - Setting breakpoints will allow for additional incentive of industry partners to include on commercial AST panels

- Clinical outcomes data to support the use of this agent for certain clinical syndromes associated with β -hemolytic streptococci (eg, cellulitis)
- Contemporary data support a single distribution associated with the wild-type and clinical success
- AHWG Vote: Unanimously to set a breakpoint for SXT and β -hemolytic streptococci
- Table 1 Test and Report Recommendation
 - Tier 2: Most likely to influence commercial AST manufacturers to prioritize
 - Tier 4
 - High rates of susceptibility
 - Often used empirically
 - Tier 4 initially and once we have a broadly available test and more clinical data then reconsider/re-evaluate placement
 - AHWG Vote: Unanimously for placement in Tier 4 initially
- Addition of comments
 - Add a comment that the clinical data limited to SSTI/UTI. Thus each institution needs to evaluate how to test and report.
 - “Trimethoprim-sulfamethoxazole might be considered for testing and/or reporting for beta-hemolytic streptococci, clinical data is lacking outside of uncomplicated skin and soft tissue infections.”
 - “Trimethoprim-sulfamethoxazole should not be routinely reported for cultures from sterile sites due to lack of clinical data for use in most clinical syndromes, other than uncomplicated skin/skin structure infections.”
- AHWG Proposal
 - Set a breakpoint at the ECV ($\leq 0.5 \mu\text{g/mL}$) for all β -hemolytic streptococci. Limite testing to BMD with a comment not to use agar-based methods with sheep blood.
 - Add SXT to Tier 4 in Table 1H-1 (re-evaluate in the future.
 - AHWG Vote: Unanimously to set a breakpoint for SXT and β -hemolytic streptococci.

Interpretation	MIC
ECV	$\leq 0.5 \mu\text{g/mL}$
Susceptible	$\leq 0.5 \mu\text{g/mL}$
Intermediate	$1 \mu\text{g/mL}$
Resistant	$\geq 2 \mu\text{g/mL}$

- Breakpoints Working Group Discussion and Recommendation
 - Setting breakpoint is potentially premature (reason for ‘no’ vote)
 - Favored setting breakpoint and avoiding an ECV only
 - Limit application to uncomplicated SSTI (based on clinical data)
 - Question on rheumatic fever as a rare outcome for pediatrics in the clinical studies
 - Motion to accept the proposed SXT breakpoints ($S \leq 0.5$, $I 1$, $R \geq 2 \mu\text{g/mL}$) for β -hemolytic streptococci with the comment “Trimethoprim-sulfamethoxazole might be considered for testing and/or reporting for β -hemolytic streptococci, clinical data is lacking outside of uncomplicated skin and soft tissue infections.” WG Vote: 7-1-1-4.
 - Motion to place SXT in to Tier 4 in Table 1H-1. WG Vote: 8-0-1-4.

Antimicrobial Agent		Disk Content	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
FOLATE PATHWAY ANTAGONISTS											
Trimethoprim-sulfamethoxazole		1.25/23.75 µg	—	—	—	—	≤ 0.5	—	1	≥ 2	

1. The only approved MIC method for testing is broth microdilution. Agar based methods using MHA with 5% Sheep Blood (e.g. disk diffusion, gradient diffusion, agar dilution) should not be performed due to increased thymidine content leading to false resistance.
2. Reporting should be limited to isolates recovered from (uncomplicated?) skin and skin structure infections – *COMMENT TBD*

SC DISCUSSION (MAIN POINTS)

- Should this apply to only group A streptococci or all β-hemolytic streptococci?
 - The AHWG wants it to apply to all β-hemolytic streptococci. They do want to set a breakpoint for SXT.
- Set a breakpoint at the ECV at ($S \leq 0.5$, $I \geq 1$, $R \geq 2$ µg/mL) plus a comment that trimethoprim-sulfamethoxazole might be considered.
- Action Item: Methods Working Group and AHWG work on method comparison studies. The next step would be to look at disk diffusion. Consider the Mueller-Hinton fastidious media.
- There must be a clear documentation not to test pharyngeal isolates and that SXT does not prevent rheumatic fever.
- This drug will be helpful for diabetic foot infections. Patients are given amoxicillin then get a rash, then their medical record lists them as having allergies to a wide range of drugs. Clindamycin and tetracycline resistance rates are high.
- Laboratories have seen clindamycin and tetracycline susceptibility decrease, so there is a need for an SXT breakpoint. Should test and know if resistance occurs or increases.
- There were a few studies in Germany and India that have resistance to trimethoprim or trimethoprim/sulfamethoxazole.
- If there is a breakpoint, that will enable world-wide surveillance.
- The Veterinary AST Subcommittee has isolates and next-generation sequencing data that they would be happy to share.
- Can the comment say “broth microdilution” instead of “reference method broth microdilution”?
 - There is lyophilized BMD data that looks acceptable, so CLSI could remove the word “reference”.
- There is concern that there is not enough clinical data.
- How do laboratories only test on skin, maybe state “skin and skin structure”?
- Should not undersell the strong clinical data here. There is strong clinical data that this works for wild-type organisms.
- Setting a breakpoint goes against the FDA package insert which is difficult.
- Due to the lack of PK/PD data and clinical data from sterile sites EUCAST went with the ECV.
- What about the intermediate and resistance breakpoints?

- Know there is resistance.
- This is the most conservative place to set a resistance breakpoint.
- Need the intermediate range to set the disk diffusion breakpoint.
- Breakpoints for STX when used for skin and soft tissue. Consider a Table 1 warning that testing is not indicated for pharyngeal isolates.
- The word “uncomplicated” should not be included. Laboratories cannot know if something is “uncomplicated”.
- Remove the first sentence in the comment, “The only approved MIC method for testing is broth microdilution.”
- Laboratories could include in their reporting that it is only for skin.

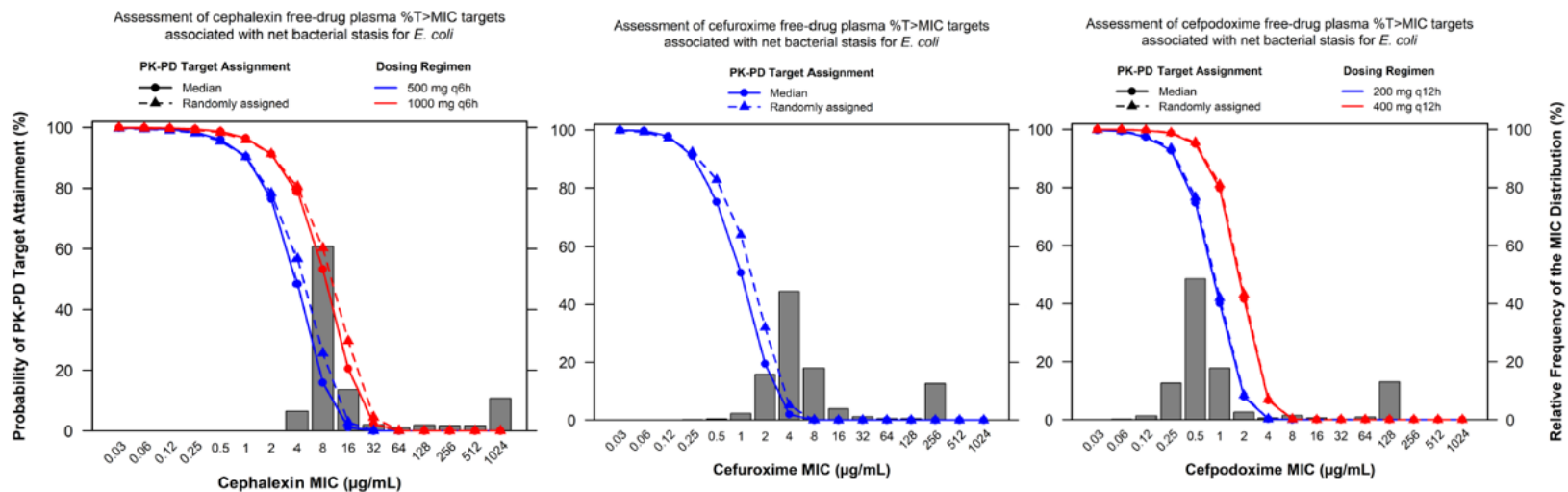
A motion to accept the trimethoprim-sulfamethoxazole MIC breakpoints ($S \leq 0.5$, $I 1$, $R \geq 2 \mu\text{g/mL}$) for β -hemolytic streptococci with the comment, “Agar based methods using MHA with 5% sheep blood (eg, disk diffusion, gradient diffusion, agar diffusion) should not be performed due to increased thymidine content leading to false resistance. Not routinely reported other than isolates recovered from skin and skin structure infections.”, the Table 1 comment, and placement in Table 1 Tier 4 was made and seconded. Vote: 10 for, 3 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:

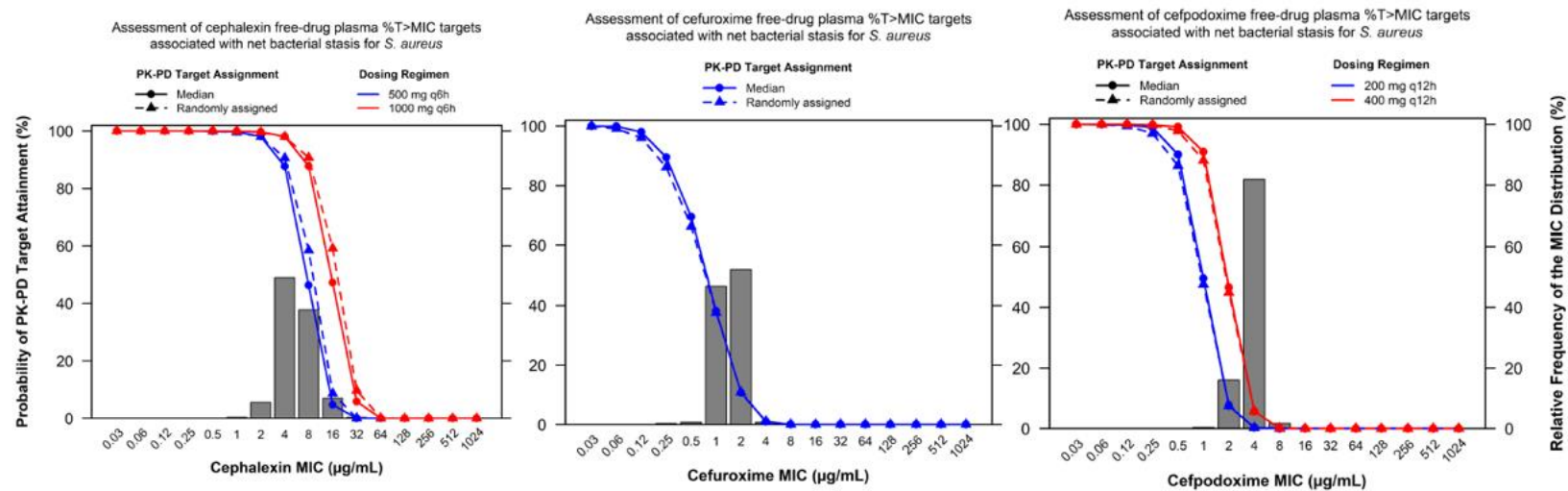
- There is not enough clinical data. The clinical data is not paired with the microbiology data.
- Need a comment also in Table 2 regarding not to use for pharyngeal isolates.

ORAL CEPHALOSPORIN BREAKPOINTS

- Bugs and Drugs Reviewed
 - *E. coli* and *S. aureus*
 - Cephalexin, cefuroxime, cefpodoxime
- *E.coli* PK/PD PTA



- *S. aureus* PK/PD PTA



- USCAST Data

Candidate Cephalosporin Susceptible Breakpoints for *E. coli* and *S. aureus* by Agent and Percentages of Susceptible Isolates

Pathogen	Antimicrobial agent	Dosing regimen		Candidate susceptible breakpoints ^a			
				Median		Randomly assigned	
				MIC (µg/mL)	%S ^b	MIC (µg/mL)	%S ^b
<i>E. coli</i>	Cephalexin	Low	500 mg q6h	1	0	1	0
		High	1000 mg q6h	2	0.1	2	0.1
	Cefuroxime	-	500 mg q12h	0.25	0.3	0.25	0.3
	Cefpodoxime	Low	200 mg q12h	0.25	14.3	0.25	14.3
		High	400 mg q12h	0.5/1 ^c	62.8/80.5	0.5/1 ^c	62.8/80.5
<i>S. aureus</i>	Cephalexin	Low	500 mg q6h	2	5.9	4	54.9
		High	1000 mg q6h	4/8 ^d	54.9/92.7	8	92.7
	Cefuroxime	-	500 mg q12h	0.12	0	0.12	0
	Cefpodoxime	Low	200 mg q12h	0.5	0	0.25	0
		High	400 mg q12h	1	0.3	0.5	0

a. Candidate susceptible breakpoints represented the highest MIC values for each cephalosporin agent at which percent probabilities of PK-PD target attainment based on median and randomly assigned %T>MIC targets associated with net bacterial stasis that were ≥ 90% were achieved.

b. %S = percent of isolates susceptible at the given candidate susceptible breakpoint.

c. Percent probabilities of PK-PD target attainment based on median and randomly assigned free-drug plasma %T>MIC targets associated net bacterial stasis were 79.7 and 81.0%, respectively, at an MIC of 1 mg/L.

d. The percent probability of PK-PD target attainment based on the median free-drug plasma %T>MIC target associated with net bacterial stasis was 87.8% at an MIC value of 8 mg/L.

Oral Cephalosporin STIC for Enterobacterales by Organization

Drug	USCAST ^a			Current STIC (µg/mL) by organization											
				US FDA ^b			CLSI ^c						EUCAST ^d (uUTI only)		
							Actual cephalosporin agent			Cefazolin as surrogate agent for oral cephalosporins and uUTI only ^e					
S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	
Cephalexin 500 mg q6h 1000 mg q6h	Not supported by the data			CLSI M100			-	-	-	≤ 16	-	≥ 32	≤16	-	≥ 32
Cefuroxime 500 mg q12h	Not supported by the data			CLSI M100			≤ 4	8–16 [^]	≥ 32	≤ 16	-	≥ 32	≤ 8	-	≥ 16
Cefpodoxime 200 mg q12h 400 mg q12h	Not supported by the data			CLSI M100			≤ 2	4 [^]	≥ 8	≤ 16	-	≥ 32	≤ 1	-	≥ 2
	≤ 1	-	≥ 2												

Note: S=susceptible, I=intermediate and R=resistant. [^], designation for agents that have the potential to concentrate in the urine.

a. The STIC for cefpodoxime 400 mg q12h are based on a net bacterial stasis endpoint and should therefore only be applied to non-severe, uncomplicated infections.

b. US FDA 2025 interpretive criteria. A notation of "CLSI M100" indicates that the US FDA recognizes the STIC described in the CLSI M100 guidance.

c. CLSI M100-ED35 (2025) interpretive criteria.

d. EUCAST 2025 clinical breakpoint tables.

e. STIC for cephalexin, cefuroxime and cefpodoxime is determined by evaluating STIC for cefazolin as the surrogate agent when used for therapy of uUTI arising from *E. coli*, *K. pneumoniae*, and *P. mirabilis*.

USCAST Oral Cephalosporin STIC for *S. aureus*

Drug	USCAST STIC for <i>S. aureus</i> ^a			Current STIC (µg/mL) for <i>Staphylococcus</i> species by organization											
				US FDA ^b			CLSI ^c						EUCAST ^d		
	Oxacillin as the surrogate agent ^e						Cefoxitin as surrogate ^e			Cefoxitin as surrogate ^e					
				S	I	R							S	I	R
Cephalexin 500 mg q6h	Not supported by the data			CLSI M100 is recognized			≤ 2	-	≥ 4	≤ 4	-	≥ 8	-	-	≥ 8
1000 mg q6h	≤ 8	-	≥ 16												
Cefuroxime 500 mg q12h	Not supported by the data			CLSI M100 is not recognized			≤ 2	-	≥ 4	≤ 4	-	≥ 8	-	-	≥ 8
Cefpodoxime 200 mg q12h 400 mg q6h	Not supported by the data			CLSI M100 is not recognized			≤ 2	-	≥ 4	≤ 4	-	≥ 8	-	-	≥ 8

Note: S=susceptible, I=intermediate and R=resistant.

a. The STIC for cephalexin 1000 mg q6h are based on a net bacterial stasis endpoint and should therefore only be applied to non-severe, uncomplicated infections.

b. US FDA 2025 interpretive criteria. A notation of "CLSI M100" indicates that the US FDA recognizes the STIC described in the CLSI M100 guidance.

c. CLSI M100-ED35 (2025) interpretive criteria. Isolates that test resistant by cefoxitin or oxacillin, when using the appropriate test method for the species, should be reported as methicillin (oxacillin) resistant. Methicillin (oxacillin)-susceptible staphylococci can be considered susceptible to cephalexin, cefuroxime, and cefpodoxime. Dosing regimens for individual agents upon which STIC were established were not described.

d. EUCAST 2025 clinical breakpoint tables. *S. aureus* with cefoxitin MIC values ≥ 8 mg/L are reported as methicillin resistant. The dosing regimen used to establish the cephalexin STIC was 250 to 1000 mg PO administered 2-3 times daily. For the cefuroxime and cefpodoxime STIC, the dosing regimens used to establish these criteria were cefuroxime 250 to 500 mg PO administered PO twice daily and cefpodoxime 100 to 200 mg PO administered twice daily.

e. STIC for cephalexin, cefuroxime and cefpodoxime against *S. aureus* is determined by evaluating STIC for oxacillin or cefoxitin as the surrogate agent as recommended by CLSI and cefoxitin as the surrogate agent as recommended by EUCAST.

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TESTING COMMITTEE FOR THE UNITED STATES

24

- Suggestions
 - Constitute ad hoc working group to review oral cephem breakpoints for Enterobacterales
 - Either remove oral cephem breakpoints entirely or make them uncomplicated UTI-specific
 - Consider adding separate cefadroxil disk breakpoint
- Breakpoints Working Group Discussion and Recommendation
 - M23 criteria for breakpoint revision (Ch. 4.2)
 - New PK/PD data indicate that existing breakpoints may have been set inappropriately high or low
 - Overall agreement to form AHWG to address oral cephalosporin breakpoints
 - Current breakpoints are unclear for systemic vs. UTI
 - Assess for clinical signal that prompts need to revise breakpoints
 - Suggestion for urinary breakpoints (more challenging to conduct but important)
 - WG Request: Approve formation of AHWG to review oral cephalosporin breakpoints

A motion to form an ad hoc working group to review oral cephalosporin breakpoints was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

CARBAPENEMASE DETECTION IN CARBAPENEM RESISTANT *PSEUDOMONAS AERUGINOSA*

- Rationale for need for criteria to recommend carbapenemase testing in carbapenem resistant *Pseudomonas aeruginosa* (CRPA)
 - CRPA is a WHO high-priority pathogen
 - Carbapenem resistance in *P. aeruginosa* can be due to a combination of outer membrane porin mutations, AmpC β -lactamase overexpression, and/or efflux pumps OR due to carbapenemases
 - Carbapenemases are common in CRPA ex-US but rare in US
 - CLSI M100 has several methods to detect carbapenemases in Enterobacterales and *P. aeruginosa*
 - M100 recommends carbapenemase testing for CRE, but not CRPA
 - Knowing a microorganism has a carbapenemase could guide use of new β -lactamase/ β -lactamase inhibitors that target specific enzymes (eg, avibactam, relebactam, taniborbactam)
 - No algorithm based on AST phenotype to guide carbapenemase testing of CRPA
- Clinical Study
 - Reyes et al. Lancet Microbe 2023. PMID: 36774938.
 - Multinational study of 972 CRPA isolates in 2019 that underwent whole genome sequencing
 - Global epidemiology of carbapenemases in CRPA
 - Mortality higher in carbapenemase producers
 - Carbapenemase-producing CRPA less likely to be susceptible to other anti-pseudomonal agents than non-carbapenemase-producing CRPA
 - Carbapenemase-producing CRPA more meropenem resistant than non-carbapenemase-producing CRPA
- CLSI M100 guidance to detect carbapenemases in *P. aeruginosa*

Introduction to Tables 3B and 3C. Tests for Carbapenemases in Enterobacterales and *Pseudomonas aeruginosa*

Institutional treatment guidelines, infection prevention procedures, or epidemiological investigations may necessitate identification of carbapenemase-producing Enterobacterales and *P. aeruginosa*.¹ **Tests that detect the type of carbapenemase are recommended to inform treatment decisions in carbapenem-resistant Enterobacterales isolates.**

Carbapenemase-producing isolates of Enterobacterales usually test intermediate or resistant to one or more carbapenems using the current breakpoints as listed in Table 2A-1 (**NOTE:** Testing not susceptible to ertapenem is often the most sensitive indicator of carbapenemase production. **Depending on local epidemiology and available resources, carbapenemase testing for *Enterobacter cloacae* complex and *Klebsiella aerogenes* isolates that are only resistant to ertapenem might not be necessary. Ertapenem resistance in these species is often due to mechanisms other than carbapenemase production and carbapenemases are currently uncommon in such isolates).** Carbapenemase-producing Enterobacterales usually test resistant to one or more agents in cephalosporin subclass III (eg, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone). However, some isolates that produce carbapenemases, such as **OXA-48**, SME, or IMI, often test susceptible to these cephalosporins.

	Tests Used for Carbapenemase Detection			
	Carba NP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Organisms	Enterobacterales and <i>P. aeruginosa</i> that are not susceptible to one or more carbapenems	Enterobacterales and <i>P. aeruginosa</i> that are not susceptible to one or more carbapenems	Enterobacterales that are positive by mCIM	Enterobacterales and <i>P. aeruginosa</i> that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by Carba NP or mCIM
Strengths	Rapid	No special reagents or media necessary	No special reagents or media necessary	Determines type of carbapenemase in addition to absence or presence of the enzyme

- Developing criteria to guide which CRPA isolates should undergo carbapenemase testing (Gill et al. Antibiotics (Basel) 2020. PMID: 33120865)
- Global ERACE-PA surveillance program to develop phenotypic algorithm for carbapenemase testing in CRPA (Gill et al. Open Forum Infect Dis 2021. PMID: 35106312)
- Request
 - Establish a group to review data and set criteria for carbapenemase testing in *P. aeruginosa* isolates based on phenotypic AST results
 - Candidate criterion: Resistance to imipenem or meropenem and non-susceptibility to cefepime and ceftazidime. Or addition of ceftolozane-tazobactam-non-susceptible when available.
- Breakpoints Working Group Discussion and Recommendation
 - Optimal definition should take into account that many laboratories do not test imipenem
 - Unlike with CRE:
 - Carbapenemases still rare in *P. aeruginosa* in the US
 - Low yield for limited clinical microbiology lab resources
 - Less prediction of *in vitro* activity based on carbapenemase type with *P. aeruginosa* compared to CRE
 - Example: Only 52% of CRPA with bla_{KPC} are susceptible to ceftazidime-avibactam
 - No data that carbapenemase presence or types adds predictive information on *in vivo* efficacy compared to AST results alone

- With CRE, efficacy of cefepime poor for KPC+ CRE even if cefepime-susceptible
- With CRE, efficacy of meropenem-vaborbactam limited for OXA-48+ CRE even if meropenem-vaborbactam susceptible
- There may be more carbapenemases in CRPA in certain regions of the country (eg, Houston) than the national average
 - Gottesdiener et al. ESCMID 2024. Manuscript under review.
 - Gill et al. J Antimicrob Chemother 2021. PMID: 33103202.
 - Fouad et al. J Antimicrob Chemother 2023. PMID: 37522258

SC DISCUSSION (MAIN POINTS)

- Testing is helpful to look for an outbreak.
- For *P. aeruginosa*, laboratories are going to have to test the new drugs phenotypically anyways.
- The eye drop outbreak was a VIM producing organism.
- It would be helpful to have guidance of the susceptibility patterns laboratories expect to see if it is a carbapenemase producer.
- If the isolate is susceptible to all the cephalosporins then probably don't need to test for a carbapenemase.

CEFTRIAXONE AND CEFIXIME MIC BREAKPOINTS FOR *NEISSERIA GONORRHOEAE*

- Current GC Breakpoints for Ceftriaxone and Cefixime

Current Recommendations	S (µg/mL)	R (µg/mL)
FDA STIC	≤0.25	No Breakpoint
CLSI M100	≤0.25	No Breakpoint
EUCAST	≤0.125	≥0.25

- CLSI also has disk diffusion breakpoints (S ≥ 35 mm) for each antimicrobial agent, but there are none from EUCAST
 - What's wrong with this?
 - Currently unable to define resistance
 - This is a problem because "resistant" isolates are no longer rare in some parts of the world and clinical failures have been reported
 - New data are available: MIC distributions, PK-PD, clinical failures
- GC Treatment Guidelines
- Epidemiological Cutoff Values for Ceftriaxone and Cefixime in *Neisseria gonorrhoeae*
 - Data sources for ECVs
 - Agar dilution
 - NML/Gonococcal Antimicrobial Surveillance Program (GASP)-Canada
 - Passive surveillance, all body sites, since 2022--WGS first, then AST
 - Includes WHO control strains
 - US-CDC Gonococcal Isolate Surveillance Program (GISP)
 - Active surveillance, mostly symptomatic men with urethritis at STD clinics
 - Other methods that are not consistently via agar dilution
 - EUCAST: calibrated to reference BMD?
 - WHO Enhanced Gonococcal Antimicrobial Surveillance Program (WHO-EGASP): Etest
 - ECV Summary: NML and US-CDC only

ECOFF 97.5%	Ceftriaxone	Cefixime
NML + WHO	0.125	0.063
US-CDC	0.032	0.063
NML+WHO+CDC	0.032	0.063

- Commercial MIC Tests
 - Ceftriaxone: Etest vs agar dilution for GC
 - Biedenbach et al. J Clin Microbiol 1996. PMID: 8940476
 - This is the only FDA-cleared MIC test for *N. gonorrhoeae* ceftriaxone AST
 - Etest and agar dilution prepared from the same inoculum
 - 96% essential agreement
 - Etest trend of +1 doubling dilution
 - Several surveillance systems rely on Etest for first tier testing. In areas with very limited agar dilution, Etest may be the primary source of MIC results.
 - Cefixime: Etest vs agar dilution for GC
 - Papp et al. J Med Microbiol 2018. PMID: 29219803
 - Etest cefixime is not FDA cleared for this species, but it is used in some places
 - There are no studies comparing Etest to agar dilution from the same inoculum, only from different inocula
 - 74% essential agreement
- Genetic mechanisms of resistance to cephalosporins
 - Cephalosporin resistance in GC: *penA* mutations
 - penA* encodes PBP2, target of cephalosporins
 - penA* mutations with elevated cephalosporin MICs: A311V, T316A, T483S
 - Incorporation of mutations to a susceptible strain with *penA35* allele increases MICs. Changing mutations to wild type in a resistant strain with the *penA41* allele causes the strain to become susceptible.
 - The *penA* mosaic allele has been shown to decrease susceptibility to cephalosporins
- PK/PD Analysis
 - Probability of target attainment (PTA) for ceftriaxone and cefixime vs GC by MIC value
 - PK-PD target from literature suggest β -lactams need a minimum of 10 and possibly 20 hours above the MIC value for effective treatment of urethritis
 - Chisholm et al. JAC 2010. PMID: 20693173
 - PD simulation for ceftriaxone 500 mg
 - MIC 0.125: 32.8 h
 - MIC 0.25: 24.3 h (current breakpoint)**
 - MIC 0.5: 15.6 h
 - PD simulation for cefixime 400 mg:
 - MIC 0.06: 22.2 h
 - MIC 0.125: 18.8 h

- MIC: 0.25: 15.3 h (current breakpoint)
 - MIC 0.5: 11.7 h
- PK/PD of ceftriaxone for *N. gonorrhoeae*: Hollow fiber model
 - Unemo et al. JAC 2024. PMID: 38497988
 - Hollow fiber model or urogenital and pharyngeal infection
 - Ceftriaxone 500 mg and 1 gm IM
 - Against WHO X, R and WHO Z reference strains (MIC 2, 0.5 and 0.5)
 - Assumptions
 - 5% free fraction in plasma, and t_{1/2} of 7.5 hours,
 - Pharyngeal: 10.9% of serum concentrations in tonsil
 - Results
 - Model of urogenital infection / ceftriaxone 1 gm failed to eradicate *N. gonorrhoeae* with ceftriaxone MIC of 2
 - Model of pharyngeal infection / ceftriaxone 500 mg and 1 gm failed to eradicate *N. gonorrhoeae* with ceftriaxone MIC of 0.5-2
- PK/PD of ceftriaxone and cefixime for *N. gonorrhoeae*: Murine model: PD target
 - Connolly et al. AAC 2019. PMID: 30642924
 - Surrogate model of cervicovaginal infection
 - Estrogenized female BALB/c mice
 - Used cephalosporin susceptible strain FA1090, and two cephalosporin-resistant strains (H041 and F89 with ceftriaxone MIC of 2 and 1-2 mg/L)
 - Results for effective killing of *N. gonorrhoeae*
 - Ceftriaxone: fT_{>MIC} of 23.6 hours
 - Cefixime: fT_{>MIC} of 36.8 hours
- USCAST Data

Assessment of Candidate Azithromycin and Ceftriaxone Susceptible Breakpoints for *N. gonorrhoeae* Based on the Results of Model-Predicted Efficacy and/or PK-PD Target Attainment Analyses

Antimicrobial agent	Dosing regimen		Candidate susceptible breakpoints ^{a,b}			
			Clinical PK-PD target		Randomly assigned non-clinical PK-PD targets	
			MIC (mg/L)	%S	MIC (mg/L)	%S
Azithromycin	1000 mg	PO	0.06	17.9	0.03	3.6
	2000 mg	PO	0.12	50.0	0.06	17.9
Ceftriaxone	500 mg	IM			0.06	96.4
	1000 mg	IM			0.12	97.6

a. Candidate susceptible breakpoints for azithromycin were assessed based on MIC values at which mean percent probabilities of microbiological cure were $\geq 95\%$ or percent probabilities of PK-PD target attainment were $\geq 90\%$.

b. Candidate susceptible breakpoints for ceftriaxone were assessed based on MIC values at which percent probabilities of PK-PD target attainment $\geq 85\%$ were achieved. For the ceftriaxone 500 mg IM dose, the percent probability of PK-PD target attainment was 89.2% at an MIC value of 0.06 mg/L. For the ceftriaxone 1000 mg IM dose, the percent probability of PK-PD target attainment was 88.7% at an MIC value of 0.12 mg/L. PK-PD target attainment analyses were based on simulations that incorporated inflated interindividual variance values of the population PK model parameters.

STIC Recommendations for Azithromycin and Ceftriaxone Against *N. gonorrhoeae* and Comparison by Organization

Drug	Dosing regimen ^a	USCAST STIC (mg/L)			Current STIC (mg/L) by organization							
					USA FDA ^b			CLSI ^c			EUCAST ^d	
		S	I	R	S	I	R	S	I	R	S	R
Azithromycin	2000 mg PO	≤ 0.25		≥ 0.5	Not recognized			≤ 1	-	-	-	
Ceftriaxone	500 mg IM	≤ 0.25			CLSI M100			≤ 0.25	-	-	≤ 0.12	≥ 0.25

Note: S=susceptible, I=intermediate and R=resistant.

- The azithromycin and ceftriaxone dosing regimens upon which STIC recommendations are based are consistent with those recommended by the CDC for uncomplicated gonococcal infections, which include administration of a single dose of 500 mg ceftriaxone IM as first-line treatment, or a single dose of azithromycin 2000 mg PO with a single dose of gentamicin 240 mg IM as an alternative dosing regimen <https://www.cdc.gov/std/treatment-guidelines/gonorrhea-adults.htm>.
- FDA 2023/2024 interpretive criteria. <https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria>. A notation of "CLSI M100" indicates that the FDA recognizes the STIC described in the CLSI M100 guidance.
- CLSI M100-ED34 (2024)/M100-ED33 (2023) interpretive criteria. The CLSI STIC for azithromycin are based on administration of a single dose of azithromycin 1g PO in conjunction with another agent.
- EUCAST 2024 clinical breakpoint tables. The EUCAST STIC for ceftriaxone are based on administration of a single dose of 0.5 to 1 g ceftriaxone IM. Azithromycin is always used in conjunction with another effective agent. For testing purposes with the aim of detecting acquired resistance mechanisms, the ECOFF for azithromycin is 1 mg/L.

- Therapeutic Outcome Data
 - USCAST Data

Microbiological Response Post-Ceftriaxone Treatment by MIC

- Microbiological response by MIC in Japanese male patients with gonococcal urethritis after administration of a single dose of 1000 mg ceftriaxone IV was assessed between 2 to 41 days post-therapy [1].
- These data demonstrated a high percentage of microbiological eradication across all MIC values observed up to 0.25 mg/L, suggesting a susceptible breakpoint that is at least as high as 0.25 mg/L.

1. Ito S, Yasuda M, Hatazaki K, et al. Microbiological efficacy and tolerability of a single-dose regimen of 1 g of ceftriaxone in men with gonococcal urethritis. J Antimicrob Chemother 2016;71:2559-2562.

MIC (mg/L)	Eradicated	Persistent
0.001	100 (1/1)	0
0.002	0	0
0.004	100 (8/8)	0
0.008	96.8 (30/31)	3.2 (1/31)
0.016	100 (19/19)	0
0.03	100 (8/8)	0
0.06	100 (22/22)	0
0.125	100 (35/35)	0
0.25	100 (12/12)	0
Not determined ^a	96.6 (56/58)	3.4 (2/58)
Overall	98.5 (191/194)	1.5 (3/194)

Note: Numbers presented as % (n/N). The CLSI M100 susceptible breakpoint for ceftriaxone is ≤ 0.25 µg/mL.

a. Isolates not cultured for MIC testing.

- Ceftriaxone *N. gonorrhoeae* treatment failures reported in literature

Site of Infection	Sex	Age	Year	Country	CRO MIC	Treatment	Outcome	PMID
Pharynx	Female	20s	2024	Vietnam	0.5 mg/L	CRO 1 g + DOX	TOC Culture Positive, failed AZM 2 g (TOC NAAT positive, culture negative), cleared with ETP 1 g IV x1	39417254
Genital/Rectal	Female	30s	2018	Spain	1.0 mg/L	CRO 1 g	Genital cleared, rectal persisted (TOC culture positive), failed GEN 240 mg + AZM 2 g (symptoms persisted), cleared with ETP 1 g IV x3	30862336
Genital/Pharynx	Male	50s	2018	Thailand	1.0 mg/L	CRO 1 g + DOX	Genital cleared, pharyngeal persisted (TOC culture positive), failed SPC (TOC culture positive), cleared with ETP 1 g IV x3	29991383
Genital/Pharynx	Male	20s	2015	Japan	0.25 mg/L	CRO 500 mg + AZM 1g	Genital cleared, pharyngeal persisted (TOC culture positive), cleared with CRO 1 g + AZM 2 g	27332921
Genital	Male	50s	2022	Australia	0.25 mg/L	CRO 500 mg + AZM 1.5g	TOC NAAT Positive (culture negative) at 2 weeks, cleared with augmentin 1 g BID x 7d	35713023
Pharynx††	Female	20s	2009	Japan	2.0 mg/L	CRO 1 g	TOC NAAT Positive at 2 weeks, cleared with repeat CRO 1 g	21192886

†† This case is NOT clearly treatment failure - could have been re-infection

- Additional literature
 - Handsfield et al. NEJM 1991. PMID: 1922235
 - Fifer et al. JAC 2024. PMID: 39417254
 - Allen et al. JAMA 2013. PMID: 23299608
- MIC Breakpoint Proposal
 - Data Summary Ceftriaxone

Data Type	Ceftriaxone MIC cutoff point (mg/L)	Considerations
ECOFF	0.03 (97.5%)	The ECOFF value is the minimum possible cutoff value. CLSI uses a 97.5% ECOFF and EUCAST uses 99.9%
Genomic Data	0.12	This value represents the lowest MIC at which <i>penA</i> A311V, associated with CRO-R has been detected
PK-PD	0.06 (500 mg dose)	- In vitro hollow fiber PK-PD model (500 mg dose) - PTA 89% at MIC 0.06 - PTA 81% at MIC 0.12
Therapeutic (Clinical)	Susceptible breakpoint at least as high as 0.25	-100% clinical cure with 1 g ceftriaxone IV at MICs of 0.25 -2 clinical failures at MICs of 0.25

○ Possible Breakpoints for Ceftriaxone Considered

Option	S	I	R	Comment
1	≤0.25	None	≥0.50	Intermediate not included because
2	≤0.25	0.5	≥1.0	This proposal includes an intermediate dilution, however clinical data and PK/PD data suggest an increased rate of therapeutic failure and the need for a higher dose.
3	≤0.125	0.25	≥0.5	Breakpoints include an intermediate range and is suitable for the 500mg ceftriaxone dose.
4	≤0.125	None	≥0.25	This breakpoint agrees with both the genomic and the therapeutic data and is one dilution higher than the breakpoint suggested by the PK-PD data

The breakpoints recommended by the AHWG

- The susceptible breakpoint is supported by mutational analysis and clinical outcome data.
- An intermediate breakpoint is included to account for technical variability. The MIC of 0.25 ug/mL is acceptable because of low rate of clinical failure and the option of a higher dose.
- The AHWG recommends a comment like this: The ceftriaxone breakpoints are based upon the clinical response of uncomplicated genital infections. Data describing drug exposure and outcome for infections at other body sites is limited.

○ Data Summary Cefixime

Data Type	MIC cutoff point (mg/L)	Considerations
ECOFF	0.06 (97.5%)	The ECOFF value is the minimum possible cutoff value. CLSI uses a 97.5% ECOFF and EUCAST uses 99.9%
Genomic Data	No clear breakpoint	
PK-PD Breakpoint	0.06 (dose of 400 mg)	Limited data, but cefixime 400 mg effectively treated <i>N. gonorrhoeae</i> with cefixime MIC of 0.06 mg/L. Cefixime 800 mg was not modeled.
Therapeutic (Clinical)	0.125	Limited data, but treatment was successful at this MIC for uncomplicated urethral infections. For 3 infections at other sites (2 rectal and 1 pharynx specimens) the TOC culture was positive after treatment. Slide 53

○ Possible Breakpoints for Cefixime Considered

Option	S	I	R	Comment
1	≤0.125	0.25	≥0.5	There is less data for cefixime than ceftriaxone because the drug is used less frequently. TOC recommendations in treatment guidelines can help to convey actions needed to minimize therapy risks if the drug is used as monotherapy. This would require a comment that the breakpoint applies to uncomplicated urethritis only.
2	≤0.06	0.12	≥0.25	This breakpoint applies to all infections.

The breakpoints recommended by the AHWG

- The susceptible breakpoint is supported by mutational analysis and clinical outcome data.
- An intermediate breakpoint is included to account for technical variability and variability in clinical outcome data by body site.
- No comment is needed because this is a breakpoint that is meant to support all infection types

• GC AHWG Discussion and Recommendation

Drug	MIC (µg/mL)			Disk Diffusion (mm)			Comment
	S	I	R	S	R	I	
Ceftriaxone (500 mg IM)	≤0.125	0.25	≥0.5	No breakpoints are proposed today because testing of ceph-R isolates is needed. The AHWG recommends publishing revised MIC breakpoints without disk diffusion breakpoints now. In the following year, recommendations for disk diffusion breakpoints are expected.			The ceftriaxone breakpoints are based upon the clinical response of uncomplicated genital infections. Data describing drug exposure and outcome for infections at other body sites is limited.
Cefixime (800 mg oral)	≤0.06	0.125	≥0.25				No Comment

- Breakpoints Working Group Discussion and Recommendation
 - CDC recommends 1 g ceftriaxone in obese patients (>150 kg)
 - Though a comment unnecessary for ceftriaxone given failures so rare and not seen at MICs ≤ 0.12 µg/mL
 - A conservative breakpoint because clinical failures necessary, but thought conservative breakpoint prudent because the infection is treated empirically without AST results
 - Motion to accept ceftriaxone MIC breakpoints for *N. gonorrhoeae* (S ≤ 0.12, I 0.25, R ≥ 0.5 µg/mL). WG Vote: 10-0-1-2.
 - Proposed AHWG comment not approved by Breakpoints Working Group
 - Motion to accept the cefixime MIC breakpoints for *N. gonorrhoeae* (S ≤ 0.06, I 0.12, R ≥ 0.25 µg/mL). WG Vote: 9-0-1-3.

SC DISCUSSION (MAIN POINTS)

- Was there any discussion about SDD? If treatment is empiric, then maybe SDD is not necessary.
- AST will likely be performed on patients with complicated infections or have failed therapy.
- The breakpoints align with WHO and EUCAST.
- Do not know if most people can be treated with ceftriaxone at an MIC 0.25 µg/mL.
- An intermediate breakpoint may inspire providers to do a test of cure to confirm treatment worked.
- Cefixime breakpoints are largely driven by clinical data.
- Disk diffusion data is coming in January 2026.
 - Disk diffusion will be deleted for now.
 - Could a comment be added that additional disk correlate data is pending.
- Patients are being treated before there is an AST result. Providers are not acting on the MIC. It might help them understand the next steps and if follow up is needed.

- What dose will be labeled for the breakpoint?
 - Ceftriaxone is based on a dose of 500 mg.

A motion to accept the ceftriaxone MIC breakpoints for *Neisseria gonorrhoeae* ($S \leq 0.12$, I 0.25, $R \geq 0.5$ µg/mL) based on a dosage of 500 mg and removal of the disk diffusion breakpoints with a comment about pending disk diffusion breakpoint review was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

A motion to accept the cefixime MIC breakpoints for *Neisseria gonorrhoeae* ($S \leq 0.06$, I 0.12, $R \geq 0.25$ µg/mL) based on a dosage of 800 mg and removal of the disk diffusion breakpoints with a comment about pending disk diffusion breakpoint review was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

TETRACYCLINE URINE BREAKPOINT FOR ACINETOBACTER SPP.

- Tetracycline breakpoints

	Previous/old breakpoints (2024 M100-ED34)			Updated breakpoints		
	S	I	R	S	I	R
Minocycline (Revised June 2024)	≤ 4	8	≥ 16	≤ 1	2	≥ 4
Doxycycline	≤ 4	8	≥ 16	Removed (Jan 2025 meeting)		
Tetracycline *Urine only	≤ 4	8	≥ 16	Archived; under review		

*Minocycline breakpoints based on dosing of 200 mg every 12 hours

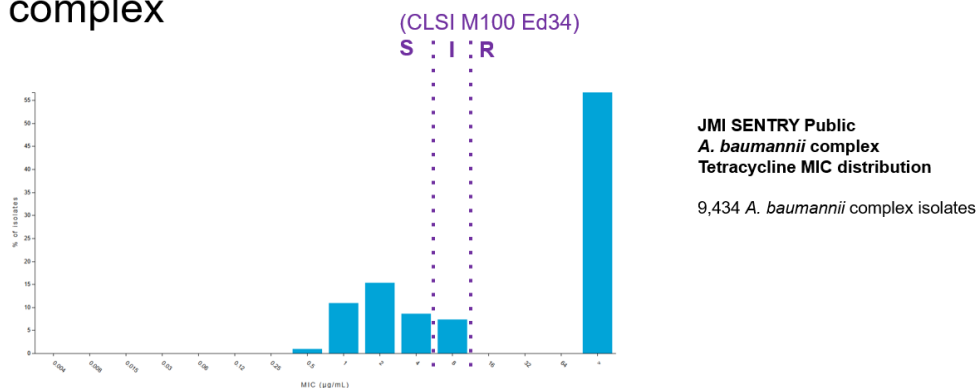
- PK and dosing comparisons
 - PK are variable with ranges throughout the older literature

Agent	Max dose per PI	Peak concentration (C_{max} ; mg/L)	Time to peak concentration (t_{max})	Half-life ($t_{1/2}$)	AUC (mg/L·h)	fAUC (mg/L·h)	Protein binding (%)
Minocycline	200 mg q12h	~3	2-3 h	~20	69.8 (0-8h)	16.8	76
Doxycycline	100 mg q12h	1.7-5.7 mg/L	2-3.5 h	~18-22 h	61	7.3	82-93 (mean, 88)
Tetracycline							55-64

- Urinary Concentrations
 - Agwuh et al. JAC 2006. PMID: 16816396.
 - Pearson et al. CMI Comms 2025.
 - Zhanel et al. Drugs 2004. PMID: 14723559.
 - Tetracycline AUC ranges from 55-75 mg·h/L
 - Urinary elimination is 30-60% depending on source
 - 150 - 300 µg/mL of a 500 mg dose?
- Tetracycline use for UTI? Not for *Acinetobacter*
 - Musher et al. J Infect Dis 1975
 - Assuming an output of urine of 1.5 liters/day, max urinary levels would be 400 µg/mL
 - Two patients with chronic *P. aeruginosa* UTI treated with tetracycline for 1.5 years with good results
 - In a pilot clinical trial, 8 of 12 hospitalized patients with UTI treated successfully with tetracycline (no other info given)
 - 171 isolates from infected urine shown to be resistant to tetracycline by Kirby-Bauer were studied for susceptibility to urinary concentrations of tetracycline
 - No *A. baumannii* isolates tested
- Tetracycline PK/PD for *Acinetobacter*: only 1 strain evaluated
 - Obana et al. JAC 1985. PMID: 4008377
 - In the untreated control mice, viable cells ranged from 107 to 108 organisms/kidney
 - Doses of 10 mg/mouse required to see reduction in kidney bacterial burden (to ~105 organisms/kidney (unsure how dose translates clinically))
 - Doxycycline and minocycline notably more effective at reducing burden compared to tetracycline
- Microbiology/ECV
 - Sources of reference BMD tetracycline MIC distribution data for *Acinetobacter baumannii* complex
 - JMI
 - SENTRY Microbiology Visualization Platform: 9,434 *A. baumannii* complex isolates as of March 7, 2025
 - Castanheira M et al. Clin Infect Dis 2014; 59: S367.
 - Huband MD et al. Antimicrob Agents Chemother 2020; 64:e02375

- Dataset provided to the AHWG by Mariana Castanheira for evaluation of tetracycline vs. minocycline MICs (presented at January 2025 meeting): 5,980 *A. baumannii* complex isolates
 - No data were available from the EUCAST website, ATLAS (Pfizer), Shionogi dataset (AMR Register/VivLi), or the IHMA dataset from the SUL-DUR studies
 - Also conducted literature search: Akers K et al. Antimicrob Agents Chemother 2009; 53:2693

Tetracycline MIC distribution: *A. baumannii* complex



Tetracycline MIC, µg/mL	0.5	1	2	4	8	≥ 16
Isolates (cumulative %)	89 (0.9)	1032 (11.9)	1444 (27.2)	815 (35.8)	702 (43.3)	5352

Data from sentry-mvp.jmilabs.com (accessed 3/7/2025)

A. baumannii complex/tetracycline ECOFF Finder summary (using a single JMI distribution at a time)

Data source(s) included	n	ECV 95.0%	ECV 97.5%	ECV 99.0%	ECV 99.5%	ECV 99.9%
JMI MVP SENTRY Public	9,434	8	8	16	16	32
JMI (2007-2011) worldwide surveillance	5,477	4	8	8	8	8
JMI (~2018) 34 countries	457	16	16	16	32	32
JMI data courtesy of M. Castanheira (for evaluation of tetra vs. mino MICs)	5,980	8	8	16	16	16

ECV (97.5%) = 8 µg/mL (?)

- *Acinetobacter* AHWG Discussion and Recommendations
 - No clinical data for UTI treatment and urinary concentrations are generally low
 - Not used clinically
 - Unclear the data used for approval of the current urinary breakpoint
 - ECV (8 mg/L) is over what the current breakpoint is now and no data to support an update
 - Motion to remove the tetracycline urine breakpoint for *Acinetobacter* spp. AHWG Vote: 6-0-0-1.
- Breakpoints Working Group Discussion and Recommendation
 - Motion to remove the tetracycline urine breakpoint for *Acinetobacter* spp. WG Vote: 8-0-1-4 (NOTE: Not a formal vote as there was not WG quorum present).

A motion to remove the tetracycline urine breakpoints for *Acinetobacter* spp. was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

MINOCYCLINE BREAKPOINT COMMENT FOR *ACINETOBACTER* SPP.

- Prediction of minocycline susceptibility in *Acinetobacter baumannii*
 - Minocycline not on many automated panels or not at a dilution that is low enough to apply susceptibility breakpoints
 - January 2025: *A. baumannii* complex isolates only (data from SENTRY)
 - 100% of isolates with tetracycline MIC ≤4 µg/mL had minocycline MIC ≤1 µg/mL
 - 99.9% of isolates with doxycycline MIC ≤1 µg/mL had minocycline MIC ≤1 µg/mL

- Comment: “When available, it is recommended to test minocycline directly. If minocycline cannot be tested, isolates with doxycycline MICs ≤ 1 $\mu\text{g/mL}$ or tetracycline MICs ≤ 4 $\mu\text{g/mL}$ are considered susceptible to minocycline. Isolates with doxycycline MICs ≥ 2 $\mu\text{g/mL}$ or tetracycline MICs ≥ 8 $\mu\text{g/mL}$ should be tested against minocycline if that result is needed for treatment.”
- What about non-*A. baumannii* complex isolates?

• Public SENTRY data (as of 5/2025): Prediction works for non-*A. baumannii* isolates too

Activity of antimicrobial agents tested against 1,110 *Acinetobacter* isolates in the SENTRY program (excluding *Acinetobacter baumannii*-calcoaceticus species complexes) with a MIC less than or equal to 1.0 $\mu\text{g/mL}$ tested against doxycycline

Organisms include *Acinetobacter beijerinckii* (8), *A. bereziniae* (96), *A. courvalinii* (22), *A. dispersus* (4), *A. gernerii* (2), *A. guillouiae* (15), *A. gyllenbergii* (2), *A. haemolyticus* (25), *A. indicus* (1), *A. johnsonii* (60), *A. junii* (96), *A. Iwoffii* (176), *A. modestus* (3), *A. parvus* (1), *A. proteolyticus* (15), *A. radioresistens* (152), *A. schindleri* (10), *A. soli* (30), *A. townneri* (1), *A. ursingii* (263), *A. variabilis* (27), *A. venetianus* (1), *A. vivianii* (11), and unspciated *Acinetobacter* (89).

Antimicrobial Agent	Dilution ($\mu\text{g/mL}$)															
	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>
Minocycline					48.3 535	77.8 326	94.4 184	99.1 52	99.7 7	99.8 1	100.0 2					

Activity of antimicrobial agents tested against 1,087 *Acinetobacter* isolates in the SENTRY program (excluding *Acinetobacter baumannii*-calcoaceticus species complexes) with a MIC less than or equal to 4.0 $\mu\text{g/mL}$ tested against tetracycline

Organisms include *Acinetobacter beijerinckii* (8), *A. bereziniae* (87), *A. courvalinii* (21), *A. dispersus* (4), *A. gernerii* (2), *A. guillouiae* (15), *A. gyllenbergii* (2), *A. haemolyticus* (27), *A. indicus* (1), *A. johnsonii* (56), *A. junii* (96), *A. Iwoffii* (174), *A. modestus* (3), *A. parvus* (1), *A. proteolyticus* (13), *A. radioresistens* (151), *A. schindleri* (10), *A. soli* (31), *A. townneri* (1), *A. ursingii* (256), *A. variabilis* (27), *A. venetianus* (1), *A. vivianii* (10), and unspciated *Acinetobacter* (90).

Antimicrobial Agent	Dilution ($\mu\text{g/mL}$)															
	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>
Minocycline					49.4 536	78.7 317	95.1 178	99.3 45	99.8 6	99.8 0	100.0 2					

• Breakpoints Working Group Discussion and Recommendation

- Motion to add the comment to minocycline breakpoint for *Acinetobacter* spp., “When available, it is recommended to test minocycline directly. If minocycline cannot be tested, isolates with doxycycline MICs ≤ 1 $\mu\text{g/mL}$ or tetracycline MICs ≤ 4 $\mu\text{g/mL}$ are considered susceptible to minocycline. Isolates with doxycycline MICs ≥ 2 $\mu\text{g/mL}$ or tetracycline MICs ≥ 8 $\mu\text{g/mL}$ should be tested against minocycline if that result is needed for treatment.” WG Vote: 8-0-1-4 (NOTE: Not a formal vote as there was not WG quorum present).

A motion to add the minocycline breakpoints comment for *Acinetobacter* spp., “When available, it is recommended to test minocycline directly. If minocycline cannot be tested, isolates with doxycycline MICs ≤ 1 $\mu\text{g/mL}$ or tetracycline MICs ≤ 4 $\mu\text{g/mL}$ are considered susceptible to minocycline. Isolates with doxycycline MICs ≥ 2 $\mu\text{g/mL}$ or tetracycline MICs ≥ 8 $\mu\text{g/mL}$ should be tested against minocycline if that result is needed for treatment.” was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

AMIKACIN MIC BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Current Aminoglycoside Breakpoints for *Acinetobacter* spp.
 - The disk correlates are the same as they were published for all organisms in 1980
 - The MIC intermediate was added sometime after 2006

AMINOGLYCOSIDES								
Gentamicin	10 µg	≥ 15	13-14	≤ 12	≤ 4	8	≥ 16	
Tobramycin	10 µg	≥ 15	13-14	≤ 12	≤ 4	8	≥ 16	
Amikacin	30 µg	≥ 17	15-16	≤ 14	≤ 16	32	≥ 64	
Netilmicin*	-	-	-	-	≤ 8	16	≥ 32	

Enterobacterales	S	I	R
Gentamicin	≤2	4	≥8
Tobramycin	≤2	4	≥8
Amikacin	≤4	8	≥16

<i>P. aeruginosa</i>	S	I	R
Gentamicin	--	--	--
Tobramycin	≤1	2	≥4
Amikacin (urine only)	≤16	8	≥16

- EUCAST aminoglycoside breakpoints for *Acinetobacter*
 - Guidance document on aminoglycosides - April 2020
 - Brackets: not true breakpoints, but ECOFF values to exclude isolates with acquired resistance mechanisms
 - Note 1/A: For systemic infections, aminoglycosides should be used in combination with other active therapy. In this circumstance, the value in brackets can be used to distinguish between wild type organisms and organisms with acquired resistance mechanisms

Aminoglycosides ¹	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	
Amikacin (systemic infections)	(8) ¹	(8) ¹		30	(19) ^A	(19) ^A		1/A. For information on how to use breakpoints in brackets, see https://www.eucast.org/eucastguidancedocuments/ .
Amikacin (infections originating from the urinary tract)	8	8		30	19	19		
Gentamicin (systemic infections)	(4) ¹	(4) ¹		10	(17) ^A	(17) ^A		
Gentamicin (infections originating from the urinary tract)	4	4		10	17	17		
Netilmicin	IE	IE			IE	IE		
Tobramycin (systemic infections)	(4) ¹	(4) ¹		10	(17) ^A	(17) ^A		
Tobramycin (infections originating from the urinary tract)	4	4		10	17	17		

- Amikacin dosing recommendations

FDA Package insert: 15 mg/kg

Intravenous Administration: The recommended daily dose for VPI-AMIKACIN is 15 mg/kg to be administered at 7.5 mg/kg every 12 hours (500 mg twice a day). The solution for intravenous use is prepared by adding the contents of a 500 mg/2 mL vial to 250 mL of sterile diluent and administered over a 30-60 minute period. Solutions for intravenous administration should be used within 24 hours after preparation.

EUCAST v15.0: 25-30 mg/kg

Aminoglycosides	Standard dosage	High dosage
Amikacin	25-30 mg/kg x 1 iv	None
Gentamicin	6-7 mg/kg x 1 iv	None
Netilmicin	6-7 mg/kg x 1 iv	None
Tobramycin	6-7 mg/kg x 1 iv	None

- amikacin dosage is most often 15 – 20 mg/kg/day, not the 25 – 30 mg/kg/day suggested by the pharmacokinetic/pharmacokinetic modelling and by the fact that amikacin is 4 times less active than gentamicin and tobramycin.

EUCAST is concerned that doses lower than those listed with the EUCAST breakpoints Dosages tab fail to deliver adequate exposure for the wild-type populations of target species, especially in serious systemic infections. This is particularly problematic for amikacin where dosing traditions are lower than in any European or FDA guideline [4 - 8] and acceptance of higher doses is lower than for other aminoglycosides [9]. EUCAST encourages the use of therapeutic drug monitoring for this drug class, which has a narrow window between efficacy and toxicity [9,10].

IDSA HAP/VAP guidelines: 15-20 mg/kg

Table 3. Suggested Empiric Treatment Options for Clinically Suspected Ventilator-Associated Pneumonia in Units Where Empiric Methicillin-Resistant *Staphylococcus aureus* Coverage and Double Antipseudomonal/Gram-Negative Coverage Are Appropriate

A. Gram-Positive Antibiotics With MRSA Activity	B. Gram-Negative Antibiotics With Antipseudomonal Activity: β -Lactam-Based Agents	C. Gram-Negative Antibiotics With Antipseudomonal Activity: Non- β -Lactam-Based Agents
Glycopeptides ^a Vancomycin 15 mg/kg IV q8-12h (consider a loading dose of 25-30 mg/kg x 1 for severe illness)	Antipseudomonal penicillins ^b Piperacillin-tazobactam 4.5 g IV q6h ^b	Fluoroquinolones Ciprofloxacin 400 mg IV q8h Levofloxacin 750 mg IV q24h
OR	OR	OR
Oxazolidinones Linezolid 600 mg IV q12h	Cephalosporins ^b Cefepime 2 g IV q8h Ceftazidime 2 g IV q8h	Aminoglycosides ^{a,c} Amikacin 15-20 mg/kg IV q24h Gentamicin 5-7 mg/kg IV q24h Tobramycin 5-7 mg/kg IV q24h

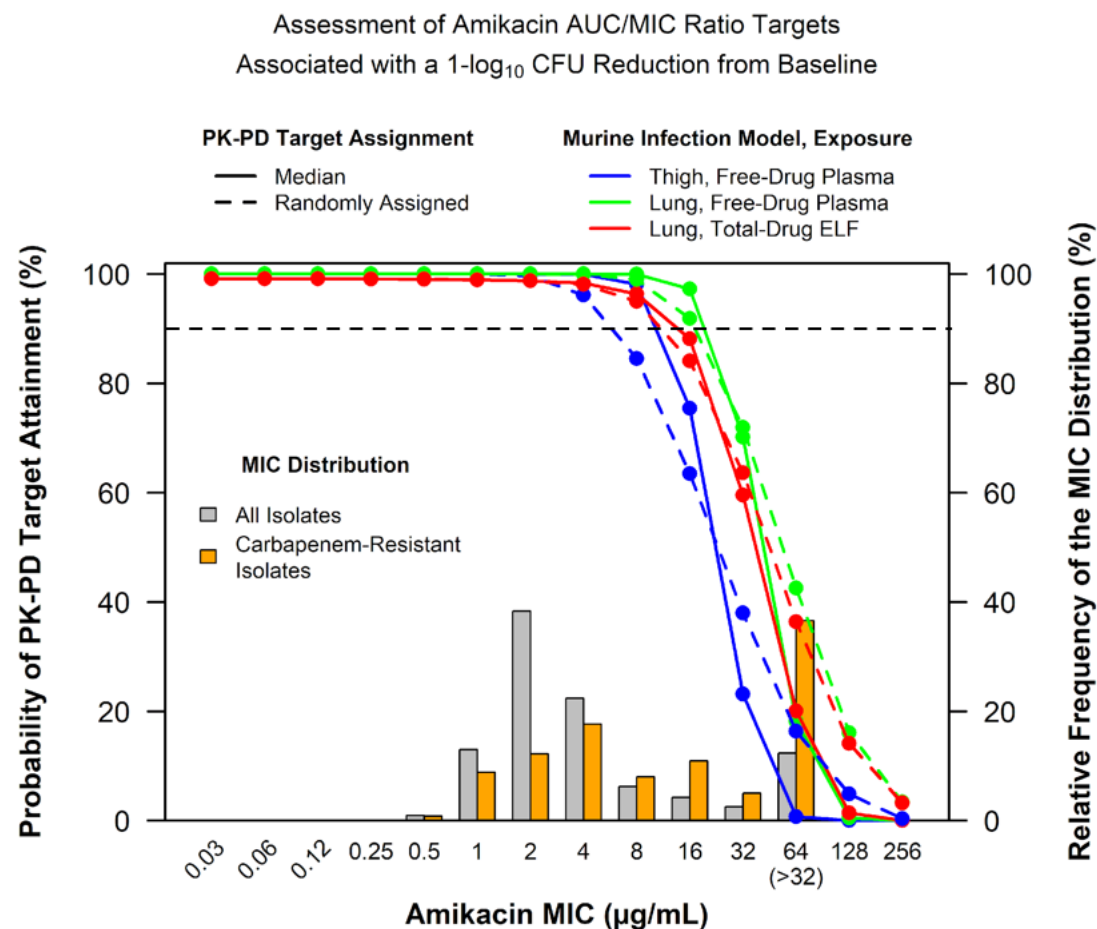
- Pk/PD
 - Data provided by Dr. Alex Lepak and USCAST Report 0002
 - Selected 5 strains: all *A. baumannii* complex
 - Thigh and lung treatment studies
 - 4 drugs X 5 ACBC strains
 - Neutropenic mouse lung and thigh
 - Emax - stasis and 1 log kill vs plasma and ELF PK/PD drivers

- Population PK and Monte Carlo Simulation
 - Simulated patients with cUTI/AP and HABP/VABP were generated using demographic data from clinical trials
 - Typical PK values for each simulated patient were calculated using demographic data for relevant covariates predictive of PK with fixed effect population PK parameter estimates
 - Simulated patients received maximal dosing regimens and free-drug concentration time-profiles were generated
 - Percent probabilities of attaining median and randomly assigned PK-PD targets associated with a 1 log₁₀ CFU reduction from baseline were assessed
- Integrating PK/PD Targets: Amikacin/*A. baumannii*
 - Utilized targets associated with 1-log kill endpoint in animal model
 - Felt this was the best target endpoint given much of *A. baumannii/calcoaceticus* complex infections are pulmonary/high burden infections with limited source control in which 1-log kill targets are likely more appropriate than stasis
 - Utilized two methods of integrating the animal model 1-log kill PK/PD targets in pharmacometric analyses of target attainment in simulated patients
 - Median PK/PD target value assigned to each simulated patient (fAUC/MIC 12.2)
 - Random assignment of individual strain PK/PD target to each simulated patient
 - Examined attainment associated with thigh model plasma exposure targets, lung model plasma exposure targets, and lung model ELF exposure targets

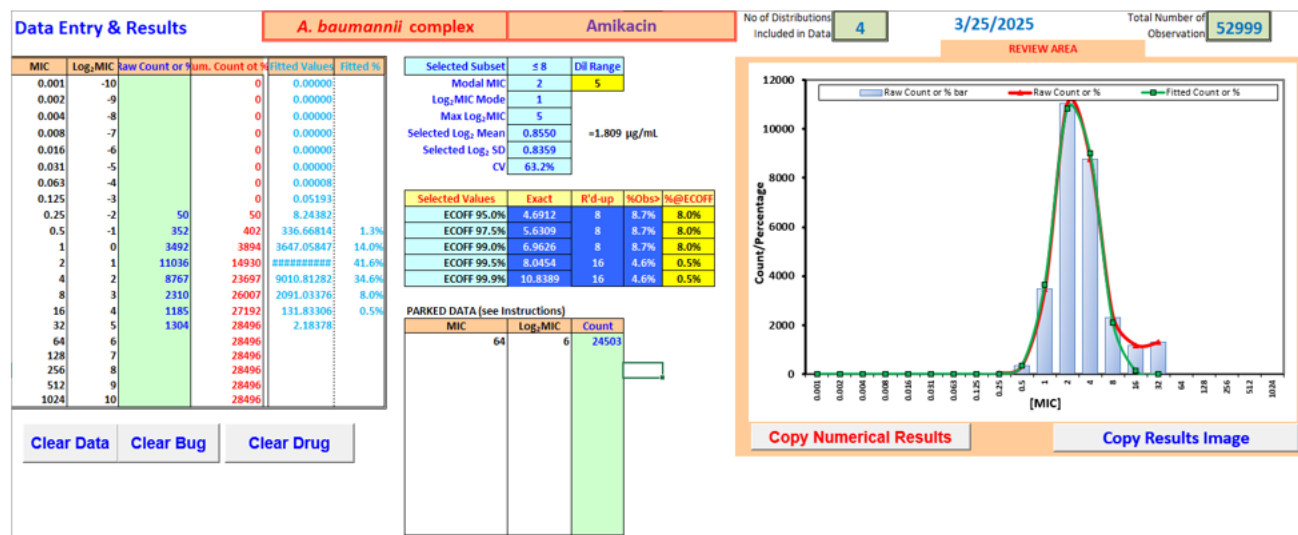
Percent Probabilities of PK-PD Target Attainment for Amikacin by MIC Overlaid Over MIC Distributions for *A. baumannii* Isolates from the USA

Drug	USCAST-recommended STIC (µg/mL) based on analysis results ^a		
	S	I	R
Amikacin	≤ 8	-	≥ 16

- Clinical Data
 - Clinical data for amikacin treatment of *Acinetobacter* spp. infections
 - Clinical data sparse
 - No outcomes by MIC data
 - Most relevant references:
 - Review: Poulidakos Eur J Clin Microbiol Infect Dis (2014) 33:1675-1685
 - Kuo, Clinical Microbiology and Infection, 2007;13:196-198



- Hernandez-Torres (2012) Multidrug and carbapenem-resistant *Acinetobacter baumannii* infections: factors associated with mortality. Med Clin (Barc) 138:650
 - Mostly used as part of combination therapy, small numbers of patients
- MIC distributions vs *Acinetobacter* spp.
 - Sources of reference BMD amikacin MIC distribution data for *Acinetobacter baumannii* complex
 - JMI
 - SENTRY Microbiology Visualization Platform: 10,742 *A. baumannii* complex isolates as of 3/25/2025
 - Lepak AJ et al. Antimicrob Agents Chemother 2023; 67: e0145222.
 - 754 *A. baumannii* complex isolates from US SENTRY surveillance 2018-2020
 - 892 *A. baumannii* complex isolates from Europe SENTRY surveillance 2018-202
 - EUCAST website: 2,019 *A. baumannii* isolates as of 3/25/2025
 - ATLAS (Pfizer): 18,746 *A. baumannii* complex isolates from all regions in the last 5 years as of 3/25/2025
 - IHMA: 21,492 *A. baumannii* complex isolates collected globally from 2018-2025 (data generously provided by Meredith Hackel at IHMA)
 - ECOFF Finder: Amikacin/*A. baumannii*
 - Input JMI SENTRY Public, EUCAST, ATLAS, and IHMA distributions
 - Lumped *A. baumannii* complex together, given that species within the complex can be difficult for clinical laboratories to distinguish



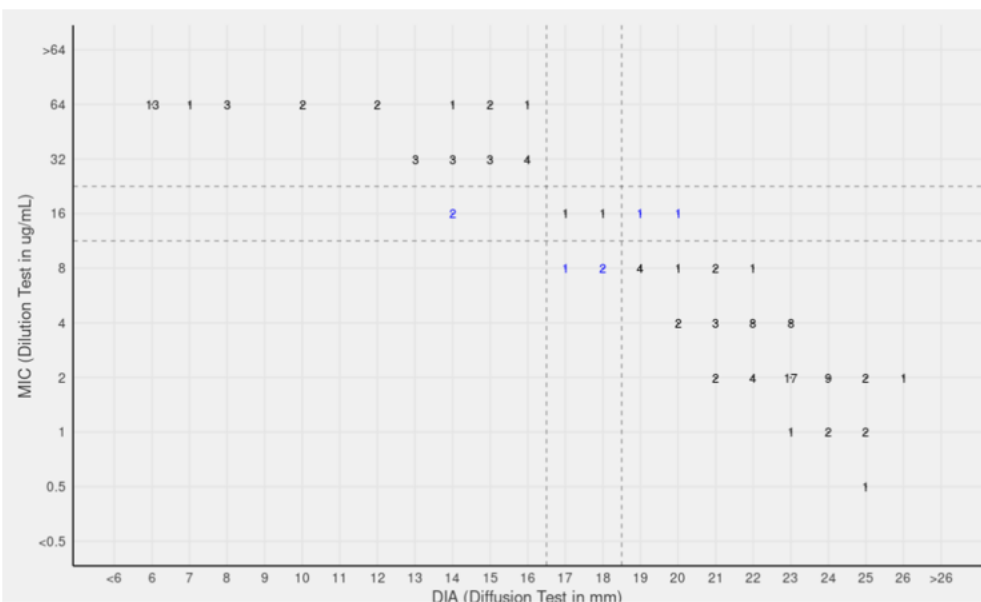
- Other considerations
 - Amikacin MICs are higher among *A. baumannii* complex than among *Acinetobacter* species not in the complex
 - Relatively few carbapenem-resistant *A. baumannii* complex isolates have amikacin MICs within the wild-type distribution. Although this is a fact to consider in breakpoint setting, it does not impact the ECV.
- *Acinetobacter* AHWG Discussion and Recommendations
 - Motion to accept the amikacin MIC breakpoints ($S \leq 8$, $I 16$, $R \geq 32$ µg/mL) for *Acinetobacter* spp. AHWG Vote: 7-0-0-0.

- Comments in support:
 - Generally will still be using combination therapy and I category may not ultimately matter
 - Intermediate might depend on the setting for which clinicians are interpreting
- Arguments against:
 - Worried intermediate could cause clinicians to push the dose
 - 16 mg/L is only ~3% of isolates
 - In the setting of pan-R isolate, if you see an intermediate for amikacin, this may be viewed differently because not many other options available
- Also considered having no intermediate category ($S \leq 8$, $R \geq 16$)
- Breakpoints Working Group Discussion and Recommendation
 - ECV: 8 µg/mL for most common pathogen (*A. baumannii* complex)
 - PK-PD: PTA of 98%/96% for MIC 8 µg/mL (for median neutropenic thigh/lung infection model 1-log kil target); PTA decreases to 75%/85% at MIC 16, 23%/63% at MIC 32
 - Clinical: sparse, small studies, usually combination therapy, no outcomes by MIC data
 - Dosage: 20 mg/kg used in PK-PD modeling (this is above what is in the FDA package insert, but frequently used clinically and in IDSA guidelines)
 - Motion to accept the amikacin MIC breakpoints ($S \leq 8$, I 16, $R \geq 32$ µg/mL) for *Acinetobacter* spp. based on a dosage of 20 mg/kg/day. WG Vote: 8-0-1-4 (NOTE: Not a formal vote as there was not WG quorum present).

A motion to accept the amikacin MIC breakpoints ($S \leq 8$, I 16, $R \geq 32$ µg/mL) for *Acinetobacter* spp. based on a dosage of 20 mg/kg/day was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

AMIKACIN DISK DIFFUSION BREAKPOINTS FOR ACINETOBACTER SPP.

- dBETS suggestion for optimized correlates (EUCAST dataset)
 - These are the disk correlates presented by the AHWG and approved by the Breakpoints WG.
 - There was discussion about potentially expanding to at least a 3 mm range based on CLSI M23 criteria.



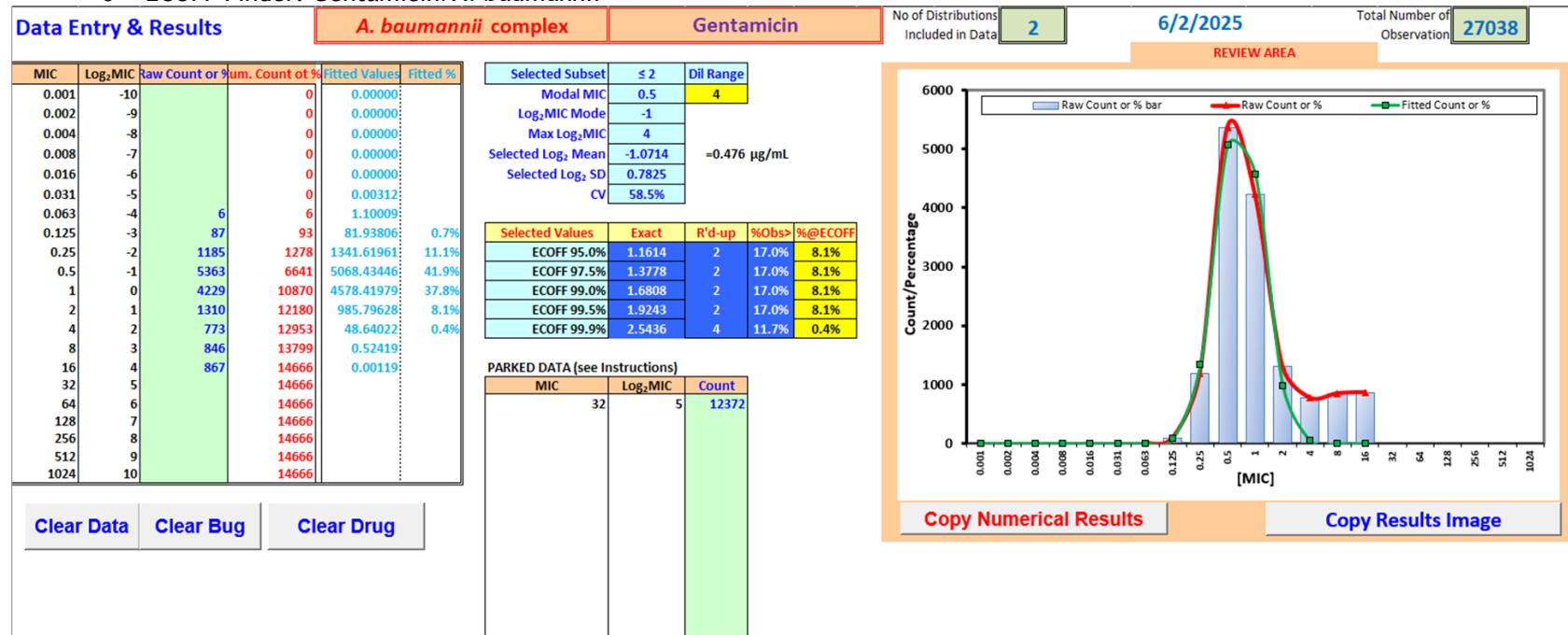
	S	I	R
MIC	≤ 8	16	≥ 32
Disk	≥ 19	17-18	≤ 16

	n	VME	ME	<u>mE</u>
≥ I+2	25	0 (0%)	NA	0 (0%)
I+1 to I-1	30	0 (0%)	0 (0%)	7 (23.3%)
≤ I-2	62	NA	0 (0%)	0 (0%)

- Language in CLSI M23 regarding the relationship between the width of an intermediate disk range and the width of QC ranges
 - “When zone diameter breakpoints are selected, consideration given to the species tested and whether they belong to the same group of organisms as one of the QC strains will help determine the range of the intermediate category, when it exists. The minimum intermediate range should equal half or be 1 mm wider than half of the relevant QC range, preferably a little wider to accommodate interlaboratory variation. The maximum intermediate range should be no wider than the relevant QC range...
 - There is no *Acinetobacter* spp. QC strain for amikacin
 - The *Pseudomonas aeruginosa* ATCC 27853 QC range is 20-26 mm (7 mm)
 - If this were an *Acinetobacter* spp. QC strain, that would suggest that the intermediate range should be at least 4 mm wide
 - Note that some other narrow (2 mm wide) amikacin intermediate ranges exist in M100
- Summary of presented options for *Acinetobacter* spp. amikacin disk correlates

	2 mm intermediate range		n	VME	ME	<u>mE</u>	Approved by breakpoint WG
	Minimizes error rates	≥ I+2	25	0 (0%)	NA	0 (0%)	
		I+1 to I-1	30	0 (0%)	0 (0%)	7 (23.3%)	
		≤ I-2	62	NA	0 (0%)	0 (0%)	
	3 mm intermediate range		n	VME	ME	<u>mE</u>	Option to expand to a 3 mm range to allow for additional interlaboratory variation; still meets M23 criteria
	Acceptable error rates	≥ I+2	25	0 (0%)	NA	0 (0%)	
		I+1 to I-1	30	0 (0%)	0 (0%)	10 (33.3%)	
		≤ I-2	62	NA	0 (0%)	0 (0%)	
	4 mm intermediate range		n	VME	ME	<u>mE</u>	Borderline, since guideline criterion is <40% ←
	Acceptable error rates	≥ I+2	25	0 (0%)	NA	0 (0%)	
		I+1 to I-1	30	0 (0%)	0 (0%)	12 (40%)	
		≤ I-2	62	NA	0 (0%)	2 (3.2%)	
• Breakpoints Working Group Discussion and Recommendation ○ Motion to accept the amikacin disk diffusion breakpoints (S ≥ 20, I 17-19, R ≤ 16 mm) for <i>Acinetobacter</i> spp. WG Vote: 8-0-1-4 (NOTE: Not a formal vote as there was not WG quorum present).							
A motion to accept the amikacin disk diffusion breakpoints (S ≥ 20, I 17-19, R ≤ 16 mm) for <i>Acinetobacter</i> spp. was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)							
GENTAMICIN AND TOBRAMYCIN MIC BREAKPOINTS FOR ACINETOBACTER SPP.							
• Background ○ No clinical data exist ○ Unlikely to get any additional PK/PD data ○ Could we borrow PK/PD target of amikacin and use gentamicin and tobramycin PTA analysis for Enterobacterales and <i>Pseudomonas aeruginosa</i>? • PK/PD ○ Target AUC/MIC exposures have been considered interchangeable/applicable to all aminoglycosides ▪ Eg. AUC/MIC target for gentamicin for <i>E. coli</i> = AUC/MIC target for tobramycin for <i>E. coli</i>							

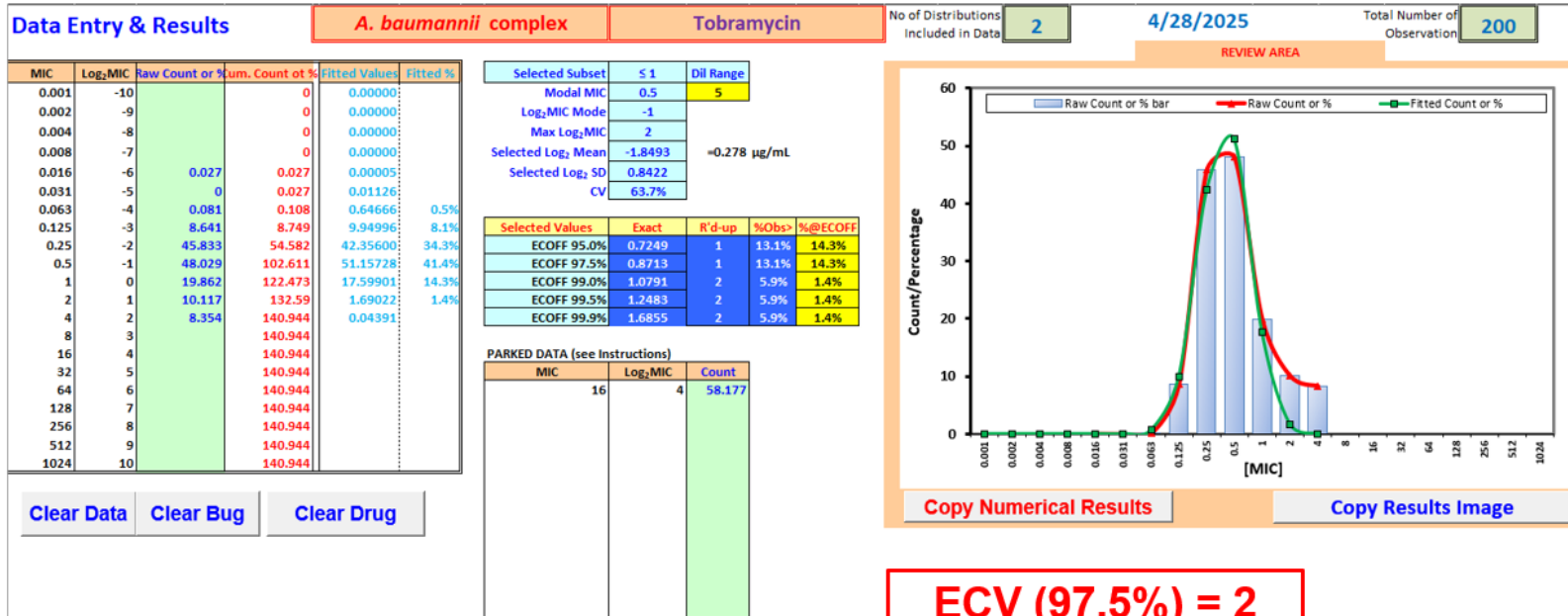
- Experimental data has backed this up from mouse studies
- 2-log kill target for amikacin and *Acinetobacter* spp. in neutropenic thigh lung model (19.2) similar to stasis target for gentamicin and Enterobacterales in same model (21.4)
 - PK models using gentamicin 7 mg/kg/day
 - Suggests > 95% PTA for gentamicin and *Acinetobacter* spp. at gentamicin MIC of 2 µg/mL
- 2-log kill target for amikacin and *Acinetobacter* spp. in neutropenic thigh lung model (19.2) similar to stasis target for tobramycin and Enterobacterales in same model (21.4)
 - PK models using tobramycin 7 mg/kg/day
 - Suggests > 95% PTA for tobramycin and *Acinetobacter* spp. at gentamicin MIC of 2 µg/mL
- Gentamicin and amikacin PTA of stasis target for Enterobacterales that is similar to amikacin/*Acinetobacter* target
- MIC distributions vs *Acinetobacter* spp.
 - Sources of reference BMD gentamicin MIC distribution data for *Acinetobacter baumannii* complex
 - EUCAST website: 1,896 *A. baumannii* isolates as of 4/8/2025
 - IHMA: 25,142 *A. baumannii* complex isolates collected globally from 2018-2025 (data generously provided by Meredith Hackel at IHMA)
 - ECOFF Finder: Gentamicin/*A. baumannii*



ECV (97.5%) = 2 µg/mL

- Page 108 of 166

Weighted dataset because 1 lab (JMI) >50% of total



**ECV (97.5%) = 2
µg/mL**

EUCAST: tobramycin ECV 4 µg/mL

- *Acinetobacter* AHWG Discussion and Recommendations
 - No clinical data for gentamicin or tobramycin
 - Removing breakpoints considered, but did not want to remove options for treatment of *A. baumannii*
 - ECV of 2 µg/mL fits with “borrowed” PK/PD data
 - Given PK and dosing considerations, having gentamicin/tobramycin breakpoints that are 4-fold less than amikacin makes sense
 - Motion to lower susceptible breakpoints for gentamicin and tobramycin 1 dilution to S ≤ 2, I 4, R ≥ 8 µg/mL for *Acinetobacter* spp. AHWG Vote: 6-1-0-0.
- Breakpoints Working Group Discussion and Recommendation
 - Intermediate of 4 µg/mL for gentamicin and tobramycin has a steep PK/PD drop-off, although this is for a 2-log kill target
 - Consideration of leaving the susceptible breakpoint at ≤ 4 µg/mL given indirect PK/PD data
 - Motion to accept the gentamicin and tobramycin MIC breakpoints (S ≤ 2, I 4, R ≥ 8 µg/mL) for *Acinetobacter* spp. based on a dosage of 7 mg/kg/mL. WG Vote: 8-0-1-4 (NOTE: Not a formal vote as there was not WG quorum present).

SC DISCUSSION (MAIN POINTS)

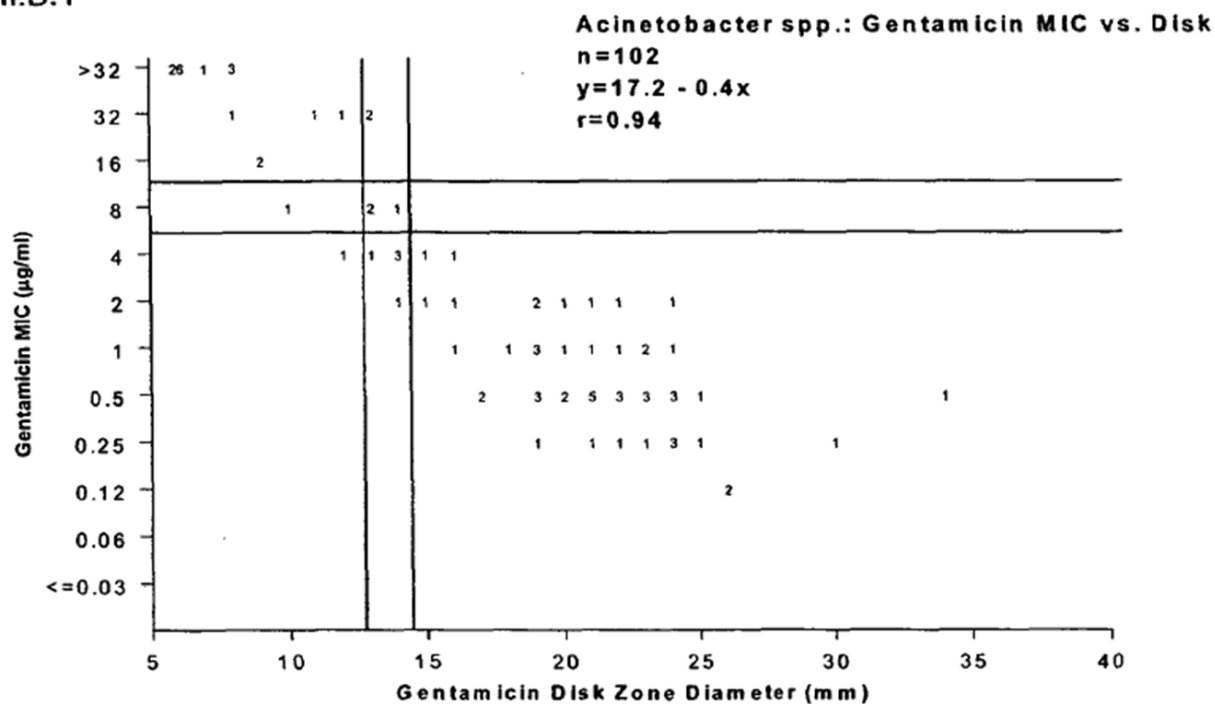
- Why are these drugs in Tier 1? Could they be moved to a different tier, so laboratories do not have to go through the hassle of updating breakpoints.
- The FDA does not currently recognize the gentamicin breakpoint for *Acinetobacter*.
- Consistency is important across the aminoglycosides so need to change these breakpoints.
- South America uses lower doses.
- It would be good for the AHWG to look at the Table 1 drugs for *Acinetobacter*
- The CAP checklist for following updated breakpoints is for FDA breakpoints, not CLSI breakpoints.

A motion to accept the gentamicin and tobramycin MIC breakpoints ($S \leq 2$, $I 4$, $R \geq 8 \mu\text{g/mL}$) for *Acinetobacter* spp. based on a dosage of 7 mg/kg/mL was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

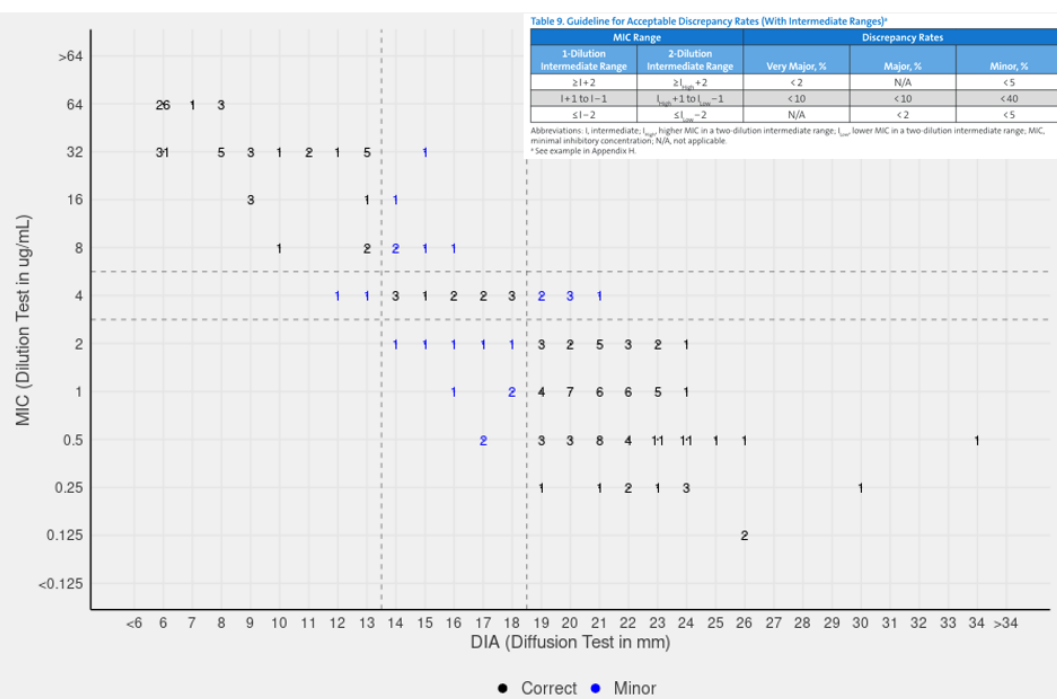
GENTAMICIN DISK DIFFUSION BREAKPOINTS FOR ACINETOBACTER SPP.

- Gentamicin MIC/disk correlates for *Acinetobacter* (2006 Agenda Book)

III.B.1



- dBets Combined Gentamicin 2/4/8 MIC Breakpoints with EUCAST data



≤ 13 R; 14-18 I; ≥ 19 S

	VME	ME	mE
I + 2	0	0	2.4
I +/-1	0	0	36.2
I -2	0	0	4.5

≤ 13 R; 14-17 I; ≥ 18 S

	VME	ME	mE
I + 2	0	0	2.4
I +/-1	0	0	46.3
I -2	0	0	0

- Breakpoints Working Group Discussion and Recommendation

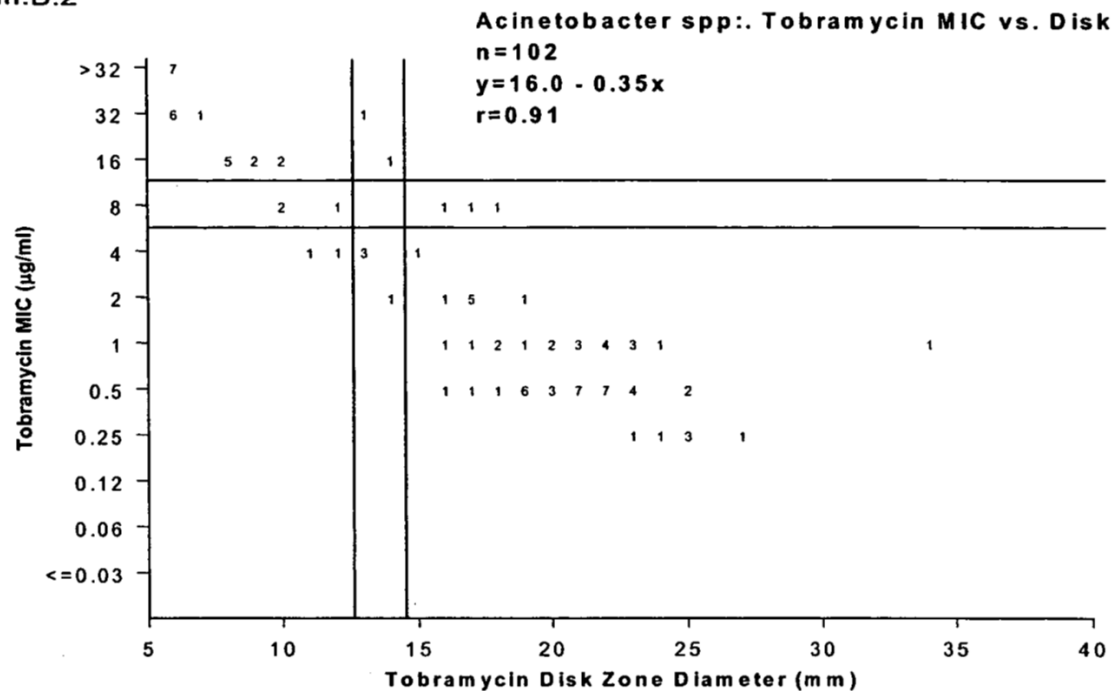
- Motion to accept the gentamicin disk diffusion breakpoints ($S \geq 19$, I 14-18, $R \leq 13$ mm) for *Acinetobacter* spp. WG Vote: 8-0-1-4 (NOTE: Not a formal vote as there was not WG quorum present).

A motion to accept the gentamicin disk diffusion breakpoints ($S \geq 19$, I 14-18, $R \leq 13$ mm) for *Acinetobacter* spp. was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

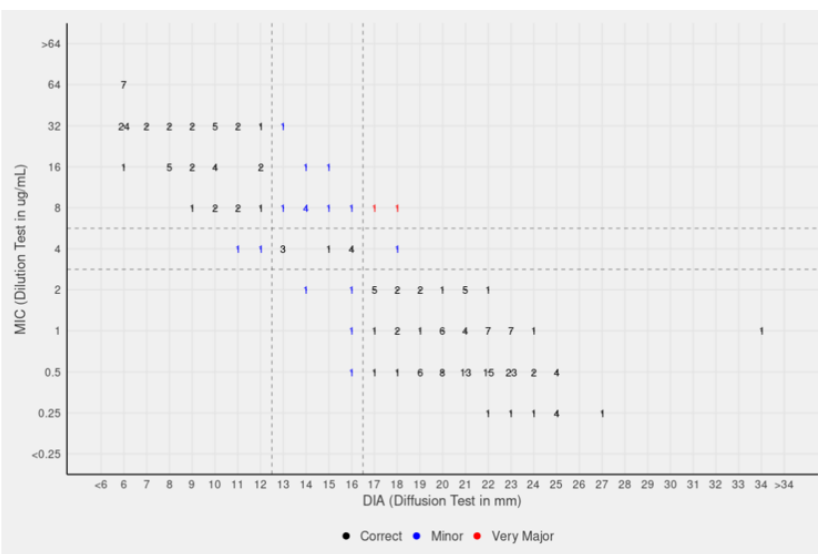
TOBRAMYCIN DISK DIFFUSION BREAKPOINTS FOR ACINETOBACTER SPP.

- Tobramycin MIC/disk correlates for *Acinetobacter* (2006 Agenda Book)

III.B.2



- dBETS suggestion for optimized correlates (combined dataset)
 - Note: *Pseudomonas aeruginosa* ATCC 27853 QC range is 20-26 mm (7 mm)



	S	I	R
MIC	≤ 2	4	≥ 8
Disk	≥ 17	13-16	≤ 12

	n	VME	ME	mE
≥ I+2	62	0 (0%)	NA	3 (4.8%)
I+1 to I-1	44	2 (4.5%)	0 (0%)	12 (27.3%)
≤ I-2	113	NA	0 (0%)	2 (1.8%)

- Breakpoints Working Group Discussion and Recommendation
 - Motion to accept the tobramycin disk diffusion breakpoints ($S \geq 17$, I 13-16, $R \leq 12$ mm) for *Acinetobacter* spp. WG Vote: 8-0-1-4 (NOTE: Not a formal vote as there was not WG quorum present).

A motion to accept the tobramycin disk diffusion breakpoints ($S \geq 17$, I 13-16, $R \leq 12$ mm) for *Acinetobacter* spp. was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

3. ADJOURNMENT

Dr. Mathers thanked the participants for their attention. The meeting was adjourned at 5:30 PM Central Standard (US) time.

2025 JUNE AST MEETING
SUMMARY MINUTES
PLENARY 3: Tuesday, 3 June 2025
7:30 AM - 12:00 PM
Central Standard Time (US)

#	Description
1.	<u>OPENING</u> Dr. Mathers opened the meeting at 7:30 AM Central Standard (US) time.

2. METHODS WORKING GROUP (T. DINGLE AND K. JOHNSON)

EARLY GROWTH AST AD HOC WORKING GROUP REPORT

- Background
 - Traditional AST methods require 18-24 subculture incubation time prior to performing AST
 - Opportunities to reduce the turnaround time of culture incubation before setting up AST
 - Approved at the June 2024 meeting to proceed with AHWG for early growth AST
- Objectives
 - Demonstrate that early growth AST (6 hours) by disk diffusion, gradient strips, and BMD compares well to standard 18-24 hour growth using current breakpoints
 - Gram positive organisms
 - Enterobacterales
 - Nonfermenters
- Study Design: Phase I
 - The Phase I study will assess different commercially available CLSI recommended media, broth, disks and gradient diffusion strips (up to 3 vendors each)
 - Goals:
 - Determine whether performance of all different manufacturers' products are consistent
 - Select a single media, broth, disk and gradient diffusion strip to use in Phase II of testing (clinical isolates)
 - Initial testing of QC organisms will be included to ensure results are acceptable per CLSI standards.
- Study Participants
 - 2 sites
 - Children's Hospital Los Angeles, Los Angeles, CA, USA (CHLA)
 - Keck Medical Center of USC, Los Angeles, CA, USA
 - Each site will perform testing on a different set of organisms
 - CHLA: Gram-negatives
 - Keck USC: Gram-positives
- QC Organisms

<i>Staphylococcus</i>	<i>Enterococcus</i>	<i>Enterobacterales</i>	<i>Pseudomonas</i>
<i>S. aureus</i> , ATCC 29213	<i>E. faecalis</i> , ATCC 29212	<i>K. pneumoniae</i> , ATCC BAA-1705	<i>P. aeruginosa</i> , ATCC 27853
<i>S. aureus</i> , ATCC 25923	<i>E. faecalis</i> , ATCC 51299	<i>E. coli</i> , ATCC 25922	
		<i>E. coli</i> , ATCC 35218	

- Test Organisms

- 12 unique clinical isolates (obtain from ARLN)
- *S. aureus* (2)
- *E. faecium* (1)
- *E. faecalis* (1)
- *E. coli* (2)
- *K. pneumoniae* (1)
- *E. cloacae* complex (1)
- *Pseudomonas aeruginosa* (2)
- *Acinetobacter baumannii* (2)

• Antibiotics Tested

- * MIC testing only

<i>Staphylococcus</i>	<i>Enterococcus</i>	<i>Enterobacterales</i>	<i>Pseudomonas</i>	<i>Acinetobacter</i>
cefoxitin (30 µg)	ampicillin (10 µg)	ampicillin (10 µg)	cefepime (30 µg)	ampicillin-sulbactam (10/10 µg)
clindamycin (2 µg)	ciprofloxacin (5 µg)	cefazolin (30 µg)	ceftazidime (20 µg)	ceftazidime (30 µg)
doxycycline (30 µg)	linezolid (30 µg)	cefepime (30 µg)	ciprofloxacin (5 µg)	cefepime (30 µg)
erythromycin (15 µg)	nitrofurantoin (300 µg)	ceftriaxone (30 µg)	tobramycin (10 µg)	ciprofloxacin (5 µg)
linezolid (30 µg)	vancomycin (30 µg)	ciprofloxacin (5 µg)	meropenem (10 µg)	tobramycin (10 µg)
nitrofurantoin (300 µg)		gentamicin (10 µg)	piperacillin tazobactam (100/10 µg)	meropenem (10 µg)
TMP/SMX (25 µg)		meropenem (10 µg)		piperacillin tazobactam (100/10 µg)
vancomycin (30 µg)*		nitrofurantoin (300 µg)		
		piperacillin tazobactam (100/10 µg)		
		TMP/SMX (25 µg)		

• Phase I Test Method

- BMD: frozen-form panels containing cation-adjusted Mueller-Hinton broth (CAMHB) (Thermo Fisher):
 - CAMHB from 3 different manufacturers: BD Difco, BD BBL, Oxoid
- Mueller-Hinton agar
 - 3 manufacturers: BD, Hardy and Remel
- Disk diffusion:
 - 2 manufacturers: BD, Hardy

- Gradient diffusion:
 - 2 manufacturers: bioMérieux (Etest) and Liofilchem (MTS)
- Quality control:
 - Performed on each day of testing
 - Colony counts will be performed on 3 of the ATCC strains tested
- Summary
 - Completion of Phase I will allow for selection of most appropriate media, disk, broth and gradient diffusion strip to use for Phase II
 - Phase II
 - Multicenter study testing clinical isolates sourced from multiple sites
 - Aim to test one type of media, disk, broth and gradient diffusion strip (as determined by Phase I)
- Methods Working Group Discussion and Recommendation
 - Concern about using a single manufacturer for Phase II
 - This is what was done for the MHF study with *Haemophilus*, so this protocol was mirrored here.
 - Piperacillin/tazobactam disk may be getting adjusted, so keep in touch with Joint CLSI-EUCAST WG
 - Why 6 hours and not another time point?
 - Associated with high agreement in published studies
 - Appropriate for a single shift in a microbiology laboratory
 - Given low number of isolates, any concern about physiological differences within a single species?
 - This would be looked at more thoroughly in Phase II
 - Motion to move forward with the early growth study as proposed. WG Vote: 8-0-1-2.

SC DISCUSSION (MAIN POINTS)

- Please look at longer time points in the Phase I study upfront. This could possibly be done with QC.
- Concern about a single manufacturer in this study. In the MHF study, the MHF was not commercially available.
- Need to document the primary media that isolates are grown on.
- Suggestion to continue reading the plates at multiple time points.
- Consider total laboratory automation incubation. Consider smart incubators.
 - AHWG are considering using a WASP for sub-cultures.
 - The smart incubator is out of scope for this AHWG. This is for a standard method subculture incubation.
- This is a lot of work. Is this something CLSI needs right now?
- This could be helpful for laboratories outside of the United States.
- This should be coordinated with the media manufacturers.
 - Andre Hsuing with Hardy is willing to assist.
- There is a concern where lag vs exponential growth could affect commercial methods such as with the Vitek.
- Consider growing up the isolates in liquid media instead of solid media.
- The piperacillin/tazobactam disk mass will be changing, so probably good to exclude this drug in the Phase I data.

A motion to approve the early growth AST methods phase 1 study as proposed, to consider additional time points for QC, and to return to the Subcommittee for approval for phase 2 was made and seconded. Vote: 12 for, 1 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:

- There needs to be multiple media manufacturers in the Phase I study.

MIC METHODS FOR EPLETTX1 (TELUM THERAPEUTICS)

- Background on EpleTTX1
 - EPLETTX1 is a novel lysin-based antimicrobial targeting *A. baumannii* and other Gram-negative bacteria
 - Challenges observed in MIC assay with standard CLSI CAMHB:
 - Lack of observed activity
 - Trailing end points
 - Protein precipitation
 - Similar issues reported with other lysin therapeutics
- MIC for EpleTTX1 against *Acinetobacter baumannii* NCTC 13304 in different media
 - Dilution of CAMHB to 20% and 10% allowed for the observation of a MIC for EPLETTX1. These lower concentrations compromised bacterial growth and blocked growth for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.
 - CAA medium, composed of 5 g/L bacto casamino acids, 5.2 mM K₂HPO₄, and 50 mL of 1 mM MgSO₄, consistently yielded clear, reproducible results.
 - Moreover, a panel of 63 Gram-negative clinical isolates, including *A. baumannii*, *Escherichia coli*, *K. pneumoniae*, and *P. aeruginosa*, demonstrated robust growth in this medium and reproducible MIC values.
 - Gibco and Formedium casamino acids yield consistent, reproducible MIC results.
- Validation plan for MIC assay in CAA
 - Institutions: Telum Therapeutics + 6 independent laboratories
 - Duration: 3 days
 - Bacterial strain: *A. baumannii* NCTC 13304 (freshly plated for each day experiment)
 - Protein: 1 lot of EpleTTX1
 - Antibiotic for QC: 1 lot of meropenem (one replicate in CAMHB included in each 96-well plate)
 - Media lots: Three CAA media lots: two using Bacto casamino acids from Gibco (lots 1 and 2), and one using Caso casamino acids from Formedium (lot 3); 1 lot of CAMHB for meropenem
 - Number of MIC replicates per laboratory: 3-4 replicates per day with each lot of media (30 in total)
 - Bacteria titration: Each day inoculum titration in CAA and CAMHB (for the QC strain)
- Proposed MIC protocol for EPLETTX1 validation
 - Bacterial Inoculum Preparation
 - Suspend isolated colonies of *A. baumannii* NCTC 13304 (18-24 h freshly plated on TSA) in CAA or CAMHB (for QC purposes).
 - Adjust to a turbidity equivalent to 0.5 McFarland standard (OD₆₂₅ between 0.08 and 0.13).
 - Dilute in the corresponding medium to achieve a final concentration of 2-8×10⁵ CFU/mL in each well.
 - Preparation of Antimicrobial Solutions and 96-Well Plate
 - Prepare meropenem at 2048 µg/mL and EpleTX1 at 256 µg/mL in CAA.
 - Fill column 1 with 200 µL of the diluted antimicrobial; columns 2 to 12 with 100 µL of medium.
 - Perform 1:2 serial dilutions from column 1 to column 11.

- Add 100 µL of the bacterial inoculum to all
- Bacterial Titer Control
 - Check daily to ensure the concentration is within $2-8 \times 10^5$ CFU/mL.
 - Take 10 µL from the growth control, perform serial dilutions in PBS, and plate on TSA.
 - Incubate at 35 ± 2 °C for 16 - 20 hours.
- Incubation and Reading of Results
 - Incubate the plate at 35 ± 2 °C for 16-20 hours, within 15 minutes of adding the inoculum.
 - Read the MIC as the lowest concentration with no visible growth.
 - Verify controls: no contamination in media-only wells, turbid growth control, and meropenem MIC within CLSI range (32-128 µg/mL).
- The only deviation from CLSI Guidelines is the media chosen, CAA instead CAMHB
- Methods Working Group Discussion and Recommendation
 - Joint CLSI-EUCAST protocol on variations from the reference method has not been finalized
 - Why did MICs vary significantly between CAA manufacturer?
 - Unknown. MICs could only be obtained with CAA from Gibco and Foremedium
 - Manufacturer was encouraged to understand why these differences occur.
 - Have any strains been identified that fail therapy in the murine model?
 - No resistant strains have been identified
 - Methods WG agreed that both susceptible and resistant strains are required to fine tune the method
 - Is the lysin sticking to the plate?
 - It is not.
 - Is there any other way of seeing activity? Is there another method that could be used to detect non-susceptibility?
 - Microscopically, you can see cells lysing and there is a reduction in CFU in time kill experiments.
 - PH-based hydrolysis assay?
 - What about the exelastase method (also a lysin)?
 - Will not work as this is a completely different lysin and a completely different mechanism of action.

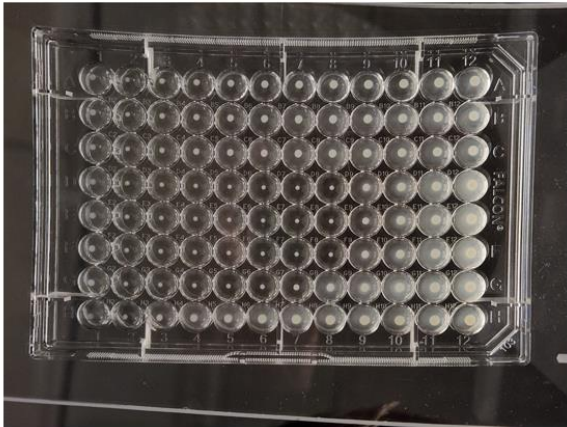
SC DISCUSSION (MAIN POINTS)

- What are the next steps?
 - The sponsor needs to understand the media manufacturer difference and they need to have both resistant and susceptible isolates.

CEFAZOLIN INOCULUM EFFECT AD HOC WORKING GROUP REPORT

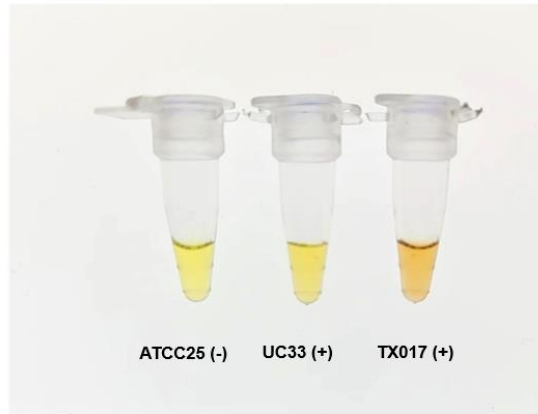
- Background
 - Cefazolin clinical failures have been reported for deep-seated methicillin susceptible *Staphylococcus aureus* (MSSA) infections, particularly infective endocarditis
 - Cefazolin failure observed in isolates with inoculum effect (CzIE)
 - Recent studies found an association of the CzIE with treatment failure and mortality.
 - Phenotype NOT detected by routine susceptibility testing
 - Gold standard assay: BMD at standard inoculum (10^5 CFU/mL) and high inoculum (10^7 CFU/mL)
 - An increase in cefazolin MIC to ≥ 16 µg/mL with the high inoculum is considered positive for CIE

- Availability of a rapid test for the CzIE phenotype could positively impact treatment decisions for CzIE-positive MSSA in select clinically appropriate scenarios (ie, high inoculum infections such as endocarditis)
- Objectives
 - PHASE 1: Assess the prevalence of CzIE phenotype in MSSA isolates in contemporary US strains. -> Done
 - PHASE 2: Evaluate the revised rapid CzIE assay. Assess suitability for multi-center evaluation. -> Done
 - PHASE 3: Perform multi-center evaluation of the revised rapid CzIE assay.
- Multicenter Study: Design and Logistics
 - Study Protocol: The Houston and Colombia research teams have formalized the protocol for the rapid CzIE test for use in the multicenter study.
 - Methodologies to Detect Cefazolin Inoculum Effect (courtesy of Jacob Dziadula)



High Inoculum Minimum Inhibitory Control Assay

- Gold standard
- Time and labor intensive
 - Takes 3 days

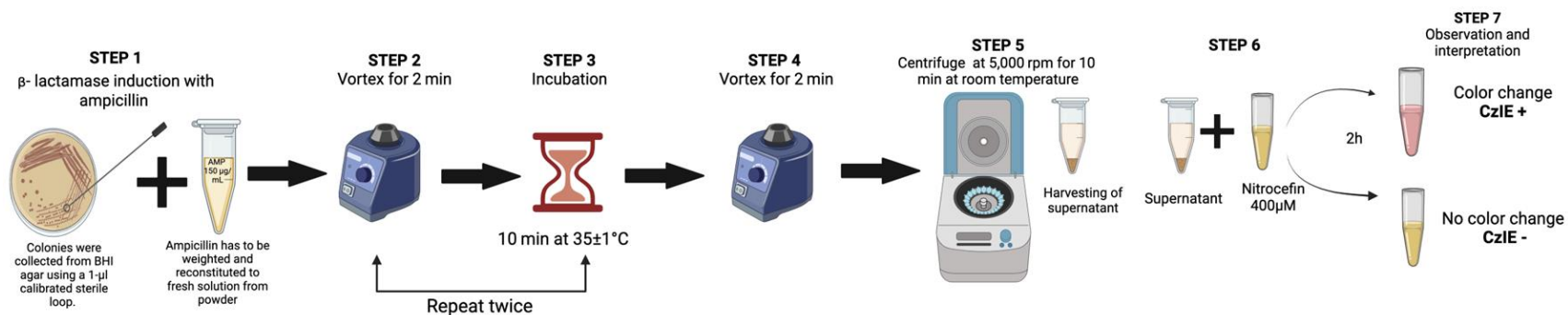


Nitrocefin-based Colorimetric Rapid Test

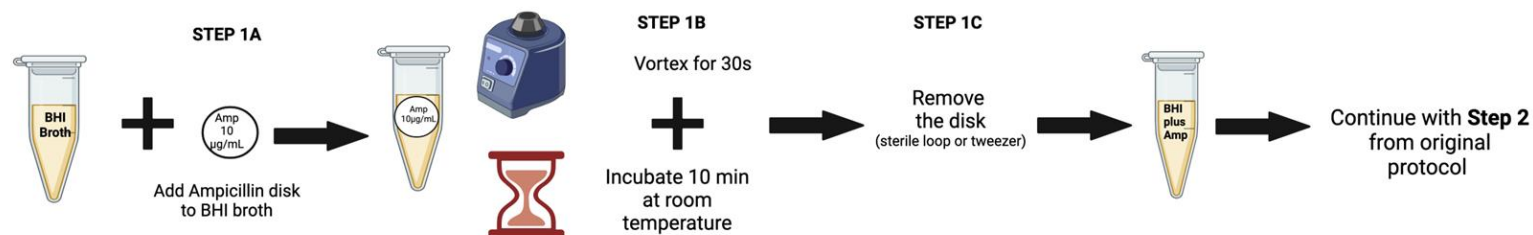
- Easy to perform and inexpensive
- Quick: ~3hrs for results

- Modified Rapid Colorimetric Test (Carvajal LP et al, AAC 2024)

Original Protocol: Nitrocefin Rapid Test



Modification Step 1 from original protocol



- Confirm study sites: CHLA, DEA, UAH, USC confirmed. Washington U pending.
- Isolate selection (all currently frozen at CHLA)

MIC High Inoculum	Number of Isolates	Percent of Isolates	CzIE +	blaZ Type	# of Isolates
≥16	55	51.4		A	24
8	20	18.7		C	29
4	7	6.5		D	1
2	3	2.8			
1	7	6.5			
0.5	5	4.7			
0.25	10	9.3			
SUM	107				

- Distribution of isolates/controls - from CHLA
- Quality control - 3 strains (TX17, UC33, ATCC 29213) - each day of testing with corresponding media/disks used for test strains
- Supplies

Material/Reagent	Manufacturer	Cost/Donation
Sterile BHI broth	Oxoid	
	BD	
Ampicillin disks	Oxoid	
	BD BBL	
DMSO	Thermo	
Nitrocefin	Thermo	
PBS	Thermo	
Eppendorf Tubes	-	Supplied by study site
0.2mL Tubes	-	Supplied by study site
1uL calibrated sterile loops	-	Supplied by study site

107 x 4 =
428
results/site

- Media Manufacturer Affects Performance of Rapid CzIE Test
 - Data courtesy of Sara Gomez-Villegas (CLSI June 2022 Meeting Materials)
 - Oxoid BHI appeared to perform better than BD BHI
 - Comparator: Standard and high inoculum cefazolin BMD
 - Comparator Definition of CzIE positivity: High inoculum MIC ≥16 µg/mL, standard inoculum MIC ≤ 8 µg/mL

	BD BHI	Oxoid BHI
Sensitivity	69.8%	96.8%
Specificity	96.7%	90.3%
FN Rate	38.8%	6.6%
FP Rate	2.2%	4.7%

- Methods Working Group Discussion and Recommendation
 - This assay needs clinical trial data to show that this is important clinically. Is the work premature? Need prospective RCT data.
 - Data currently available is retrospective.
 - SNAP trial is ongoing and could potentially answer some of these questions.
 - Concern over color change for Type C BlaZ control strain (UC33) and ability of laboratories to detect this change
 - Training on the subtleties of the colour change will be important. Have had success with implementing this assay in a clinical laboratory in Colombia.
 - Type A BlaZ more associated with clinical failures than Type C, and Type A CzIE is more reproducible in the test.
 - Have been discussing this for some time: How many laboratories would actually use this test? With challenges with color change, not sure this can be an M100 method. Current data do not support this test for clinical practice.
 - Motion to not further pursue a CzIE method due to lack of data and difficulties in reading. WG Vote: 6-2-1-2 (Did not pass).
 - Encouraged to continue pursuing this work outside of the CLSI framework.

SC DISCUSSION (MAIN POINTS)

- There was a recent systematic review that showed the cefazolin inoculum effect does not have a clinical impact and it is hard to read tests like this.
- There were four publications. There was a meta study but it really only included two papers.
- There is a difference in the endocarditis patients, so there is not enough data to make a conclusion.
- The type A's have a clear color change and the type C's are hard to read.
- The SNAP subset that looked at endocarditis did not show a difference between patients treated with cefazolin versus those that were not. However, inoculum effect wasn't specifically evaluated. Given volunteer time constraints, CLSI is hesitant to push this forward at this time.
- There are higher rates of the inoculum effect in other countries, particularly in South America.
- If the assay incubation is extended, does that improve the color change?
 - If the assay is held longer, then false positives are seen.
- The decision to make is to either pause the work, or discontinue it and disband the AHWG.

A motion to discontinue the high cefazolin inoculum effect method study and disband the ad hoc working group until more data is available was made and seconded. Vote: 10 for, 3 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:

- There has been a lot of work with this group. It would be better to pause rather than disband to keep people involved and given credit for the work already done.

RIFABUTIN REFERENCE SUSCEPTIBILITY TESTING METHOD AGAINST *ACINETOBACTER BAUMANNII*

- A subgroup of Methods WG members/advisors have been meeting with EUCAST to provide joint responses to BioVersys on their rifabutin method.
- Most recent data requests sent on 19 May and a CLSI/EUCAST/BioVersys meeting is set for 10 June.

CEFIDEROCOL AD HOC WORKING GROUP REPORT

- Topics of Discussion

Topic	Update
Cefiderocol QC strain for Iron-Depleted media	• Discussed during QCWG Plenary Summary
Disk Mass Studies	• Discussed briefly during Joint CLSI-EUCAST Plenary Summary • Work is ongoing

- Identification of Possible QC Candidate Strains

- Testing of three additional media sources showed MIC differences between ID-CAMHB and CAMHB for Hardy* and Teknova** medium, but not for HiMedia

Species	Strain	Source	Media	Cefiderocol MIC (µg/mL) in ID-CAMHB or CAMHB												
				0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32		
<i>P. aeruginosa</i>	SR27001 (IMP-1)	In-house PK/PD	BD BBL						3		2	1				
			BD Difco							3		2	1			
			Hardy*					3			3					
			Teknova**		3					3						
			HiMedia									3	6 3			
<i>P. aeruginosa</i>	1606608 (IMP-7) JMI1134143	SIDERO-WT	BD BBL						3		3					
			BD Difco							3		3				
			Hardy*				3			2	1					
			Teknova**		3					3						
			HiMedia								3	3	3 3			
<i>K. pneumoniae</i>	ATCC BAA-2814 (KPC-3)	ATCC	BD BBL						1	2	1	2				
			BD Difco							3		3				
			Hardy*					3		1	2					
			Teknova**		3					3						
			HiMedia								2 1	4 5				
<i>K. pneumoniae</i>	AR Bank 550 JMI 1061995	AR Bank	BD BBL						3		2	1				
			BD Difco							3		2	1			
			Hardy*						3		3					
			Teknova**				1	2		3						
			HiMedia									2	1 2	3 4		

* *E. coli* ATCC 25922 QC out of range **Showed out of range QC results and poor intra-lab reproducibility amongst different lots

ID-CAMHB: iron-depleted cation adjusted Mueller-Hinton broth; CAMHB: cation adjusted Mueller-Hinton broth



- P. aeruginosa* SR27001 MIC Across Different Media and Lots

Manufacturer Lot# (Test Site)		N	Cefiderocol MIC (µg/mL) in ID-CAMHB or CAMHB										
			≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
BD BBL	0252334 (SHQ)	33, 3						33			3		
	9015952 (SHQ)	3, 3						3			3		
	9324795 (SHQ)	3, 3						1	1		1, 3		
	3131124 (SHQ)	12, 12					3	9		10	2		
	2343309 (JMI)	3, 3						3		2	1		
	All	54, 24					3	49	1	12	1, 12		
BD Difco	0245118 (SHQ)	33, 3						1	32				3
	1123327 (SHQ)	3, 3							3		2		1
	9156832 (SHQ)	3, 3							3				3
	1285575 (SHQ)	12, 12							4	8			12
	0314278 (JMI)	3, 3							3		2		1
	All	54, 24						1	45	8	4		20
Oxoid	2216267 (SHQ)	3, 3								2	1		3
	30000775 (SHQ)	33, 3								11	22		3
	3555379 (SHQ)	12, 12								7	5		12
	All	48, 18								20	28		18
Merck	BDDF7733 (SHQ)	30, NT						30*					
	BCCF8485 (SHQ)	12, 12	1	5	1, 3	4, 7		1, 2					
	All		1	5	1, 3	4, 7		31*, 2					
HiMedia	0000349752 (JMI)	6, 6									3	6, 3	
Hardy	524817 (JMI)	3, 3					3		1	2			
Teknova	M588720B2301 (JMI)	3, 3		3				3					

• Cefiderocol AHWG Discussion and Recommendation

- Proposed solution could be to add language that this strain can be used for troubleshooting in the IDMHMB media preparation instructions and that results should be 2 dilutions higher in CAMHB compared to IDCAMHB but that each user needs to determine their baseline values
- This strain has not been deposited on ATCC or other repositories, but Shionogi is exploring this
- Other QC strains do not show consistent or large enough differences in CAMHB vs IDCAMHB
- Participants agreed that the cefiderocol disk mass should be re-evaluated
- Erika Matuschek is performing a study with 30 ug disks cut in quarter and half
- JMI is doing a phase 1 study for mass determination (10 disk masses and 130 isolates including 50 *A. baumannii-calcoaceticus* species complex)

SC DISCUSSION (MAIN POINTS)

- Is it intended for media manufactures to use routinely or in troubleshooting?
 - It is meant to be for troubleshooting.

- Laboratories are not going to make their own media, so this is something that could be done by an end user.

INTRINSIC RESISTANCE DEFINITION AD HOC WORKING GROUP REPORT

- Objectives
 - Revisit the CLSI definition of intrinsic resistance (IR) for possible adjustments
 - Attempt to standardize the definition amongst Subcommittees on Antifungal Susceptibility Tests, AST, and Veterinary AST
 - Explore a concept of “reduced susceptibility” when IR not achieved but antimicrobial should not be used
- Background
 - First meeting April 2024
 - Discussions prior to 2024 at Subcommittee on Antifungal Susceptibility Tests meetings noted differences between the concepts of IR and “reduced susceptibility” (when IR was not achieved by strict definition)
 - Antifungal IR AHWG had adopted the bacterial IR definition and had been using that for a few years to define and apply IR
 - EUCAST representation on IR Definition AHWG
- Why revisit the IR definition?
 - Recent application of the IR definition by the Subcommittee on Antifungal Susceptibility Tests has brought up questions regarding the IR definition (terminology and arbitrariness of 97% cutoff, among others)
 - Recent CLSI M45 discussions concerning certain antimicrobial agent-organism combinations which shouldn’t be used clinically but don’t quite fit the IR definition
 - EUCAST recently revisited their definition of IR (stopped using the term January 2023)
- Current Intrinsic Resistance Definition (Appendix B, M100 35th Edition)

Intrinsic resistance is defined as inherent or innate (not acquired) antimicrobial resistance, which is reflected in the wild-type antimicrobial patterns of all or almost all representatives of a species. Intrinsic resistance is so common that susceptibility testing is unnecessary. For example, *Citrobacter* spp. are intrinsically resistant to ampicillin.

These tables can be helpful in at least three ways: 1) they provide a way to evaluate the accuracy of testing methods; 2) they aid in recognition of common phenotypes; and 3) they can assist with verification of cumulative antimicrobial susceptibility test data. In the tables, an “R” occurring with an antimicrobial agent-organism combination means that strains should test resistant. A small percentage (1% to 3%) may appear susceptible due to method variation, mutation, or low levels of resistance expression.

Each laboratory should decide which agents to test and report in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. If tested, the result for an antimicrobial agent-organism combination listed as having intrinsic resistance should be reported as resistant. Consideration may be given to adding comments regarding intrinsic resistance of agents not tested.
- Recap of January 2025 AST SC IR Discussions
 - A revised IR definition was proposed by the IR AHWG at the AST Subcommittee
 - But the revised IR definition had not been included in the agenda materials for AST Subcommittee meeting and therefore did not proceed to voting
 - Main proposed changes by the IR AHWG to the definition included:
 - Allowance to consider PK/PD and to consider clinical efficacy data when determining IR
 - Removal of the percentage cutoff since the cutoff was arbitrary

- Removal of the words “resistant” and “susceptible” in the definition (since no breakpoints exist, one cannot truly use those terms)
- IR Definition Proposed at the January 2025 Meeting

Intrinsic resistance (IR) is defined as inherent or innate (not acquired) antimicrobial resistance, evidenced by high MIC or reduced zone diameter values for specific antimicrobial agent-organism combinations for all or nearly all isolates ($\geq 90\%$) of a microbial species or organism group. The MIC distribution for antimicrobial agent-organism combinations exhibiting IR generally displays a high modal MIC well above the expected clinically achievable antimicrobial concentrations.

IR may also include antimicrobial agent-organism combinations for which available PK/PD data show insufficient antimicrobial exposure or when available clinical data demonstrate lack of efficacy.

Susceptibility testing is unnecessary for organisms considered intrinsically resistant to an antimicrobial. However, if testing is performed, they should be reported as resistant or intrinsically resistant. MIC and zone diameter values should not be reported as some isolates may exhibit low MIC or wide zone diameter values due to method variation, mutation or low levels of resistance gene expression.
- Methods Working Group and Plenary Discussions at AST Subcommittee January 2025 Meeting
 - Practical application of the proposed definition will be difficult
 - Evaluating MIC results without a defined % cutoff is difficult
 - Inclusion of the PK/PD component and the clinical outcome component in the definition makes applying the proposed definition difficult
 - PK/PD will differ depending on the patient population and body site
 - PK/PD should not be included (strongly voiced by some people). Should be independent of site of infection. Intrinsic resistance is a characteristic of the organism.
 - However, some felt that including PK/PD is important to consider when determining IR, especially for clinical treatment failures for antimicrobial agent-organism combinations that do not fit into the IR definition as it currently stands (eg, *P. aeruginosa* and gentamicin)
 - Why does the current definition even need updates?
 - Per CLSI M45 Chairholder feedback: there are concerns for some antimicrobial agent-organism combinations where most MICs are high and fall above achievable therapeutic concentrations... but they are not IR by the current published definition in M100.
 - Intrinsic resistance is an inherent characteristic of the organism.
 - Does the definition of “reduced susceptibility” being discussed by the Subcommittee on Antifungal Susceptibility Tests (see Jan 2025 AST Subcommittee meeting minutes update by the Antifungal Susceptibility Tests Subcommittee) meet some of these needs for further clarification?
 - Could consider 2 definitions - one for intrinsic, and one for when the antimicrobial does not work clinically
 - Please do not make this too complicated for laboratories
 - Final decision of AST Subcommittee: could not reach consensus on proposed definition
 - Final recommendation of AST SC: AHWG should add background on the definition and add examples. Test example organisms to determine how they stand up to the proposed definitions.
- IR Definition AHWG Discussion and Recommendation
 - Maintain one concept (eg, IR or something similar) rather than introducing yet another category for laboratories to use for reporting
 - Standardization is important to set a definition across subcommittees
 - Cutoff of 97% is arbitrary; what about 90% or 95%?
 - MICs for many of these organism/antimicrobial combinations have not been published much in the literature. It is hard to obtain enough isolates for assessment, and the 97% cutoff makes it difficult to qualify for IR

- EUCAST uses 90%
- How much of a driver of this definition should clinical use of the drug be?
 - *In vivo* intrinsic resistance (ie, clinical treatment failures/PK/PD) [example may be ceftriaxone vs. *P. aeruginosa*] vs *in vitro* intrinsic resistance (ie, based on MIC distributions and resistance mechanisms; a property which is “in the bones of the organism - whether veterinary or human”)
- Is it okay to report something as resistant if you haven’t even tested it? AHWG believes that it is (and current IR definition supports this)
- Proposed expected resistance definition:

The concept of “expected resistance” (ER) includes both intrinsic resistance (IR), which is a property inherent to an organism, and expected clinical failure (ie, treatment failure or insufficient antimicrobial exposure in a host species). Antimicrobial agent-organism combinations qualify for ER if they display either IR or expected clinical failure.

IR is defined as inherent or innate (not acquired) antimicrobial resistance, evidenced by high MICs or reduced zone diameter values for specific antimicrobial agent-organism combinations for all or nearly all isolates ($\geq 90\%$) of a microbial species or organism group (e.g. species complex). The MIC distribution for an antimicrobial agent-organism combination exhibiting IR generally displays a high modal MIC.

Expected clinical failure includes antimicrobial agent-organism combinations for which available PK/PD data show insufficient antimicrobial exposure at clinically achievable concentrations, or when available microbiologic or clinical data demonstrate lack of efficacy against a species *in vivo*.

Antimicrobial susceptibility testing (AST) is unnecessary for organisms considered to have ER to an antimicrobial agent. However, if testing is performed, the AST result should be suppressed or reported as resistant. MIC and zone diameter values should not be reported, as some isolates may exhibit low MICs or large zone diameters despite lack of *in vivo* utility of the antimicrobial agent.

- Advantages of Expected Resistance Definition
 - Umbrella term that encompasses intrinsic resistance and expected clinical failure
 - This definition is inclusive of antimicrobial agent/organism combinations which should not be used clinically but which did not quite meet the criteria of the IR definition currently published in M100
 - Clearer definition of IR is included
 - These combinations should be reported as “resistant”
- Definition Comparison

M100 Intrinsic Resistance Definition (Currently Published)	Proposed Revision of Expected Resistance
Only intrinsic resistance addressed	Includes intrinsic resistance and expected clinical failure
Does not address PK/PD or clinical failure; restricted definition	Addresses PK/PD and clinical failure for more comprehensive assessment of clinically applicable failure of an antimicrobial agent
Includes wording “susceptible” and “resistant” despite lack of breakpoints	Descriptive use of values or MICs without using terms associated with breakpoints (e.g., “displays a high modal MIC”)
Cutoff of 97%	Cutoff of 90%
States that IR is seen in “almost all representatives of a species”	Further clarifies by stating “all or nearly all isolates of a species or organism group (eg, species complex”
Addresses only MICs	Includes MICs and zone diameter values
Report as “resistant”	Report as “resistant”

- Future item: Each subcommittee will reevaluate their own antimicrobial agent/organism combinations which did not meet the original definition of IR and will update their tables accordingly.
- Methods Working Group Discussion and Recommendation
 - Agree with reporting as resistant (R) but will the table distinguish between IR and expected clinical failure?
 - Source specific should not be added to the table (eg, nitrofurantoin and urine).
 - New definition aligns with CLSI M45.
 - 90% is arbitrary. Aligns with EUCAST and is seen in some package inserts on not utilizing a drug for certain species.
 - Not reporting the MIC had some concerned, mainly LIS/manufacture issues.
 - Motion to accept the expected resistance definition but will not be placed in M100 until the tables are reconciled. WG Vote: 9-1-1-1.

SC DISCUSSION (MAIN POINTS)

- What are examples of clinical failure?
 - The aminoglycosides do not get into the human cells for *Salmonella* and *Shigella*.
- What about *Enterobacter* and cefuroxime? In places where there is no MALDI, need to check the identification.
- There might be value in calling out intrinsic resistance vs. expected clinical failure for laboratories.
- CLSI should use intrinsic resistance and expected clinical failure as they apply. Use intrinsic resistance in the intrinsic resistance tables and then use expected clinical failure when it applies.
- What about ertapenem and *P. aeruginosa*? The modal MIC is 4, so can give 6 µg to treat it. Cannot give that much ertapenem to a patient.
- Intrinsic resistance is a quality check for the laboratory.
- Moxifloxacin eye drops work for *P. aeruginosa* because of the concentration. But would not work for a systemic infection. CLSI needs to specify this information is for systemic infections.
 - Already have guidance for the lab in Table 1.

- Laboratories use the intrinsic resistance and need guidance. For *E. coli* and doxycycline, the organism is resistant in a dog. But if humans get the same organism, it is susceptible.
- For the Antifungal subcommittee, the intrinsic resistance definition is not working. The intrinsic resistance table will not go away. It will add additional bug/drug combinations that should not be used. It is important for laboratories to convey the clinical failures to providers.
- It is good to have laboratories differentiate intrinsic resistance vs. clinical failure.
- This is a good opportunity to apply a consistent definition. The clinical failure is something that can be re-evaluated as doses change over time.
- This will not go into M100 36th Edition, this needs to be approved by VAST and Antifungal Subcommittees.
- Expected clinical failure is harder to prove.

A motion to accept the expected resistance definition was made and seconded. Vote: 10 for, 3 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:

- 90% has a problem. It gets dicey to say expected clinical failure when really trying to say, “do not report”.
- There is no discussion for laboratories on how to check their work.
- The language could be tweaked.

NOTE: The expected resistance definition will not be placed in the M100 36th Edition. Definition is pending approval from the Subcommittee on Antifungal Susceptibility Tests and the Subcommittee on Veterinary AST.

INTRINSIC RESISTANCE AD HOC WORKING GROUP REPORT

- As the definition of intrinsic resistance changes, there are some discussion items:
 - Apply warnings for *Salmonella/ Shigella* in table as “R”, the same as we currently do for Enterococci
 - Should there be a difference between systemic and urinary if breakpoints are only for one? (example of ampicillin and *E. coli/P. mirabilis* - there are others)
 - What about removal of breakpoints? (example of *P. aeruginosa* and gentamicin) Should this be R?
 - What about a “commonly confused” antibiotic?
 - From a microbiology perspective, could/should the human and vet Appendices list the same bugs if both could be found?
- Apply warnings for *Salmonella/ Shigella* in table as “R”
 - Current Appendix B for *Enterococcus*, which get an R with their warnings.

Appendix B. (Continued)

B4. *Enterococcus* spp.

Antimicrobial Agent →	Cephalosporins	Vancomycin	Teicoplanin	Aminoglycosides	Clindamycin	Quinupristin-dalfopristin	Trimethoprim	Trimethoprim-sulfamethoxazole	Fusidic acid
Organism ↓									
<i>E. faecalis</i>	R ^a			R ^a	R ^a	R	R	R ^a	R
<i>E. faecium</i>	R ^a			R ^a	R ^a		R	R ^a	R
<i>E. gallinarum</i> / <i>E. casseliflavus</i>	R ^a	R		R ^a	R ^a	R	R	R ^a	R

Abbreviation: R, resistant.

Footnote

- a. **WARNING:** For *Enterococcus* spp., cephalosporins, aminoglycosides (except for high-level resistance testing), clindamycin, and trimethoprim-sulfamethoxazole may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.

- Current Appendix B, note that *Salmonella* and *Shigella* have a warning but no R for aminoglycosides, 1st and 2nd generation cephalosporins, and cephamycins

CLSI M100-Ed35

Appendix B. (Continued)

B1. Enterobacterales (Continued)

Antimicrobial Agent →	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporins II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
Organism ↓													
<i>Proteus vulgaris</i>	R				R		R ^d	R	R	R	R		
<i>Providencia rettgeri</i>	R	R			R		R ^d	R	R	R	R		
<i>Providencia stuartii</i>	R	R			R		R ^d	R	R	R	R		
<i>Raoultella</i> spp. ^f	R			R									
<i>Salmonella</i> and <i>Shigella</i> spp.	There is no intrinsic resistance to β-lactams in these organisms; refer to WARNING below for reporting.												
<i>Serratia marcescens</i>	R	R	R		R	R	R				R	R	
<i>Yersinia enterocolitica</i>	R	R		R	R								

Abbreviations: AST, antimicrobial susceptibility testing; MIC, minimal inhibitory concentration; R, resistant.

WARNING: For *Salmonella* and *Shigella* spp., aminoglycosides, first- and second-generation cephalosporins, and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.

- *Salmonella* and *Shigella* line could just match *Enterococcus* for warning. R with correct footnote for the warning.

For Use With CLSI M02 and CLSI M07

Organism	Ampicillin	Amoxicillin-clavulanate	Ampicillin-Sulbactam	Cephalosporin I	Cephamecins	Cephalosporin II		Aminoglycosides (AK, GM, TO)
Salmonella and Shigella spp.	There is no intrinsic resistance to β -lactams in this organism.			R*	R*	R*		R*

WARNING: For *Salmonella* and *Shigella* spp., aminoglycosides, first- and second-generation cephalosporins, and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.

- Methods Working Group Discussion and Recommendation
 - The same information is also found in Table 1A
 - Make it clear and match *Enterococcus* spp. table
 - Although not currently the IR definition, it will match with the new definition which will not updated until 2027.
 - Motion to include R for cephalosporins, cephamycin, and aminoglycosides for *Salmonella/Shigella*. WG Vote: 9-0-1-2.

A motion to include the resistance (R) for cephalosporins, cephamycin, and aminoglycosides for *Salmonella/Shigella* with a footnote warning in the intrinsic resistance table and Table 1A was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

NOTE: The edits in the motion above will not be placed in the M100 36th Edition. The decision was made to wait to include with the new expected resistance definition revisions.

INTRINSIC RESISTANCE AD HOC WORKING GROUP REPORT CONTINUED

- Example of when breakpoints only work for certain organisms and certain sources
 - The breakpoints for ampicillin are only for uncomplicated UTIs due to *E. coli* and *P. mirabilis*; should other sources be R?
 - For clinical resistance, maybe adding some wording when specimen source needs to be considered

B1. Enterobacterales

Antimicrobial Agent →	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamecins: Cefoxitin, Cefotetan	Cephalosporins II: Cefuroxime
Organism ↓							
<i>Citrobacter freundii</i>	R	R	R		R	R	R
<i>Citrobacter koseri</i> , <i>Citrobacter amalonaticus</i> group ^a	R			R			
<i>Enterobacter cloacae</i> complex ^b	R	R	R		R	R	
<i>Escherichia coli</i>	There is no intrinsic resistance to β -lactams in this organism.						

Ampicillin	10 µg	≥ 17	—	14–16 [^]	≤ 13	≤ 8	—	16 [^]	≥ 32	(5) Results of ampicillin testing can be used to predict results for amoxicillin. (6) Breakpoints when oral ampicillin is used are only for therapy of uncomplicated UTIs due to <i>Escherichia coli</i> and <i>Proteus mirabilis</i> .
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Organism	Ampicillin	Amoxicillin-clavulanate	Ampicillin-Sulbactam	Cephalosporin I	Te
<i>Escherichia coli</i>	Clinically R from sources other than uUTI			Cefazolin....	
<i>Escherichia coli</i>	There is no intrinsic resistance to penicillins and Cephalosporins I if source is uUTI				

- Example of when breakpoints are removed

<i>Pseudomonas aeruginosa</i>				
Amikacin	March 2023 (M100-Ed33)	X	X	Report only on organisms isolated from the urinary tract
Ciprofloxacin	January 2019 (M100, 29th ed.)	X	X	
Colistin	January 2017 (M100, 27th ed.)		X	
	January 2020 (M100, 30th ed.)		X	
Gentamicin	March 2023 (M100-Ed33)			Removed disk diffusion and MIC breakpoints

B2. Non-Enterobacterales

Antimicrobial Agent →	Ampicillin, amoxicillin	Piperacillin	Ticarcillin	Amoxicillin-sulbactam	Amoxicillin-clavulanate	Piperacillin-tazobactam	Cefotaxime	Ceftazidime	Cefepime	Aztreonam	Imipenem	Meropenem	Ertapenem	Polymyxin B	Colistin	Aminoglycosides	Tetracyclines	Tigecycline	Trimethoprim	Trimethoprim-sulfamethoxazole	Chloramphenicol	Fosfomycin
Organism ↓																						
<i>Acinetobacter baumannii</i> / <i>Acinetobacter calcoaceticus</i> complex	R				R					R			R						R		R	R
<i>Burkholderia cepacia</i> complex ^a	R	R	R	R	R	a	a	a	a	a	a		R	R				a				R
<i>Pseudomonas aeruginosa</i>	R			R	R		R	R					R				R	R	R		R	
<i>Stenotrophomonas maltophilia</i>	R	R	R	R	R	R	R	R		R	R	R	R	R		R	b		R			R

Abbreviations: MIC, minimal inhibitory concentration; R, resistant.

- Example of an inappropriate antibiotic that are commonly confused..."Will clindamycin work for that *E. coli*?"

Appendix B. (Continued)

NOTE 1: Cephalosporins III, cefepime, cefiderocol, aztreonam, ticarcillin-clavulanate, piperacillin-tazobactam, imipenem-rele meropenem-vaborbactam, and carbapenems are not listed because there is no intrinsic resistance in Enterobacterales.

NOTE 2: Enterobacterales are also intrinsically resistant to clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin, teicoplanin, telavancin), linezolid, tedizolid, quinupristin-dalfopristin, rifampin, and macrolides (erythromycin, clarithromycin). There are some exceptions with macrolides (eg, *Salmonella* and *Shigella* spp. with azithromycin).

- From a microbiology perspective, should the human and VET Appendixes list the same bugs if they could be isolated?

<i>Escherichia hermannii</i>	(in M100, not VETSED07)
<i>Providencia rettgeri</i>	(in M100, not VETSED07)
<i>Yersinia pseudotuberculosis</i>	(in VETSED07 not M100)

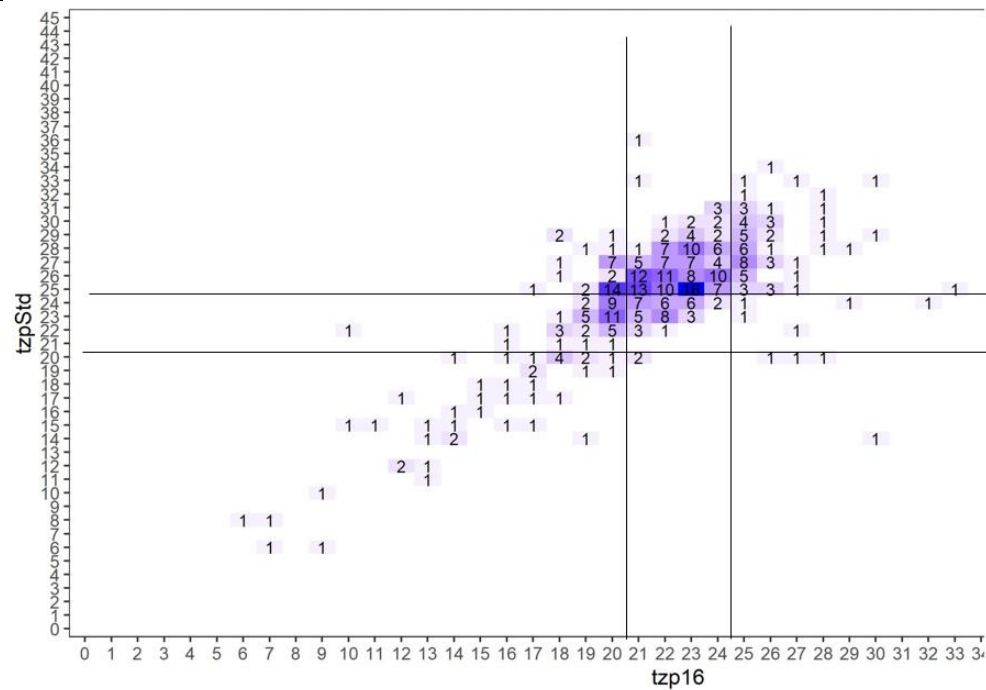
DIRECT DISK DIFFUSION SUSCEPTIBILITY TESTING AD HOC WORKING GROUP REPORT

- Objectives
 - Define disk diffusion breakpoints for applicable gram-negative rods direct from positive blood culture bottle broth
 - 16-18h (overnight reads)
 - 8-10h (early reads)
 - Review data from:
 - Direct Susceptibility Testing of Gram-negative Rods from Blood Cultures (ARLG DISK Study) - clinical isolates
 - Seeded isolate testing
- Piperacillin-tazobactam
 - In the past, reviewed performance of direct disk for piperacillin-tazobactam with old breakpoints (unable to set direct disk breakpoints at that time due to poor categorical agreement and high error rates)
 - In April 2025, reviewed performance of direct disk at 16-18h and at 8-10h with updated breakpoints for Enterobacterales and *P. aeruginosa*
 - Performance of direct disk was unacceptable due to high error rates for all except Enterobacterales at 16-18h
- Piperacillin-tazobactam 16-18h vs. Std DD Enterobacterales

	Std DD			
16-18 hr	S	SDD	R	Grand Total
S	65	5	4	74
SDD	149	41	2	192
R	32	44	43	119
Grand Total	248	90	49	385

CA	149/385	38.7%
VME	4/49	8.2%
ME	32/248	12.9%
mE	200/385	51.9%

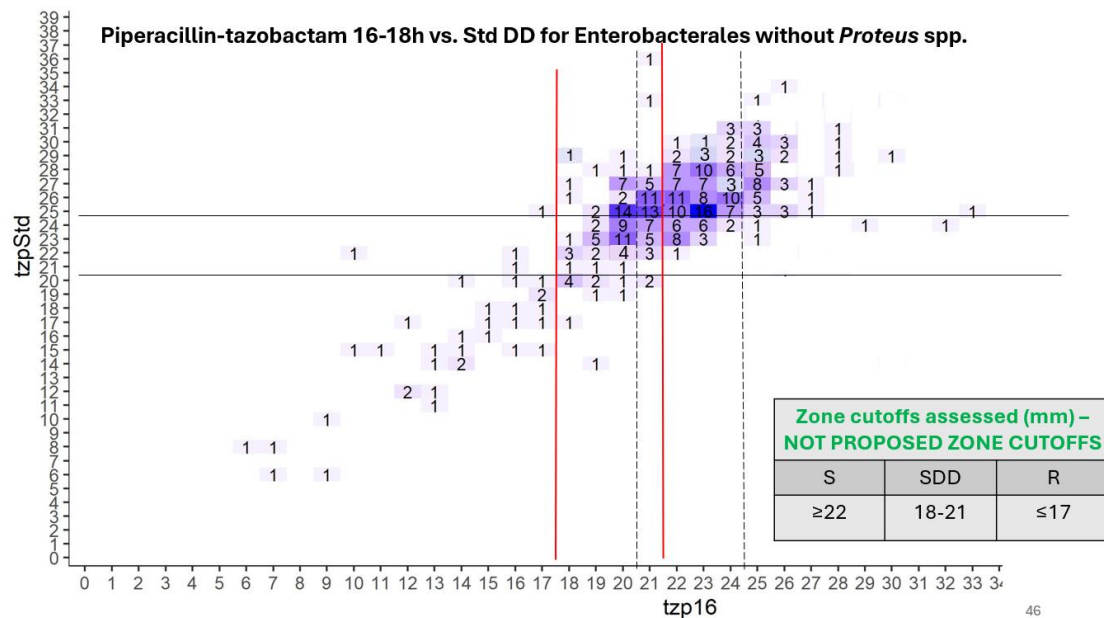
High Error Rates



- Piperacillin-tazobactam 16-18h vs. Std DD Enterobacteriales (excluding *Proteus* spp.)

	Std DD			
16-18 hr	S	SDD	R	Grand Total
S	146	30		176
SDD	63	55	13	131
R	1	3	32	36
Grand Total	210	88	45	343

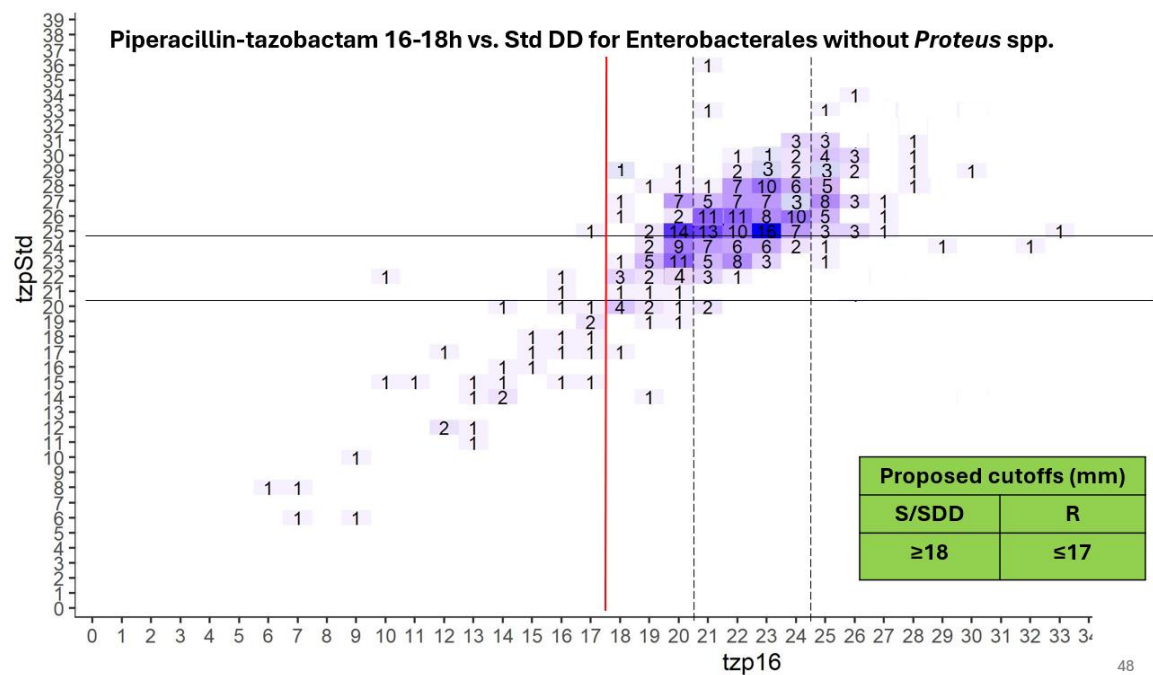
CA	233/343	67.9%
VME	0/45	0
ME	1/210	<1%
mE	109/343	31.8%



- Piperacillin-tazobactam 16-18h vs. Std DD Enterobacterales (excluding *Proteus* spp.) - Proposed
 - Proposed with the following caveat: Confirmatory MIC testing is indicated for isolates with zones of ≥18 mm to avoid falsely reporting isolates as susceptible.

	Std DD		
16-18 hr	S/SDD	R	Grand Total
S/SDD	294	13	307
R	4	32	36
Grand Total	298	45	343

CA	326/343	95.0%
VME	13/45	28.8%
ME	4/298	1.3%



- Direct Blood Disk Diffusion AHWG Discussion and Recommendation
 - Can't reliably distinguish between susceptible and SDD
 - Clinical implications
 - Piperacillin-tazobactam is frequently used as empiric therapy so it's useful to try to set a breakpoint
 - A resistant-only breakpoint is helpful to encourage an escalation in therapy
 - Reporting susceptible/SDD and resistant (vs. only reporting resistant)
 - Less confusing to clinicians to report susceptible/SDD than no result
 - Method overcalls SDD
 - Empiric pip-tazo is used at the higher dose for bloodstream infections anyway so little clinical concern with potentially using too high dose for isolates that are actually susceptible
 - High possibility that laboratories will need to correct the SDD to a susceptible once the result from the standard method is available → will result in revisions, possibly by a method other than disk diffusion
 - Could confirmatory testing include disk diffusion (not restricted to MIC)? If so, comment could state "Confirmatory antimicrobial susceptibility testing by standard methods is indicated..."
 - Piperacillin-tazobactam 100/10 µg is likely going to change in the future; studies are underway by Joint CLSI EUCAST Working Group indicating that disk content lower than 100/10 performs better
 - Proposed piperacillin-tazobactam (16-18 hour read) for Enterobacterales (excluding *Proteus* spp.)
 - NOTE: Confirmatory MIC testing is indicated for isolates with zones of ≥ 18 mm to avoid falsely reporting isolates as susceptible. A comment like this should be included to aid users in what to do when they get this result. Can model after cefiderocol comment in Table 2B-2.

Proposed cutoffs (mm)	
S/SDD	R
≥ 18	≤ 17

- Methods Working Group Discussion and Recommendation
 - Piperacillin-tazobactam is commonly used as empiric therapy, which could be helpful to have results.
 - Can a resistant only breakpoint be used or will this confuse clinicians without susceptible/SDD results
 - What is the value of a confirmatory test for an adjunctive test?
 - Confirmatory testing will be the majority of isolates.
 - 13 resistant isolates would fall into the susceptible/SDD category and would be considered VME
 - Motion to accept the resistant only breakpoint ($R \leq 17$ mm) piperacillin-tazobactam 16-18 hour direct blood culture disk diffusion for Enterobacterales (excluding *Proteus* spp.). WG Vote: 9-1-1-1.

SC DISCUSSION (MAIN POINTS)

- Will there be a comment to exclude *Proteus*?
 - The *Proteus* isolates were VME, so would not need to exclude them for a resistant only.

- The vote at the Methods Working group went through excluding *Proteus*.
- This antibiotic is so commonly used that giving providers a resistant breakpoint is clinically helpful.
- Rapid antibiotic stewardship is most helpful for antibiotic escalate therapy. Telling a provider it is resistant early is very helpful.
- The updated disk formula is likely years away so do not need to wait for the updated disk.

A motion to accept the piperacillin-tazobactam 16-18 hour direct blood culture disk diffusion breakpoint ($R \leq 17$ mm) for Enterobacterales with *Proteus* spp. included was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

BURKHOLDERIA CEPACIA COMPLEX AD HOC WORKING GROUP REPORT

- *Burkholderia cepacia* complex (BCC) MIC breakpoints were removed in M100 35th Edition
- BCC ECVs

Table F1. ECVs for *Burkholderia cepacia* Complex^a

Antimicrobial Agent	Interpretive Category and MIC, µg/mL	
	WT ^{b,c}	NWT
Ceftazidime	≤ 16	≥ 32
Levofloxacin	≤ 8	≥ 16
Meropenem	≤ 16	≥ 32
Minocycline	≤ 8	≥ 16
Trimethoprim-sulfamethoxazole	≤ 2	≥ 4

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild

Footnotes

- Insufficient data were available to establish ECVs for individual species within the *B. cepacia* complex. Although more than 50% of the data were contributed by a single laboratory for minocycline and trimethoprim-sulfamethoxazole, the data were not weighted before pooling and analysis. The ECVs are under review and will be updated if appropriate.
- The ECV is the highest MIC that defines the WT population of isolates (eg, the ECV for ceftazidime is 16 µg/mL and the WT population is ≤ 16 µg/mL).
- The ECVs for ceftazidime, levofloxacin, meropenem, and minocycline are above MICs typically achievable by routine antimicrobial dosing for similar organisms and are higher than the archived susceptible breakpoints (8, 2, 4, and 4 µg/mL, respectively).

ECVs listed in Table F2 are applicable only to the species indicated. Currently, there are insufficient data to support their use with other species.

- Limitations to currently published ECVs
 - $\geq 50\%$ of MIC data was contributed from one laboratory
 - CLSI M23 recommends normalizing data in this situation. The data supporting ECVs in M100 35th Edition had not been normalized
 - ECVs were not reviewed by the ECV AHWG of the AST Subcommittee.

- ECVs are higher than clinically achievable concentrations
- MIC data for ECVs
 - CLSI BCC AHWG studies
 - 100 isolates from persons with cystic fibrosis (CF)
 - 105 isolates from persons without CF (non-CF)
 - Used mode MIC value of replicate CLSI reference BMD
 - Mandy Wootton, published data:
 - 159 CF isolates
 - Mode MIC value of ISO BMD performed in triplicate
 - MIC values for BCC generated at IHMA, JMI, and Microbiologics using CLSI reference BMD
 - Used ECOFFinder to determine ECVs
 - Entered data following the user instructions
 - Truncated data (eg, > 64 µg/mL became 64 µg/mL)
- BCC species by laboratory/study

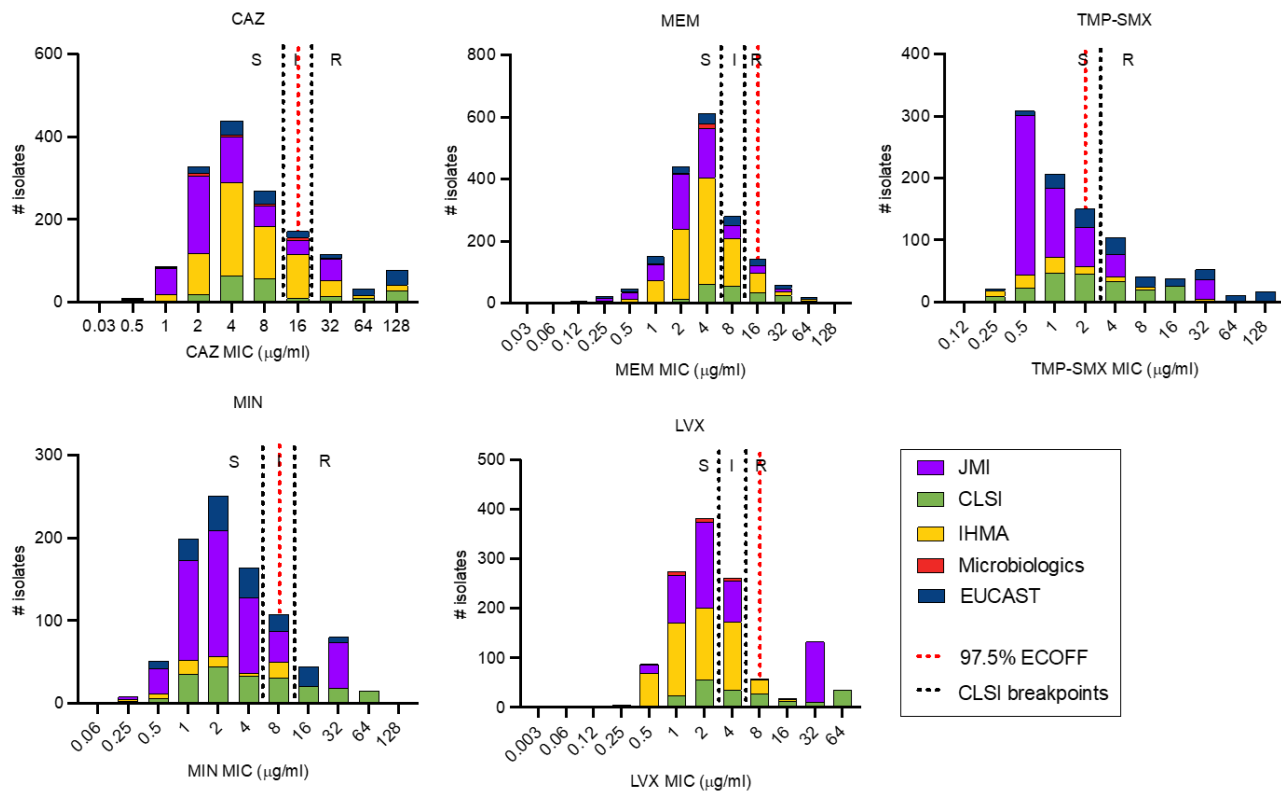
Species	Laboratory/Study					
	CLSI	EUCAST	IHMA	JMI	Microbiologics	Total
<i>Burkholderia ambifaria</i>	0	5	0	0	0	5
<i>Burkholderia anthina</i>	0	3	0	0	0	3
<i>Burkholderia cenocepacia</i>	108	63	0	14	6	191
<i>Burkholderia cenocepacia</i> IIID	0	1	0	0	0	1
<i>Burkholderia cepacia</i>	3	10	0	0	10	23
<i>Burkholderia cepacia</i> complex	0	0	1011	459	0	1470
<i>Burkholderia cepacia</i> group K	0	2	0	0	0	2
<i>Burkholderia cepacia</i> gv I	0	9	0	0	0	9
<i>Burkholderia cepacia</i> group	0	1	0	0	0	1
<i>Burkholderia contaminans</i>	2	0	0	0	0	2
<i>Burkholderia diffusa</i>	0	1	0	0	0	1
<i>Burkholderia dolosa</i>	0	4	0	0	0	4
<i>Burkholderia lata</i>	0	3	0	0	0	3
<i>Burkholderia multivorans</i>	92	36	0	26	8	162
<i>Burkholderia pyrrocinia</i>	0	1	0	0	1	2
<i>Burkholderia pyrrocinia</i> gv IX	0	3	0	0	0	3
<i>Burkholderia seminalis</i>	0	1	0	0	0	1
<i>Burkholderia stabilis</i>	0	4	0	0	0	4
<i>Burkholderia vietnamiensis</i>	0	8	0	0	1	9
Total	205	155	1011	499	26	1896

- Distribution of isolates by laboratory/study

- ≥ 50% of isolates came from one lab for MEM, MIN, and TMP-SMX
- CLSI M23 recommends normalizing data before analysis

Laboratory/study	# Isolates					% Isolates				
	CAZ	LVX	MEM	MIN	TMP-SMX	CAZ	LVX	MEM	MIN	TMP-SMX
CLSI	205	205	205	205	205	13%	16%	11%	22%	22%
Wootton <i>et al.</i>	155	0	155	155	155	10%	0%	9%	17%	16%
IHMA	642	534	901	62	85	42%	42%	50%	7%	9%
JMI	498	499	499	491	498	33%	39%	28%	54%	53%
Microbiologics	26	26	26	0	0	2%	2%	1%	0%	0%
Total	1526	1264	1786	913	943	100%	100%	100%	100%	100%

- BCC MIC distributions
 - Note low truncated JMI data for TMP-SMX



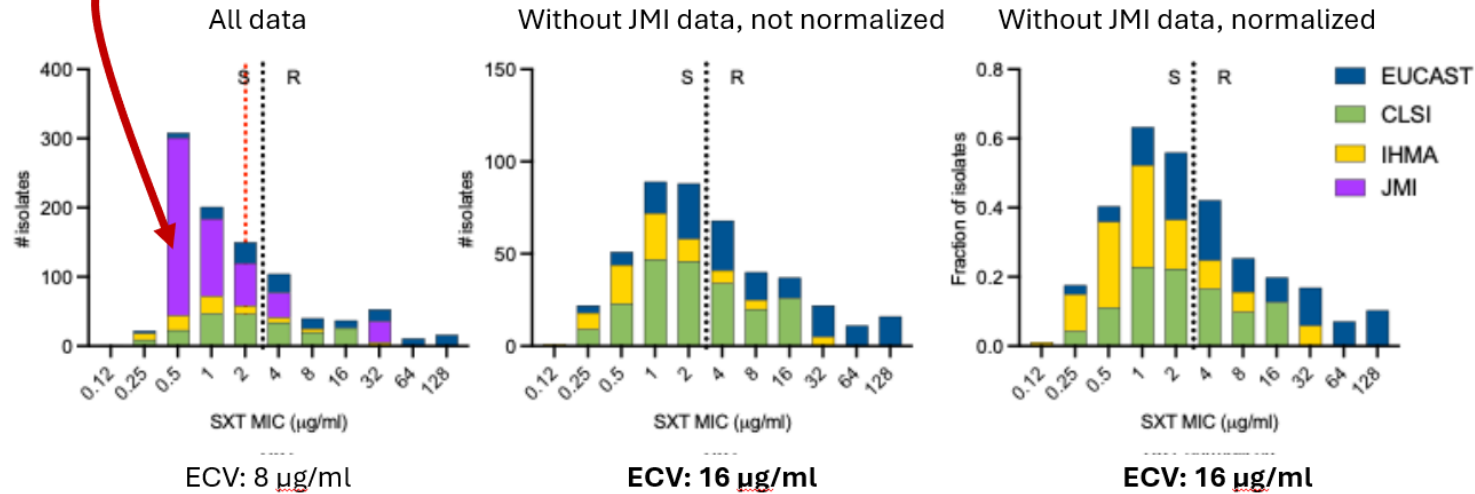
- BCC ECVs-normalized vs not normalized
 - The TMP-SMX ECV increases from 2 µg/mL to 8 µg/mL after normalization

Antimicrobial	Published ECV in M100-ED35 (µg/mL) (Not normalized)	Normalized ECVs (µg/mL)
CAZ	16	NA*
LVX	8	NA*
MEM	16	16
MIN	8	8
TMP-SMX	2	8

*NA=not applicable. CAZ and LVX ECVs did not require normalization.

- ECV AHWG Discussion and Recommendation
 - John Turnidge input
 - TMP-SMX data had been left truncated by one lab
 - Normalization changed TMP-SMX ECV after truncated data removed (based on John's suggestion), with ECV changing from 2 µg/mL to 16 µg/mL
 - EUCAST ECV method analysis, including additional data from the EUCAST MIC database, resulted in TMP-SMX ECV of 32 µg/mL
 - Combining agar and microbroth dilution methods may introduce ECV estimation errors
 - Additional ECV AHWG input
 - TMP-SMX a problem on several fronts, including method of testing, endpoint reading issues
 - No final recommendation on an ECV to set for TMP-SMX (almost impossible to set an ECV)
 - Many concerns voiced by John and the ECV AHWG about the ECV for TMP-SMX
 - Discussion that 2 µg/mL is probably not right, but ECV AHWG discussed whether it should be 4, 8, or 16 µg/mL
- TMP-SMX re-analysis, after having removed JMI data

PROBLEM: JMI data were truncated at 0.5 µg/ml on the low end of the distribution.



- Methods for normalizing data
 - CLSI M23 instructions: normalize the total data points in each dataset to 100 and then pool the data if one lab contributes $\geq 50\%$ of the MIC values

MEM-non-normalized data (n)

MEM	Column Labels					
Row Labels	CLSI	IHMA	JMI	Microbiologics	EUCAST	Grand Total
0.03		2			1	3
0.06		2	1			3
0.12		5	3			8
0.25		7	10		7	24
0.5		14	20	3	5	42
1	3	70	52	4	20	149
2	14	225	178	4	20	441
4	63	341	161	15	32	612
8	55	155	42		28	280
16	36	62	23		21	142
32	25	14	9		11	59
64	9	4			8	21
128					2	2
Grand Total	205	901	499	26	155	1786
% isolates from each lab	11%	50%	28%	1%	9%	1

MEM-normalized data (%)

Normalized MEM						
Row Labels	CLSI	IHMA	JMI	Microbiologics	EUCAST	Grand Total
0.03	0	0	0	0	1	1
0.06	0	0	0	0	0	0
0.12	0	1	1	0	0	2
0.25	0	1	2	0	5	8
0.5	0	2	4	12	3	21
1	1	8	10	15	13	47
2	7	25	36	15	13	96
4	31	38	32	58	21	180
8	27	17	8	0	18	70
16	18	7	5	0	14	44
32	12	2	2	0	7	23
64	4	0	0	0	5	9
128	0	0	0	0	1	1
Grand Total	100	100	100	100	100	500
% isolates from each lab after normalizing	6%	6%	6%	6%	6%	0.279955207

- Summary
 - Review by ECV AHWG confirmed ECVs for CAZ, LVX, MEM, and MIN
 - ECV for TMP-SMX varies based on analysis

Antimicrobial	Published ECV M100-ED35 (µg/mL)	Normalized ECV (µg/mL)	ECV with removal of JMI data for TMP- SMX (µg/mL)
CAZ	16	NA	NA
LVX	8	NA	NA
MEM	16	16	NA
MIN	8	8	NA
TMP-SMX	2	8	16

- BCC AHWG Discussion and Recommendation
 - Discussed whether to update TMP-SMX ECV to 8 or 16 µg/mL, but concerns that clinical application of either ECV would result in undertreatment
 - Concerns for ECVs: not clinically achievable and may be used as breakpoints
 - Discussed whether BCC should be moved to CLSI M45 either with ECVs as breakpoints or using old MIC breakpoints

- Consensus was neither should be done due to performance issues with AST methods, and old breakpoints are not correct
- BCC AHWG recommendations:
 - Remove ECVs from M100
 - Clearly state in the M100 that Non-Enterobacterales breakpoints should not be used to interpret BCC MICs
 - Recommend that testing should not be performed, even upon provider request, due to performance issues with AST methods and lack of prediction of therapeutic response
 - This recommendation aligns with EUCAST and recommendations of ASM's cystic fibrosis Practical Guidance in Clinical Microbiology (updated Cumitech), which suggest that testing should not be performed
- Methods Working Group Discussion and Recommendation
 - Discussion around adding to CLSI M45
 - Concerns around ECVs not clinically achievable.
 - Concerns around the recommendation of not testing
 - Motion to remove the ECVs, state Non-Enterobacterales breakpoints should not be used to interpret BCC MICs, and recommend testing should not be performed. WG Vote: 8-2-1-1. Rejections were concerns around not testing.

SC DISCUSSION (MAIN POINTS)

- The CMR says that CLSI is expected to say do not test.
- How do we take care of patients? Example lung transplant?
- The most recent data is that reference method BMD is reproducible, but do not know if there is any clinical benefit.
- The laboratory does not have a clinical context. There are a lot of issues with testing.
- Etest and agar dilution are not reproducible; however, need to keep in mind that reference method BMD is reproducible.
 - To clarify, within a method testing is reproducible, all the methods don't correlate to each other.
 - Further clarification that agar dilution and Etest reproducibility was not tested.
- Do not know what the PK/PD targets actually are for *Burkholderia*.
- Outside of the US there is limited access to reference BMD, so they will use other systems.
- IDSA/AMR group is planning to put together treatment suggestions for these organisms.
- Based on the data here, this would not meet even the CLSI M45 criteria and these isolates would get an asterisk.
- The Cochran review says to test by the reference method.
- Intent for manufacturers is to not test and not provide an MIC.

A motion to remove the *Burkholderia cepacia* complex ECVs was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

REVISIONS TO CLSI M100 TABLE 6A

- Not an official AHWG. Work is being done by Laura Koeth and Stephen LaVoie.
- Background
 - Table 6A has been updated in recent years when new agents are added but it has not been routinely updated with regard to older/generic agents
 - Many agents are available as different compounds (eg, salts or hydrate forms), some of which may require different solvents and/or diluents

- CAS numbers are assigned and do not deviate when the compounds are similar. These numbers should be added to the table for clarity.
- Following the January 2025 meeting, the table was sent as a template to several laboratories that make AST drug solutions and responses were received from 9 laboratories.
- Some Challenges
 - Not all laboratories provided specific chemical compounds and/or CAS numbers. If different solvents and/or diluents were used by these laboratories, it may have been because the compound was not the same.
 - In some cases, the CAS numbers for specific compounds were not matching (eg, noted with Med Chem Express sourced powders from one laboratory).
 - There were some inconsistencies in drug compounds, solvents, and diluents. NOTE: The current footnote B states “Although these solvents and diluents are recommended, users should always confirm with the manufacturer.”
- Options for Table Updates
 - Update the table for those agents that are clear/consistent between the laboratories. This would include addition of verified CAS numbers specific compounds, consolidation of several footnotes and placement of the footnotes next to each compound (instead of as a separate page). Can this be accomplished for the January 2026 edition?
 - Continue to query laboratories to obtain additional information so that more edits can be made at one time. Table would not be updated in January 2026.
- Table Example

Antimicrobial active agent	Antimicrobial compound options	CAS Registry Number	Solvent ^b	Diluent ^b	Footnotes?
			Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Finish diluting the final stock solution as stated below.	
Amikacin	amikacin disulfate amikacin hydrate amikacin sulfate salt FN1	39831-55-5 1257517-67-1 149022-22-0	Water	Water	FN1: Heating may be required to dissolve amikacin sulfate salt
Amoxicillin	amoxicillin anhydrous FN2 amoxicillin trihydrate	26787-78-0 61336-70-7	Phosphate buffer, pH 6, 0.1 mol/L or Sodium bicarbonate 7.5% solution	Phosphate buffer, pH 6, 0.1 mol/L	FN2: Extended time to dissolve (20-30 minutes) is typical when using amoxicillin anhydrous and solvent/diluent phosphate buffer, pH 6, 0.1 mo
	amoxicillin sodium	34642-77-8	Water	Water	
Ampicillin	ampicillin trihydrate	7177-48-2	Phosphate buffer, pH 8, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L	
	ampicillin sodium salt	69-52-3	Sodium bicarbonate	Phosphate buffer, pH 6, 0.1 mol/L	
Avibactam	avibactam sodium	1192491-61-4	Water	Water	
Azithromycin	azithromycin dihydrate	117772-70-0	95% ethanol or glacial acetic acid ^{a,c}	Broth media	Footnote? Recommend to make on day of pour.
Azlocillin			Water	Water	
Aztreonam		78110-38-0	Saturated solution sodium bicarbonate solution	Water	Footnote? How to make saturated sodium bicarbonate solution
Besifloxacin			Methanol	Water	
Biapenem			Saline ^d	Saline ^d	
Cadazolid			DMSO ^a	Water or broth	
Carbenicillin			Water	Water	
Cefaclor			Water	Water	
Cefadroxil			Phosphate buffer, pH 6, 0.1 mol/L	Water	
Cefamandole			Water	Water	
Cefazolin	cefazolin sodium salt	27164-46-1	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L	
Cefdinir			Phosphate buffer, pH 6, 0.1 mol/L	Water	
Cefditoren			Phosphate buffer, pH 6, 0.1 mol/L	Water	

- Future
 - Still work to be performed on determining
 - Which compound is correct, is there a way to determine this.
 - Some concerns for standardization retroactively could require revalidation
 - Concerns about removing versions that work because need redundancy for back orders
 - How to know if something doesn't work? Working with device manufacturers can help because they have an interest in knowing which versions work.

3. TEXT AND TABLE WORKING GROUP (S. CAMPEAU)

TEMPORARY VS PERMANENT REMOVAL OF BREAKPOINTS

- 2024:
 - During review of *Acinetobacter* minocycline breakpoint, doxycycline and tetracycline were voted to be removed (from 35th) while they underwent re-review
 - Doxycycline/tetracycline breakpoints removed in 35th ed but no details to indicate these were being reviewed and/or the removal could potentially be temporary
- 2025:
 - Doxycycline breakpoints reviewed at January 2025 meeting with a vote/approval to remove doxycycline breakpoints permanently
 - Confusion because they had already been removed/archived prior year and were not in the document
 - Tetracycline breakpoints discussed at yesterday's plenary and voted for permanent removal in 36th ed
- Potential issues/questions for users (including manufacturers) on how to handle drugs undergoing re-review
 - Is there a clinical concern for use of these breakpoints undergoing review? Maybe not always, but sometimes?
 - Can laboratories continue to use the 'old' breakpoints in the interim or no?
 - For example, with the temporary removal of the ceftriaxone/*N. gonorrhoeae* disk diffusion breakpoints discussed yesterday, by removing them, is the intention that laboratories should not do disk diffusion? Or okay to continue as is with old breakpoint?
 - Should manufacturers remove or stop reporting on their systems?
- Summary of discussion on whether 'archived' vs 'removed' vs 'eliminated' was used consistently and/or meant the same thing
 - The 'archived' material contains everything (whether permanent or temporary)
 - Archived Breakpoint table resources titled as "Breakpoints eliminated from M100..."
 - Want to update title to say "Archived" instead (eliminated felt to be too permanent)
 - Additionally, we would like to add the M100 years into the table vs just the edition
 - For example: January 2020 (M100-S20) vs M100-S20, similar to how we list in Breakpoint Additions/Revisions tables in M100)
- Summary of discussion for adding information to the archived breakpoint table and/or M100:
 - Prospectively, add details (as standardized as possible) into 'Rationale' column in archive table to make clear that breakpoints were removed because they're under review
 - Include pertinent details in M100, where appropriate, to indicate they're under review
 - eg, address in Overview of Changes and/or as comment next to drug like discussed yesterday "Additional disk correlate data are pending before disk diffusion breakpoints with the dosage regimen listed in Tables 2 Dosages can be established"
- Archived Breakpoint File - Proposed Edits
 - Update title name to say 'archived' to align with language of how these are referred to in M100
 - Include publication month/year

Archived
Breakpoints Eliminated From CLSI Document M100 Since 2010

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			M100 Edition in Which Breakpoints Were Last Included/Comments	Rationale
		S	I	R	S	I	R		
Enterobacteriales									
Cephalothin (surrogate test for uncomplicated UTI)	30 µg	≥ 18	15-17	≤ 14	≤ 8	16	≥ 32	M100-S25 January 2015 (M100-S25)	Cefazolin is a more reliable surrogate than cephalothin for predicting results for oral cephalosporins that might be used for treatment of uncomplicated UTIs.
Nalidixic acid	30 µg	≥ 19	14-18	≤ 13	≤ 16	-	≥ 32	M100S, 26th ed. Deleted for <i>Salmonella</i> spp. only	Nalidixic acid does not perform reliably in predicting susceptibility to fluoroquinolones that might be used for treatment of <i>Salmonella</i> infections. It has been shown to produce both false-resistant and false-susceptible results. ^{1,2}
Piperacillin	100 µg	≥ 21	18-20	≤ 17	-	-	-	M100-Ed31	Disk diffusion breakpoints deleted because disk correlates for revised MIC breakpoints were reassessed.
Ticarcillin	75 µg	≥ 20	15-19	≤ 14	≤ 16	32-64	≥ 128	M100-S25	This agent is no longer available.
<i>Pseudomonas aeruginosa</i>									
Cefoperazone	75 µg	≥ 21	16-20	≤ 15	≤ 16	32	≥ 64	M100-S20	These agents are no longer available or have limited indications for <i>P. aeruginosa</i> .
Cefotaxime	30 µg	≥ 23	15-22	≤ 14	≤ 8	16-32	≥ 64	M100-S20	
Ceftizoxime	30 µg	≥ 20	15-19	≤ 14	≤ 8	16-32	≥ 64	M100-S20	
Ceftriaxone	30 µg	≥ 21	14-20	≤ 13	≤ 8	16-32	≥ 64	M100-S20	
Gentamicin	10 µg	≥ 15	13-14 ^a	≤ 12	≤ 4	8 ^a	≥ 16	M100-Ed32	
Moxalactam	30 µg	≥ 23	15-22	≤ 14	≤ 8	16-32	≥ 64	M100-S20	
Ticarcillin	75 µg	≥ 24	16-23	≤ 15	≤ 16	32-64	≥ 128	M100-S25	
<i>Acinetobacter</i> spp.									
Doxycycline	30 µg	≥ 13	10-12	≤ 9	≤ 4	8	≥ 16	M100-Ed34	MIC and disk diffusion breakpoints were removed based on data showing that two CLSI reference AST methods, BMD and AD, do not correlate.

SPRING 36TH EDITION REVIEW COMMENTS

- Inclusivity of examples
 - Drugs listed are not exhaustive of the class
 - Discussed/considered either adding an 'eg,' before the drugs or include a reference to the glossaries
 - Text and Tables Working Group preferred to add reference statement (and do not add 'eg'). Place at beginning of the Appendix.

Appendix B. Intrinsic Resistance

Intrinsic resistance is defined as inherent or innate (not acquired) antimicrobial resistance, which is reflected in wild-type antimicrobial patterns of all or almost all representatives of a species. Intrinsic resistance is so common that susceptibility testing is unnecessary. For example, *Citrobacter* spp. are intrinsically resistant to ampicillin.

These tables can be helpful in at least three ways: 1) they provide a way to evaluate the accuracy of testing methods; 2) they aid in the recognition of common phenotypes; and 3) they can assist with verification of cumulative antimicrobial susceptibility test data. In the tables, an "R" occurring with an antimicrobial agent–organism combination means that strains should test resistant. A small percentage (1% to 3%) may appear susceptible due to method variation, mutation, or low levels of resistance expression.

Each laboratory should decide which agents to test and report in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. If tested, the result for an antimicrobial agent–organism combination listed as having intrinsic resistance should be reported as resistant. Consideration may be given to adding comments regarding intrinsic resistance of agents not tested. See Appendix A, footnote a.

- Refer to Glossary I and II for individual agents within the drug classes listed below

- Clarity for *S. pneumoniae* QC and MH-F
 - Initial confusion brought up about lack of QC guidance when performing *S. pneumoniae* MH-F testing and whether the ranges in 4B applied to both MHA with 5% sheep blood AND MH-F
 - This was brought to Methods Working Group and ultimately, the QC information was found in Table 2G comment (6) but felt it could be standardized with what is noted for *H. influenzae* QC
 - One sentence/comment in Tables 2 for the QC ranges/strains
 - Very little in Table 4B on which QC strains are appropriate when testing MH-F
 - Solution is to include new footnotes for the 2 QC strains in Table 4B

Table 4B. Disk Diffusion QC Ranges for Fastidious Organisms

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm			
		<i>Haemophilus influenzae</i> ATCC® 49247	<i>Haemophilus influenzae</i> ATCC® 49766	<i>Neisseria gonorrhoeae</i> ATCC® 49226	<i>Streptococcus pneumoniae</i> ATCC® 49619
Amoxicillin-clavulanate ^c	20/10 µg	15–23	–	–	–
Ampicillin	10 µg	13–21	–	–	30–36
Ampicillin-sulbactam	10/10 µg	14–22	–	–	–

Footnotes

H. influenzae footnote

New footnote uses similar language in testing conditions box in Table 2E

a. ATCC® is a registered trademark of the American Type Culture Collection.

b. Disk diffusion QC ranges for *H. influenzae* ATCC® 49247 apply to testing using either HTM (for all agents when testing *H. influenzae* or *H. parainfluenzae*) or MH-F (for selected agents when testing *H. influenzae*).

bc. Despite the lack of reliable disk diffusion breakpoints for *S. pneumoniae* with certain β-lactams, *S. pneumoniae* ATCC® 49619 is the strain designated for QC of all disk diffusion tests with all *Streptococcus* spp.

S. pneumoniae footnote

Uses similar language to *H. influenzae* footnote above and mirrors the testing conditions box on previous slide

d. Disk diffusion QC ranges for *S. pneumoniae* ATCC® 49619 apply to testing using either MHA supplemented with 5% sheep blood (for all agents when testing streptococci and *N. meningitidis*) or MH-F (for selected agents when testing *S. pneumoniae*).

- Solution is to also include additional information to clarify to spell out when MHA+5% sheep blood in Table 4B

Disk Diffusion Testing Conditions for Clinical Isolates and Performance of QC

Organism	<i>H. influenzae</i>	<i>N. gonorrhoeae</i>	Streptococci and <i>Neisseria meningitidis</i>
Medium	HTM MH-F agar	GC agar base and 1% defined growth supplement. The use of a cysteine-free growth supplement is not required for disk diffusion testing.	MHA supplemented with 5% defibrinated sheep blood for streptococci (including <i>S. pneumoniae</i>) and <i>N. meningitidis</i> MH-F agar for <i>S. pneumoniae</i> only
Inoculum	Colony suspension	Colony suspension	Colony suspension
Incubation conditions	5% CO ₂ ; 16–18 h; 35°C ± 2°C	5% CO ₂ ; 20–24 h; 36°C ± 1°C (do not exceed 37°C)	5% CO ₂ ; 20–24 h; 35°C ± 2°C

- Direct Disk Diffusion Correction
 - During Direct Disk AHWG agenda preparation, it was noted that this confirmatory testing comment did not need to be restricted to only MIC testing, but rather by another method (MIC, not necessarily reference BMD, or standard disk diffusion)

Jan 2023 vote

A motion to retain the current cefepime *P. aeruginosa* disk diffusion zone cutoffs (S_≥18, I 15–17, R_≤14) with a comment to confirm intermediate readings with an additional testing method for the 16–18h direct blood disk diffusion method was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)

Table 3F-3. Zone Diameter Disk Diffusion Breakpoints for *Pseudomonas aeruginosa* Direct From Blood Culture

General Comments

(1) Organism identification must be known before interpreting and reporting results.

(2) For additional testing and reporting recommendations, refer to Table 2B-1.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Read Times, h	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm			Comments
			S	I	R	
CEPHEMS (PARENTERAL) (including cephalosporins I, II, III, and IV. Please refer to Glossary I.)						
Cefepime	30 µg	8–10 16–18	— ≥ 18	— 15–17	— ≤ 14	(3) Confirmatory MIC testing using an MIC or standard disk diffusion method is indicated for isolates with zones of 15–17 mm to avoid reporting false-susceptible or false-resistant results.

Modification for 36th to align with the minutes

OTHER WORKING GROUP ITEMS TO TRACK

- Anaerobe - *Lactobacillus*

- The Anaerobe Working Group, M56 Document Development Committee (DDC), and M45 Working Group have been talking about *Lactobacillus* that grow poorly aerobically. The M45 Working Group is adding the following comment for AST testing of these *Lactobacillus* species “Anaerobic antimicrobial susceptibility testing methods and interpretations have not been developed for these organisms.”
- At the M56 DDC, discussed the need to add note to the anaerobe breakpoints in M100 that is similar to the note above.

Table 2J. MIC Breakpoints for Anaerobes

Testing Conditions		QC Recommendations
Medium:	Agar dilution (for all anaerobes): Brucella agar supplemented with hemin (5 µg/mL), vitamin K ₁ (1 µg/mL), and laked sheep blood (5% v/v) Broth microdilution (for <i>Bacteroides fragilis</i> and <i>Bacteroides thetaiotaomicron</i> only): Brucella broth supplemented with hemin (5 µg/mL), vitamin K ₁ (1 µg/mL), and LHB (5% v/v)	Refer to the following: • Tables 5D and 5E that list acceptable QC ranges applicable for each method • Appendix I to develop a QC plan When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC strains and QC ranges.
Inoculum:	Broth culture method or colony suspension, equivalent to 0.5 McFarland suspension Agar: 10 ⁵ CFU per spot Broth: 10 ⁶ CFU/mL	
Incubation:	36°C ± 1°C, anaerobically Broth microdilution: 46–48 hours Agar dilution: 42–48 hours	

General Comments

- (1) Refer to Table 1J for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.
- (2) For isolates for which the antimicrobial agent MICs fall within the intermediate category, maximum dosages, along with proper ancillary therapy, should be used to achieve the best possible levels of drug in abscesses and/or poorly perfused tissues. If this approach is taken, organisms for which the antimicrobial agent MICs fall within the susceptible range are generally amenable to therapy. Organisms for which the antimicrobial agent MICs are in the intermediate range may respond, but in such cases, efficacy as measured by patient clinical response should be carefully monitored. Ancillary therapy, such as drainage procedures and debridement, are of great importance for proper management of anaerobic infections.
- (3) Refer to CLSI M11¹ for examples of reading end points.
- (4) MIC values using either Brucella blood agar or Wilkins Chalgren agar (former reference medium) are considered equivalent.
- (5) Broth microdilution is recommended only for testing *Bacteroides* spp. and *Parabacteroides* spp. MIC values for agar or broth microdilution are considered equivalent for those species.
- (6) Until additional studies are performed to validate broth microdilution for testing other organisms, it should be used only for testing members of *Bacteroides* spp. and *Parabacteroides* spp.

➔ **(7) Anaerobic antimicrobial susceptibility testing methods and interpretations have not been developed for these organisms**

- Anaerobe - Updated Antibigrams
 - Anaerobe Working Group presented their tables at Monday's plenary
 - Updates needed for all the Appendix D tables and for select ECVs in Appendix F
 - Anaerobe Working Group will work with Text and Tables Working Group for implementing/updating Appendix D

- Text and Tables Working Group will help review new content, retention/removal of old footnotes/content, etc. with Anaerobe Working Group
- May need to do between end of June meeting and prior to Text and Tables Working Group review period to minimize post-review edits

- Antibigram Introduction

“The antibiogram data in this appendix were generated from unique patient isolates submitted to three reference laboratories (Mayo Clinic College of Medicine and Science, Rochester, Minnesota; UK Anaerobe Reference Unit, Public Health Wales, Cardiff, UK; and International Health Management Associates Inc., Schaumburg, Illinois), and includes isolates from the United States and outside of the United States. Antimicrobial susceptibility testing was performed by the reference agar dilution method. Antibiograms represent available historical data based on the routine testing practices of each reference laboratory and is not exhaustive for all antimicrobial agents that have anaerobic therapeutic indications. Refer to Table 1J and Table 2J for antimicrobial agents appropriate for anaerobes.

NOTE 1: Isolates from three reference laboratories collected from 1 January 2017 to 31 December 2024.

NOTE 2: Information and analysis of previous versions of this table have been published.”

- Plug
 - Reminder - please feel free to engage Text and Tables Working Group (reach out to April Bobenchik and Shelley Campeau) between meetings for any proposed ideas or help with mock ups being considered or planned for the meeting
 - Help with considerations for document placement/wording/etc.
 - Provides visuals for SC to review/evaluate in real-time
 - Streamlines any potential post-meeting edits/work

5. OUTREACH WORKING GROUP (A. SCHUETZ)

WORKING GROUP GOALS

- Educate practicing clinical microbiologists and health care professionals about AST practices and recommendations.
- Provide resources to facilitate individuals in their understanding and implementation of CLSI AST recommendations.
- Solicit suggestions from members of other CLSI Working Groups for educational activities; encourage AST Subcommittee volunteers to engage in these educational activities.
- Note: it is beyond the purview of Outreach Working Group to interpret data or provide technical recommendations that may be highly controversial, inconsistent with current or prior AST Subcommittee decisions, or that have not been confirmed by the AST Subcommittee.

PRODUCTS OF WORKING GROUP

- Education Workshops
- News updates
- Webinars
 - CLSI/Society of Infectious Diseases Pharmacists (SIDP)/American College of Clinical Pharmacy (ACCP)
 - CLSI/College of American Pathologists (CAP)
 - Other
- Programs at other meetings (eg, ASM, IDWeek)
- Other educational products
 - CLSI M100 Educational Program
 - Breakpoint Implementation Toolkit (BIT) and accompanying materials
- Other publications
 - Annual mini review of new CLSI M100
 - Other

ATTENDEE ORIENTATION

- Updated June 2024
- On demand via YouTube as CLSI New Member Orientation

WEBINARS/PRESENTATIONS

- CLSI Annual Update (23rd)
 - What's New in the 2025 CLSI Standards for Antimicrobial Susceptibility Testing (AST)?
 - February 26, 2025
 - Speakers: April Bobenchik and Romney Humphries
 - Moderator: Janet Hindler
 - Stats:
 - 468 live attendees from the webinar (450 for 2024 annual webinar)
 - 883 registrations (989 for 2024)
 - 1,074 views on-demand (not unique)

- CLSI-SIDP-ACCP Annual Webinar
 - Mind the Gap: CLSI M100 Updates to Optimize Stewardship and Patient Care Outcomes
 - May 15, 2025
 - Speakers: Jeannette Bouchard and Trish Simner
 - SIDP liaison: Lindsay Donohue
 - Stats:
 - 119 CLSI members registered (337 for 2024 CLSI/SIDP/ACCP annual webinar)
 - 766 registrants
 - 482 joined the live webinar (569 for 2024)
- CLSI-CAP Annual Webinar
 - Preliminary Title: Antimicrobial Susceptibility Testing Challenges in Proficiency Testing
 - October 9, 2025
 - Speakers: Laurel Glaser and Samia Naccache
- ASM Microbe 2025
 - CLSI Updates of New Beta-lactam Combination Agents and Other Novel Antimicrobials
 - June 22, 2025
 - Speaker: Romney Humphries
 - Moderator: Priyanka Uprety
- IDWeek 2026
 - Plan to submit a session on taxonomy and the impacts of these changes on AST and reporting
 - Amir Seyedmousavi will work with others to submit
- CLSI June 2025 Education Workshop
 - From Reads to Resistance: The Cutting Edge of Whole Genome Sequencing In Epidemiological and Antimicrobial Resistance Investigations
 - May 31, 2025
 - Speakers: Amy Mathers, David Hess, and Trish Simner
 - Moderator: Stella Antonara
- CLSI January 2026 Education Workshop
 - Ideas discussed: Phage Therapy
 - January 25, 2026

PODCAST/SOCIAL MEDIA OPPORTUNITIES

- Let's Talk Micro with Luis Plaza
 - 180: CLSI M100-Ed35 Updates and More
 - Guest: April Bobenchik
- Hot topics, easy listening and topic-focused
- Seek volunteers to present

CLSI M100 EDUCATIONAL PROGRAM

- No fee

- Enhance user ease of access
- Great for laboratory directors, training technologists and other trainees in laboratory
- Released March 2025
- June 2024 to May 2025 Stats:
 - 1531 registrations (392 prior year)
 - 779 learners accessed the course (177 prior year)
 - 225 users completed the course

QUALITY CONTROL

- On-demand QC material
 - Rationale document, implementation tools, other material types
 - Updating ASM-CAP-CLSI MIC and disk diffusion individualized quality control plans (IQCPs)
- Working closely with CAP to align with upcoming CAP checklist items regarding QC frequency
- Case scenarios to explain options available to decrease unnecessary work, if possible
 - For instance, decrease QC frequency or number of QC strains needed to test
- Annual M100 Webinar recorded extract of QC section - short video
- Anticipate posting on CLSI website

BURKHOLDERIA CEPACIA GUIDANCE

- Guidance document from the *Burkholderia cepacia* complex AHWG
- *Burkholderia cepacia* FAQs for clinical laboratories
 - FAQs with practical advice for implementation and communication tips for working with patient-facing colleagues
 - Some questions we aim to include in this document:
 - Should the laboratory provide AST results for *Burkholderia cepacia* complex (BCC) organisms?
 - What is the risk of using the “old” breakpoints and “old methods” in my lab?
 - How can I communicate this change to my clinicians?
 - What about *Burkholderia* species other than BCC?
 - Where can I find more information on BCC AST and antimicrobial therapy?
- Inclusion in News Update
- Post on CLSI website?

CLSI M02 /M07 EDUCATIONAL PROGRAM

- Based on CDC Antimicrobial Susceptibility Testing Training “Master” CD ROM from 2002
 - Removed 2019 due to lack of resources to maintain the program
- Interactive overview of M02/M07
- In progress

BREAKPOINT IMPLEMENTATION TOOLKIT (BIT)

- Launched June 2023

- Updated in March 2025
- Align with upcoming CLSI M68 (Validation of AST System Breakpoints) and CLSI M52 (Verification of AST Systems) document updates

PUBLICATIONS

- Schuetz, A, A Ferrell, J Hindler, R Humphries, A Bobenchik. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100 32nd and 33rd Editions. JCM. In Press.
- Bobenchik, A, A Ferrell, J Hindler, A Schuetz. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100 34th & 35th Edition.

NEWS UPDATE

- Publication goals: March, September
- AST News Updates are located under “Resources” tab of CLSI homepage
- April 2025
 - Feature: *Stenotrophomonas maltophilia*
 - Case: Reporting cefepime for carbapenemase producers
 - Practical tips: Linezolid/tedizolid
 - Hot topic: New antifungal drug rezafungin
 - Recent developments: *Mycobacterium chelonae* extended incubation for clarithromycin
- September 2025
 - Feature: *Burkholderia cepacia*
 - Case: *Candida auris* AST
 - Practical tips: QC - new recommendations
 - Hot topics: FDA breakpoint developments
 - More News!: Anaerobe Working Group AST status and CLSI M11
 - Recent developments
 - Disk diffusion not a reference method
 - Voriconazole *A. fumigatus* breakpoint recognized by FDA, other new breakpoints from Antifungal Tests Subcommittee
 - CLSI M100 Educational Program (how access)

VOLUNTEER OPPORTUNITIES

- News Update
 - Provide feedback on content, delivery, and structure
 - Suggest content
 - Partner with others to write articles (case studies and more)
- Other Publications
 - Assorted topics
- Webinars / Workshops / Lectures
 - Suggest content
 - Speakers

	• Other Projects • If anyone is asked to talk about CLSI, please coordinate with Outreach Working Group. SC DISCUSSION (MAIN POINTS) • Should CLSI consider their own podcast? ○ This is being discussed internally. • There was a SHEA session on CLSI, should all these AST Subcommittee CLSI-related sessions be brought to the CLSI Outreach Working Group? ○ The Outreach Working Group thinks that each individual talk related to CLSI topics does NOT need to be sent to the working group for approval or review.
7.	<u>ADJOURNMENT</u> Dr. Mathers thanked the participants for their attention. The meeting was adjourned at 12:00 PM Central Standard (US) time.

PLENARY ATTENDEES

Plenary 1	Plenary 2	Plenary 3
Adams Jennifer K.	Adams Jennifer K.	Adams Jennifer K.
Alby Kevin	Alby Kevin	Alby Kevin
Ambler Jane E.	Ambler Jane E.	Ambler Jane E.
Andermann Tessa	Andermann Tessa	Andermann Tessa
Antonara Stella	Antonara Stella	Antonara Stella
Arbefeville Sophie	Arbefeville Sophie	Arbefeville Sophie
Atkinson Dunn Robyn	Atkinson Dunn Robyn	Atkinson Dunn Robyn
Bala Shukal	Bala Shukal	Bala Shukal
Balbuena Rocio	Balbuena Rocio	Balbuena Rocio
Barlow Brooke	Barlow Brooke	Barlow Brooke
Bennett Jill	Bennett Jill	Bennett Jill
Bensman Timothy J.	Bensman Timothy J.	Bensman Timothy J.
Bhalodi Amira	Bhalodi Amira	Bhalodi Amira
Bhatnagar Amelia	Bhatnagar Amelia	Bhatnagar Amelia
Bhatti Micah M.	Bhatti Micah M.	Bhatti Micah M.
Bixby Morgan	Bixby Morgan	Bixby Morgan
Blosser Sara	Blosser Sara	Blosser Sara
Bobenchik April M.	Bobenchik April M.	Bobenchik April M.
Boswell Malcolm	Boswell Malcolm	Bowden Robert
Bowden Robert	Bowden Robert	Bradford Patricia
Bradford Patricia	Bradford Patricia	Brown Carrine
Brown Carrine	Brown Carrine	Bryan, MD, PhD Andrew
Bryan, MD, PhD Andrew	Bryan, MD, PhD Andrew	Bryson Alexandra Lynn
Bryson Alexandra Lynn	Bryson Alexandra Lynn	Bulman Zackery P.
Bulman Zackery P.	Bulman Zackery P.	Bulnes Rene
Burgess David S	Bulnes Rene	Burgess David S
Burgos-Garay Maria	Burgess David S	Burgos-Garay Maria
Burnham Carey-Ann	Burnham Carey-Ann	Burnham Carey-Ann
Campbell Davina	Bush Karen	Bush Karen
Campeau Shelley	Campbell Davina	Campbell Davina
Campodonico Victoria	Campeau Shelley	Campeau Shelley
Capraro Gerald A.	Campodonico Victoria	Campodonico Victoria
Carpenter Darcie E.	Capraro Gerald A.	Capraro Gerald A.
Carvalhaes Cecilia	Carpenter Darcie E.	Carpenter Darcie E.
Castanheira Mariana	Carvalhaes Cecilia	Carvalhaes Cecilia
Chandler Courtney	Castanheira Mariana	Castanheira Mariana
Chandrasekaran Sukantha	Chandler Courtney	Chandler Courtney
CHEN YAMIN	Chandrasekaran Sukantha	Chandrasekaran Sukantha
Cole Nicolynn	CHEN YAMIN	CHEN YAMIN
Copsey-Mawer Sarah	Cole Nicolynn	Cole Nicolynn
Cullen Sharon K.	Copsey-Mawer Sarah	Copsey-Mawer Sarah

DeJonge Boudewijn
Dial Courtney
Dien Bard Jennifer
Dieppois Guennaelle
Dingle Tanis
Donohue Lindsay
Dressel Dana C.
Duncan Elaine
Edelstein Paul
Esparza German
Fedorenko Marianna
Ferrell Andrea L.
Filkins Laura
Fisher Mark A.
Fratoni Andrew
Fu Yakun
Gadiraju Saipriya
Gancarz Barb
Gandhi Mukesh
Gandhi Ronak
Garg Rahul
Garrett Elizabeth
Gatermann Sören
Gefroh Sarah
Geyer Chelsie
Ghosh Mayurika
Giske Christian G.
Glasgow Heather
Gold Howard
Goldstein Beth P.
Gomez Emily J.
Gray Alice
Gray Kamisha
Griffin Natasha
Haddock Christopher
Hahnemann Katie
Hamilton Lauren
Hara Takafumi
Hendrix Megan
Herrera Elide
Hill Brandon
Hindler Janet A.
Hirsch Elizabeth

Cullen Sharon K.
DeJonge Boudewijn
Dial Courtney
Dien Bard Jennifer
Dieppois Guennaelle
Dingle Tanis
Donohue Lindsay
Dressel Dana C.
Duncan Elaine
Edelstein Paul
Esparza German
Fedorenko Marianna
Ferrell Andrea L.
Filkins Laura
Fisher Mark A.
Fratoni Andrew
Fu Yakun
Gadiraju Saipriya
Gancarz Barb
Gandhi Mukesh
Gandhi Ronak
Garg Rahul
Garrett Elizabeth
Gatermann Sören
Gefroh Sarah
Geyer Chelsie
Ghosh Mayurika
Giske Christian G.
Glasgow Heather
Gold Howard
Goldstein Beth P.
Gomez Emily J.
Gray Alice
Gray Kamisha
Griffin Natasha
Haddock Christopher
Hahnemann Katie
Hamilton Lauren
Hara Takafumi
Hendrix Megan
Herrera Elide
Hill Brandon
Hindler Janet A.

Cullen Sharon K.
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Dial Courtney
Dien Bard Jennifer
Dieppois Guennaelle
Dingle Tanis
Donohue Lindsay
Dressel Dana C.
Duncan Elaine
Edelstein Paul
Esparza German
Fedorenko Marianna
Ferrell Andrea L.
Filkins Laura
Fisher Mark A.
Fratoni Andrew
Fu Yakun
Gancarz Barb
Gandhi Mukesh
Gandhi Ronak
Garg Rahul
Garrett Elizabeth
Gatermann Sören
Gefroh Sarah
Geyer Chelsie
Ghosh Mayurika
Giske Christian G.
Glasgow Heather
Gold Howard
Goldstein Beth P.
Gomez Emily J.
Gray Kamisha
Griffin Natasha
Haddock Christopher
Hahnemann Katie
Hamilton Lauren
Hara Takafumi
Hendrix Megan
Herrera Elide
Hill Brandon
Hindler Janet A.
Hirsch Elizabeth
Howe Zachary

Howe Zachary
Hsiung Andre
Huband Michael D.
Hufnagel David
Iguchi Mitsutaka
Jean Sophonie
Jimenez Pearson Antonieta
Johnson Kristie
Karlow sky James
Kato Seiji
Kersh Ellen N.
Khalid Haziq
Khan Ayesha
Kilic Abdullah
Killian Scott B.
Kirn Thomas
Klavins Anna
Koeth Laura M.
Kuti Joseph
Lam Christine M.
LaVoie Stephen
Leung Beth
Lewis James S.
Li Xian-Zhi
Liesman Rachael
Livesay Hannah
Luna Brian
Lutgring Joseph
Machado Maria Jose
Maddock Kelli
Madon Andrew
Malysa Michelle
Maripini Nihaal
Martin Isabella
Mathers Amy J
Matuschek Erika
mcclain jennifer
McCurdy Sandra
McLeod Sarah
Miller Jennifer
Miller Linda A.
Miller Melissa
Miller William

Hirsch Elizabeth
Howe Zachary
Hsiung Andre
Huband Michael D.
Hufnagel David
Iguchi Mitsutaka
Jean Sophonie
Jimenez Pearson Antonieta
Johnson Kristie
Karlow sky James
Kato Seiji
Kersh Ellen N.
Khalid Haziq
Khan Ayesha
Kilic Abdullah
Killian Scott B.
Kirn Thomas
Klavins Anna
Koeth Laura M.
Kuti Joseph
Lam Christine M.
LaVoie Stephen
Lee Tien-Yi
Leung Beth
Lewis James S.
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 Moeck Greg
 Morales Yesenia
 Moussa Samir
 Muntha Kesava
 Naccache Samia N.
 Narayanan Navaneeth
 Nicolau David P.
 Ohkusu Kiyofumi
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 Oyarzun Sebastian Cifuentes
 Palavecino Elizabeth
 Patel Jean B.
 Patel Rinal
 Perez Katherine
 Pierce Virginia M.
 Pillar Chris
 Pischel Kelsey
 Rajeev Lara
 Redell Mark A
 Renick Paul
 Riccobene Todd
 Rice Felicia
 Rossi Flavia
 Rotunno Will
 Sabour Sarah
 Sanchez Belkys
 Sanchez Susan
 Satlin Michael
 Scangarella-Oman Nicole
 Scheetz Marc H.
 Schuermeyer Linda
 Schuetz Audrey N.
 Seyedmousavi Amir
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Trabold Peter
Trauner Andrej
Trebosc Vincent
Truong Thao
Turng Ben
Uprety Priyanka
van Nuenen Marc
Van Tam T.
Wehr Collette
Weingarten Rebecca
Weinstein Melvin P.
Westblade Lars F.
Wiederhold Nathan P.
Wikler Matthew A.
Winkler Marisa
Wootton Mandy
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