Meeting Title:	**Subcommittee on Antimicrobial Susceptibility Testing (AST)**	**Contact:**	egomez@clsi.org
Meeting Location:	Tempe, Arizona, USA		
Meeting Dates and Times: All times are Mountain Standard (US) time.	**Plenary 1:** Monday, 26 January 2026, 7:30 AM – 12:00 PM **Plenary 2:** Monday, 26 January 2026, 1:00 – 5:30 PM **Plenary 3:** Tuesday, 26 January 2026, 7:30 AM – 12:00 PM		
Meeting Purpose:	The purpose of this meeting is to review and discuss AST WG and SC business in preparation for publication of the next edition of M100 (37th).		
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**
Patricia J. Simner, PhD, D(ABMM) AST Subcommittee Secretary – January 2026	**Mayo Clinic**
Members Present:	
Kevin Alby, PhD, D(ABMM)	Brown University Health
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
Joseph D. Lutgring, MD	Centers for Disease Control and Prevention
William Miller, MD	Methodist Hospital
Stephanie L. Mitchell, PhD, D(ABMM)	Beckman Coulter
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
Paula Snippes Vagnone, MT(ASCP)	Minnesota Department of Health
Pranita D. Tamma, MD, MHS	The Children's Hospital of Philadelphia
Members Absent:	
Alexandra L. Bryson, PhD, D(ABMM) AST Subcommittee Secretary	Virginia Commonwealth University Health
Advisors Present:	
Mariana Castanheira, PhD	Element/JMI Laboratories
Sharon K. Cullen, BS, RAC	Beckman Coulter, Inc. Microbiology Business
Boudewijn DeJonge, PhD	Shionogi & Co
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
Andrew Fratoni, PharmD	Hartford Hospital
Elizabeth Garrett, PhD, D(ABMM)	Penn State Health, Milton S. Hershey Medical Center
Sören Gatermann, PhD	EUCAST

Elizabeth Hirsch, PharmD	University of Minnesota
Andre Hsiung, M(ASCP), MS, MBA	Hardy Diagnostics
Romney M. Humphries, PhD, D(ABMM), FIDSA, FAAM	Vanderbilt University Medical Center
Antonieta Jimenez Pearson, MQC, PhD	INCIENSA
Kristie Johnson, PhD, D(ABMM)	University of Maryland
Samia Naccache, PhD, M(ASCP), D(ABMM)	LabCorp
Mike Satlin, MD	Weill Cornell Medicine
Patricia J. Simner, PhD, D(ABMM)	Mayo Clinic
Jolyn Tenllado	bioMérieux
Melvin P. Weinstein, MD	Robert Wood Johnson University Hospital
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
Dmitri Iarikov, MD, PhD	FDA Center for Drug Evaluation and Research
Thomas J. Kirn, MD, PhD	Rutgers Robert Wood Johnson Medical School
Ribhi Shawar, PhD, D(ABMM), F(AMM)	FDA Center for Devices and Radiological Health
Reviewers and Guests (Non-SC-roster attendees): see Plenary Attendee List below	
Staff:	
Jennifer Adams, MT(ASCP), MSHA	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, 26 January 2026 7:30 AM – 12:00 PM Mountain Standard Time (US)			
Time	**Item**	**Presenter**	**Page**
7:30 AM – 7:40 AM (10 min)	Opening Remarks	A. Mathers	6
7:40 AM – 7:45 AM (5 min)	Approval of Meeting Agenda	A. Mathers	6
7:45 AM – 7:55 AM (10 min)	CLSI Welcome and Update	B. Jones	6
7:55 AM – 8:05 AM (10 min)	CLSI Awards	B. Jones	6
8:05 AM – 8:15 AM (10 min)	AST Subcommittee Update	E. Gomez	7
8:15 AM – 8:35 AM (20 min)	CLSI Subcommittee Document Processes	E. Gomez	10
8:35 AM – 8:45 AM (10 min)	Subcommittee on Antifungal Susceptibility Tests (AFST) Update	N. Wiederhold	15
8:45 AM – 8:55 AM (10 min)	EUCAST Update	S. Gatermann	19
8:55 AM – 9:25 AM (30 min)	Investigational Breakpoints AHWG	A. Khan M. Wikler	22
9:25 AM – 9:45 AM (20 min)	Break		
9:45 AM – 10:15 AM (30 min)	Anaerobes WG	D. Carpenter S. Copsey-Mawer	28
10:15 AM – 10:35 AM (20 min)	Text and Tables WG	A. Bobenchik S. Campeau	29
10:35 AM – 12:00 PM (1 hr 30 min)	Quality Control WG	S. Cullen C. Pillar	34

PLENARY AGENDA: SESSION 2 – IN PERSON Monday, 26 January 2026 1:00 PM – 5:30 PM Mountain Standard Time (US)			
Time	**Item**	**Presenter**	**Page**
1:00 PM – 3:00 PM (2 hr)	Breakpoints WG: Part 1	N. Narayanan M. Satlin	53
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	53

PLENARY AGENDA: SESSION 3 – IN PERSON Tuesday, 27 January 2026 7:30 AM – 12:00 PM Mountain Standard Time (US)			
Time	**Item**	**Presenter**	**Page**
7:30 AM – 9:30 AM (2 hr)	Methods WG	T. Dingle K. Johnson	104
9:30 AM – 9:50 AM (20 min)	Break		
9:50 AM – 10:20 AM (30 min)	Outreach WG	J. Hindler A. Schuetz	129
10:20 AM – 11:20 AM (1 hr)	Joint CLSI-EUCAST WG	J. Hindler E. Matuschek	134
11:20 AM – 11:50 AM (30 min)	Miscellaneous	A. Mathers	144
11:50 AM - 12:00 PM (10 min)	Closing Remarks	A. Mathers	144

Summary of Voting Decisions and Action Items

Summary of Passing Votes			
#	**Motion Made and Seconded**	**Results**[a]	**Page**[b]
1.	To approve the January 2026 meeting agenda.	13-0-0-1	6
2.	To accept the proposed comment "Only approved reference MIC method for testing is agar dilution." or a similar comment for mecillinam in Table 2A-1.	13-0-0-1	31
3.	To accept the Debio 1453 MIC QC range for *Neisseria gonorrhoeae* ATCC 49226 (0.016-0.06 µg/mL).	13-0-0-1	39
4.	To accept the current azithromycin susceptible MIC breakpoint (≤ 1 µg/mL) and the proposed resistant MIC breakpoint (≥ 2 µg/mL) based on a dosage of 2g PO for *Neisseria gonorrhoeae*.	13-1-0-0	69
5.	To accept the azithromycin disk diffusion breakpoints (S ≥ 30 mm, I 27-29 mm, R ≤ 26 mm) for *Neisseria gonorrhoeae*.	14-0-0-1	70
6.	To accept the cefixime disk diffusion (5 µg disk) breakpoints (S ≥ 37 mm, I 32-36 mm, R ≤ 31 mm) for *Neisseria gonorrhoeae*.	13-0-0-1	71
7.	To approve the ceftriaxone disk diffusion (5 µg disk) and QC study for *Neisseria gonorrhoeae* using CLSI M23 criteria.	14-0-0-0	74
8.	To remove the ceftriaxone and cefotaxime breakpoints for *Acinetobacter* spp. in Tables 1 and 2.	14-0-0-0	82
9.	To maintain the current ceftazidime MIC breakpoints (S ≤ 8, I 16, R ≥ 32 µg/mL) for *Acinetobacter* spp. based on a dosage of 2g IV q8h.	12-2-0-0	92
10.	To move forward with the CAMHB method for cefiderocol-xeruborbactam at 100% inhibition.	13-0-1-0	107
11.	To accept the cefiderocol-xeruborbactam MIC supplemental QC for *Acinetobacter baumannii* #1134488 (1/4-4/4 µg/mL).	13-0-1-0	112
12.	To accept the cefiderocol-xeruborbactam MIC routine QC for *Klebsiella pneumoniae* ATCC BAA-2814 (0.5/4-4/4 µg/mL).	13-0-1-0	112
13.	To accept the cefiderocol-xeruborbactam MIC supplemental QC for *Pseudomonas aeruginosa* ATCC 27853 (0.25/4-2/4 µg/mL).	13-0-1-0	113
14.	To approve the modified method for rifabutin and *Acinetobacter baumannii* complex using CA-MHA supplement with 100 µM PIH in Appendix H.	13-1-0-0	126
15.	To add the proposed revisions to CLSI M23S disk sources for Phase 1 and Phase 2.	14-0-0-1	135

[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.

2026 JANUARY AST MEETING SUMMARY MINUTES PLENARY 1: Monday, 26 January 2026 7:30 AM – 12:00 PM Mountain Standard Time (US)	
#	**Description**
1.	**OPENING REMARKS (A. MATHERS)** Dr. Mathers opened the meeting at 7:30 AM Mountain Standard (US) time by welcoming the participants to the hybrid CLSI meeting in Tempe, Arizona.
2.	**APPROVAL OF MEETING AGENDA** **A motion to approve the June 2025 meeting agenda was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)**
3.	**CLSI WELCOME AND UPDATE (B. JONES)** Dr. Jones provided an update on CLSI activities. She highlighted the global impact and the good the subcommittee does during a challenging time. She thanked everyone for being in attendance.
4.	**CLSI AWARDS (B. JONES)** Dr. Jones communicated that the Standards Development Award was awarded to Dr. Robert Bowden at the CLSI VAST Subcommittee meeting. She presented the Russell J. Eliers Award for exceptional contributions to Dr. Victor Waddell.

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

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

- 2026 Roster Changes
  - 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)
    - January 2026 Secretary: Patricia J. Simner, PhD, D(ABMM)
  - AST Subcommittee Members
    - Completing Service
      - Audrey Schuetz
      - Patricia Simner
    - New Members
      - William Miller
      - Paula Snippes Vagnone
  - AST Subcommittee Advisors
    - Completing Service
      - Joe Kuti
      - Maria Machado
      - Brigit Quinn
    - New Advisors
      - Boudewijn DeJonge
      - Andrew Fratoni
      - Elizabeth Garrett
      - Patricia Simner
- Ad Hoc Working Group (AHWG) Changes
  - Disbanded Ad Hoc Working Groups
    - Methods Working Group
      - *Burkholderia cepacia* complex AST AHWG
      - High Inoculum Cefazolin Testing AHWG
  - New Ad Hoc Working Groups
    - AST Subcommittee
      - Investigational Breakpoints AHWG
    - Breakpoints Working Group
      - Oral Cephalosporins AHWG
    - Methods Working Group
      - Solvents Table AHWG
    - Antimycobacterial AST Working Group
      - Antimycobacterial AST Working Group

- Subcommittee Voting Rules
  - 2/3 of the subcommittee eligible voters must vote affirmatively
  - There is not a requirement that 1 member from each of the three constituencies must vote affirmatively

Subcommittee on Antimicrobial Susceptibility Testing
Chairholder's Rules on Voting

January 2026 AST Subcommittee Roster
14 voting members (excludes Chairholder and Vice-chairholder)

Committee Status	"Pass" Vote
All members present and voting	14-0; 13-1; 12-2; 11-3; 10-4
One member not present or abstaining	13-0; 12-1; 11-2; 10-3
Two members not present or abstaining	12-0; 11-1; 10-2
Three members not present or abstaining	11-0; 10-1
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.

- AST Subcommittee Documents
  - 2026 Publications
    - M100-36th Edition published in January 2026
  - Active Documents
    - M45, Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria, 4th Edition

- Chairholders: Trish Simner and Romney Humphries
- M24, Susceptibility Testing of Mycobacteria, *Nocardia* spp. and Other Aerobic Actinomycetes, 4th Edition
  - Chairholders: Nancy Wengenack and Nikki Parrish
- Call for Volunteers
  - M40, Quality Control of Microbiological Transport Systems, 3rd Edition
    - Chairholders: Karissa Culbreath and Yvette McCarter
    - Apply by 9 February 2025
  - Committee for Mitigating Changes in Prokaryotic Nomenclature (CoMICProN)
    - CLSI has been invited to provide a representative to CoMICProN, an ad hoc committee of the International Committee on Systematics of Prokaryotes (ICSP).
    - CoMICPRoN was formed by ICSP and a diverse group of microbiologists to promote taxonomic accuracy and understanding of the impact of updated nomenclature on clinical and applied microbiology.
    - The CLSI representative is required to regularly report on the group's progress and consult CLSI as appropriate regarding decision-making.
    - New members must be approved by a vote of existing members, and candidates need to provide a current CV to be considered.
    - If you are interested in representing CLSI or have questions, please contact Kathy Castagna, Director of External Affairs at kcastagna@clsi.org.

- Next Meeting
  - 31 May – 2 June 2026 in Chicago (Rosemont), Illinois
  - Meeting materials due 1 May 2026
  - Virtual Only Working Group Meetings in weeks of 11 May and 18 May 2025
- CLSI M100 37th Edition
  - Publishing January 2027
  - January and June 2026 meeting decisions
  - No additional decisions after the June 2026 meeting
- Celebration of Dedication (Presented by A. Mathers)
  - Karen Bush, PhD
  - Christian G. Giske, MD, PhD

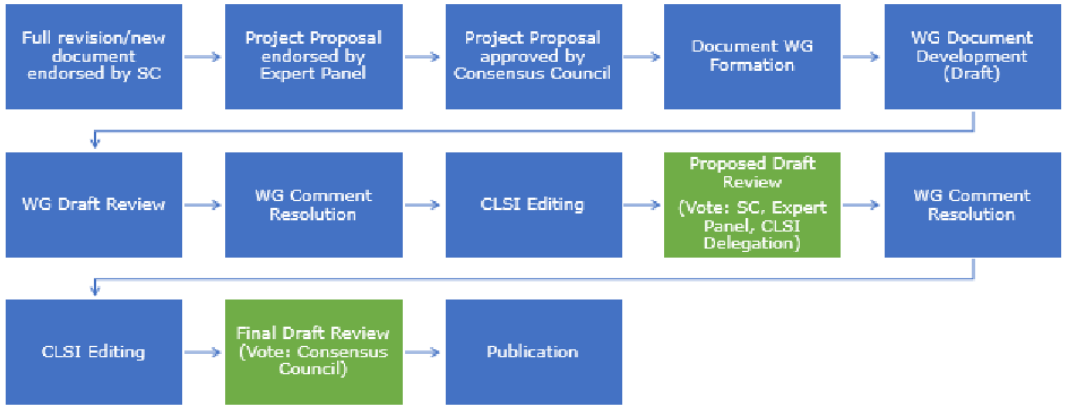
6. **CLSI SUBCOMMITTEE DOCUMENT PROCESSES (E. GOMEZ)**

Ms. Gomez provided an update on the CLSI subcommittee document processes. The main points included:

- CLSI Governance Structure
  - Subcommittees (SCs) report to the Expert Panel on Microbiology
  - No Document Development Committees (DDCs). Working groups (WGs) are formed for document development.
  - Subcommittees focus solely on antimicrobial/antifungal susceptibility testing
  - Expert Panel also focuses on microbiology identification and other testing methods and laboratory guidance
- CLSI Subcommittees
  - Antimicrobial Susceptibility Testing (AST SC)
  - Antifungal Susceptibility Tests (AFST SC)
  - Veterinary Antimicrobial Susceptibility Testing (VAST SC)
- Subcommittee Processes Guidance Documents
  - M23, Development of *In Vitro* Susceptibility Test Methods, Breakpoints, and Quality Control Parameters, 6th Edition
    - AST and AFST Subcommittees
    - Provides guidance for developing breakpoints and QC ranges for AST according to CLSI human AST documents. It describes the data used to establish these breakpoints and QC ranges for antimicrobial agents, including microbiological data, PK/PD characteristics, and clinical data.
  - M23S, Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria CLSI, 2nd Edition
  - M23S2, Process to Submit Disk Content (Potency) Data for Joint CLSI-EUCAST Working Group Review and Approval, 2nd Edition
  - M23S3, Procedure for Confirming the Acceptability of Mueller-Hinton Agar Sources for Subsequent Use in CLSI and/or EUCAST Studies to Establish Disk Diffusion Quality Control Ranges, 1st Edition
  - VET02, Development of Quality Control Ranges, Breakpoints, and Interpretive Categories, for Antimicrobial Agents Used in Veterinary Medicine, 4th Edition
    - VAST Subcommittee
    - Provides recommendations for developing QC ranges, agar disk diffusion zones of inhibition breakpoints, and dilution MIC breakpoints for AST for aerobic bacteria isolated from animals and performed by CLSI AST standards. It describes the data used to establish these QC ranges, breakpoints, and interpretive categories for antimicrobial agents intended for veterinary use.
- Subcommittee Decision Processes
  - Process 1
    - Ad Hoc Working Groups (AHWGs) hold discussions to complete assigned task. AHWGs may work closely with a sponsor. A vote (poll) may be taken among AHWG members to reach consensus.
    - AHWG Chairholders present a detailed summary of AHWG's efforts to the WG. WG conducts preliminary vote (poll) on WG recommendation to the SC.
    - WG Chairholder presents a detailed summary and the WG recommendation to the SC. SC conducts the final and official vote.
    - NOTE: At the discretion of the SC Chairholder, an AHWG may present directly to the SC.
  - Process 2 (No AHWGs)
    - Sponsors or WG participants present a detailed summary of efforts to the WG.
    - WG discusses and conducts preliminary vote (poll) on WG recommendation to the SC.

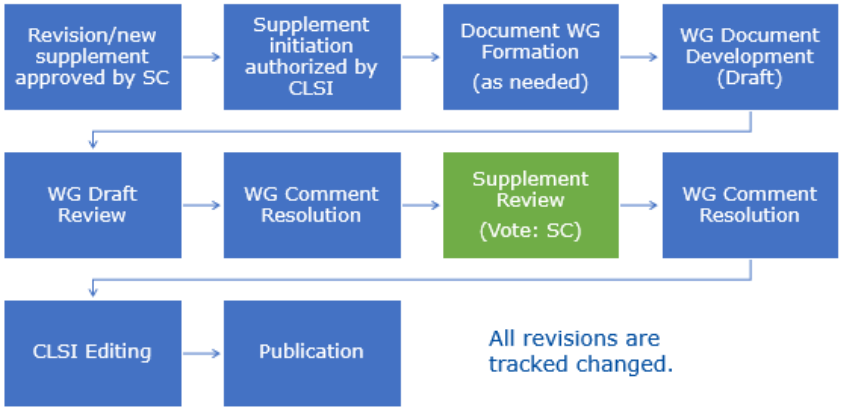
- WG Chairholder presents a detailed summary and the WG recommendation to the SC. SC conducts the final and official vote.
- NOTE: At the discretion of the SC Chairholder, a sponsor or CLSI participant may present directly to the SC.

- Document Types
  - Standards and Guidelines
    - Standards
      - Identifies specific, essential requirements for materials, methods, or practices for voluntary use in an unmodified form
      - May also contain discretionary elements, which are clearly identified
    - Guidelines
      - Describes criteria and recommendations for a general operating practice, method, or material for voluntary use
      - Can be used as written or modified by the user to fit specific needs
    - Developed through the CLSI Document Development Consensus Process
  - Supplements
    - Supplements to published microbiology standards or guidelines support the scope, purpose, methodology, and performance of the associated approved consensus document by providing information that updates or refines its use
    - Are developed by WGs with review and comments by the applicable SC
    - Due to the extremely detailed and technical nature of their contents, supplements are developed through a process that has limited consensus
  - Reports and Derivative Products
    - Reports
      - Summarizes factual information without providing specific recommendations
      - Published for informational purposes only
      - Do not contain technical or procedural recommendations
      - May become guidelines through the Consensus Document Development Process
    - Developed through the Derivative Product Development Process
  - Rationale Documents
    - Important for medical laboratories, drug and device manufacturers, and regulatory and accreditation organizations to understand how AST breakpoints and interpretive categories are determined
    - Provide the scientific reasons behind the SC's decisions, along with documentation of the standardized data and methods used to determine breakpoints
    - Used for FDA review and recognition of CLSI breakpoints
      - CLSI and FDA have a separate process for FDA recognition of breakpoints
      - Meet quarterly with FDA
- Subcommittee Document Processes
  - Revisions
    - Two types of revisions: Full and Limited
    - Limited Revisions:
      - No changes to the document's scope, purpose, or intended audience
      - Shall not significantly alter the methodology used or application of the consensus document
  - Standards and Guideline Process (Full Revision)

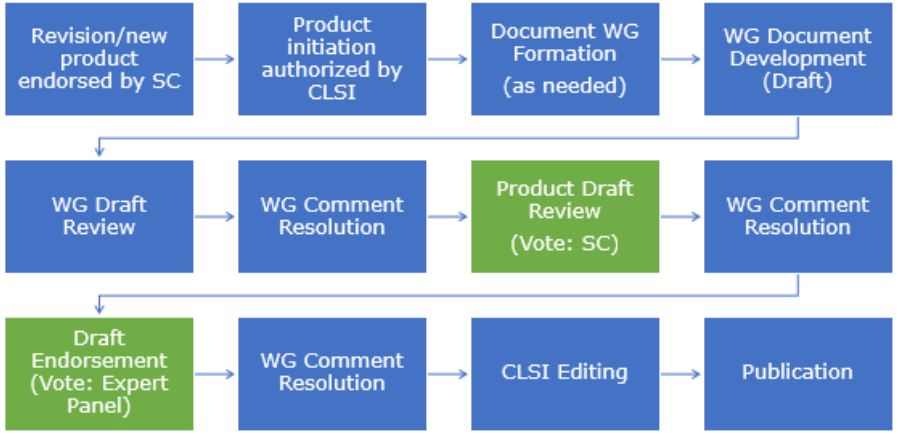


- Standards and Guidelines Process (Limited Revision)

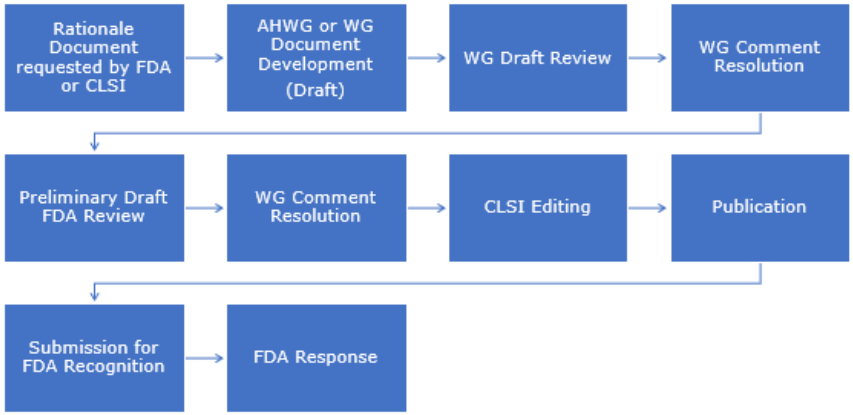
- Supplement Process



- Reports and Derivative Products Process



- Rationale Document Process


Rationale Document requested by FDA or CLSI
AHWG or WG Document Development (Draft)
WG Draft Review
WG Comment Resolution
Preliminary Draft FDA Review
WG Comment Resolution
CLSI Editing
Publication
Submission for FDA Recognition
FDA Response

7. **SUBCOMMITTEE ON ANTIFUNGAL SUSCEPTIBILITY TESTS UPDATE (N. WIEDERHOLD)**

Dr. Wiederhold provided an update on the activities of the Subcommittee of Antifungal Susceptibility Tests (AFST SC). The main points included:

- Document Status (Methods) – 5 year review

Document #	Document Type	Edition	Publication Date	Final Due Date for Next Review	Reaffirm/ Revise/ Archive	Comments
M27, *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*	Standard	4th	11/2017	2022	To be revised	• Revision in progress
M38, *Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*	Standard	3rd	11/2017	2022	To be revised	• Revision in progress
M44, *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts*	Guideline	3rd	12/2018	2023	To be revised	• Revision in progress
M51, *Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi*	Guideline	1st	10/2010	N/A	Archived 2021	• Archiving approved by Council in Fall 2021 • Can be revised if needed.
M57, *Principles and Procedures for the Development of Epidemiologic Cutoff Values for Antifungal Susceptibility Testing*	Guideline	1st	4/2016	2026	Reaffirmed 2021	• Reaffirmation approved by Consensus Council in Fall 2021 • Must be reviewed in 2026

Next

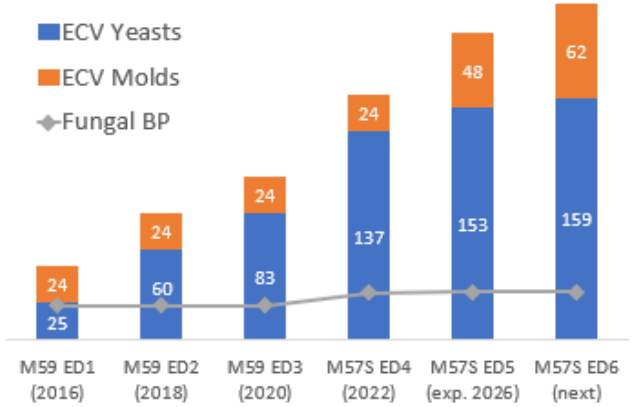
- Document Status (Supplements) – yearly review possible

Document #	Document Type	Edition	Publication Date	Final Due Date for Next Review	Reaffirm/ Revise/ Archive	Comments
M57S (formerly M59), *Epidemiological Cutoff Values for Antifungal Susceptibility Testing*	Supplement	4th	8/2022	Yearly/As needed	N/A	• Revision recoded as M57S • Published August 2022 • Revision WG formed, revision to begin in near future
M27M44S (formerly M60), *Performance Standards for Antifungal Susceptibility Testing of Yeasts*	Supplement	3rd	8/2022	Yearly/As needed	N/A	• Revision recoded as M27M44S • Revision in progress
M38M51S (formerly M61), *Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi*	Supplement	3rd	8/2022	Yearly/As needed	N/A	• Revision recoded as M38M51S • Revision in progress

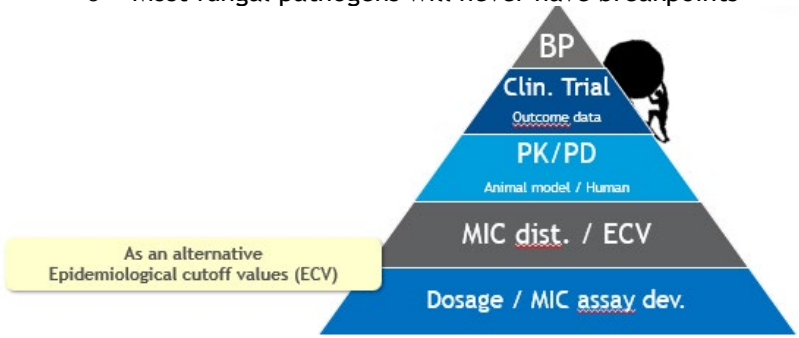
Priority

With CLSI editors - both M27M44S and M38M51S on track for Spring publication

- CLSI Antifungal ECVs and Breakpoints
  - Next M57S 5th Edition will cover 201 ECVs
  - 4X increase in available ECVs (breakpoints relatively stable)
  - Effort to close ECV gap with molds



- Antifungal Outcome Data is Lacking for Breakpoints
  - Often no clinical outcome – PK/PD data for less common species
  - Not enough cases or isolates with MIC
  - Patient with other disease and underlying conditions
  - Not economically feasible for released antifungals
  - Most fungal pathogens will never have breakpoints



- New ECVs Established (Dallas Smith and Shawn Lockhart)
  - 13 new ECVs accepted

Fonsecaea monophora	*Purpureocillium lilacinum*	Ibrexafungerp vs. *Candida*
• Itraconazole (2 µg/ml) • Posaconazole (0.25 µg/ml) • Voriconazole (1 µg/ml)	• Voriconazole (1 µg/ml) • Posaconazole (2 µg/ml) • Isavuconazole (4 µg/ml) • Terbinafine (2 µg/ml)	*C. albicans* (0.25 µg/ml) *C. auris* (1 µg/ml) *C. glabrata* (0.5 µg/ml) *C. orthopsilosis* (0.5 µg/ml) *C. parapsilosis* (0.5 µg/ml) *C. tropicalis* (0.25 µg/ml)

    - Votes on several ECVs tabled
        - *Phialophora verrucosa* species complex (can an ECV be set for SC?)
        - *Paecilomyces variotii*
    - Discussion on targeting ECVs for dermatophytes
- New Methods Appendix A AHWG
    - Appendix A. Suggestions for Confirming Antimicrobial Susceptibility Test Results and Organisms Identification for Agents Approved by the US Food and Drug Administration for Clinical Use
    - Mission - To review Appendix A with Bacterial, Fungal, and VET groups to align definitions for Category I, II, III
    - AFST does not have an equivalent to M100 Appendix A
        - SC was supportive of creating an Appendix A for AFST documents
            - Yeast – M27M44S
            - Filamentous fungi – M38M44S
        - Will establish AHWG under Reporting Working Group for this purpose
- Summary of Reporting Working Group
    - Intrinsic Resistance (IR)
        - New Expected Resistance Definition – AST, AFST, and VAST SCs approved
            - Moving forward with new definition
            - IR may be reported as IR or R
            - Expected Clinical Failure (ECF) = R* (pushback from SC members and advisors)
            - Need to educate CLSI users (labs and clinicians)
    - Body Site Reporting
        - Updates on testing and reporting MICs against *Candida* for:
            - Amphotericin B vs CNS and ocular isolates
            - Also revision to rationale language for lipid Amphotericin B for urine isolates
            - Echinocandins vs CNS, ocular, and urine isolates
            - 5-flucytosine vs CNS, ocular, and urine isolates
- Summary of Breakpoint Working Group
    - Breakpoints for voriconazole and isavuconazole vs *A. fumigatus* established and accepted by FDA
    - Posaconazole breakpoints in process
        - Collection of *A. fumigatus* strains (WT and defined Cyp51A mutations) being tested by different labs for reproducibility
    - Breakpoints for rezafungin vs *Candida* spp. established

	▪ Rationale document with FDA for pre-IND review and comments ○ *Candida auris* Breakpoint AHWG established **SC DISCUSSION (MAIN POINTS)** • There were discussions how the subcommittees should work together on how to adopt the new "expected resistance" definitions. It was recommended that the 3 subcommittees meet to come to consensus on how to approach table creation and standardization across the various subcommittees. • The Intrinsic Resistance Definition AHWG could take this on as there are representatives from all subcommittees on this working group. • There should be consensus on what the ECV means and the definition from the various subcommittees. • The antimycobacterial document (M24S) should also consider adding an Appendix A and standardizing with the other documents.

8. **EUCAST UPDATE (S. GATERMANN)**

Dr. Gatermann provided an update on the activities of EUCAST. The main points included:

- Gepotidacin
  - Triazaacenaphthylen
  - Inhibits gyrase and topoisomerase IV but mechanism different from fluoroquinolones
  - uUTI
  - MHRA approval August 2025
  - Dosage: 2 x 1500 mg oral

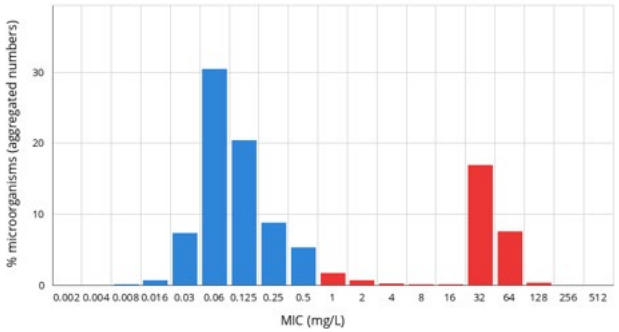
	S ≤	R >
E. coli	8	8
E. faecalis	8	8
S. saprophyticus	0.25	0.25

- Trimethoprim-sulfamethoxazole: Revised breakpoints

Species	(T)ECOFF	breakpoints V 15.0 S ≤ / R>
Enterobacterales[1]	0.5	2/4
E. coli	0.5	2/4
K. pneumoniae	0.5	2/4
Acinetobacter spp[2]	0.5	2/4
Staphylococcus spp.[3]	(0.25)	2/4
S. aureus	(0.25)	2/4
Streptococcus A/C/G	0.5[4]	1/2
S. pneumoniae	1	1/2
H. influenzae	0.5	0.5/1
M. catarrhalis	1	0.5/1
Aeromonas spp.[5]	(1)	2/4

Species	breakpoints V16.0 (based on ECOFFs) S ≤ / R>
Enterobacterales except *Serratia*	0.5/0.5
Serratia spp.[1]	0.001/2
Acinetobacter spp.	0.5/0.5
Staphylococcus spp.[2]	0.5/0.5
Streptococcus A/C/G	0.5/0.5
S. pneumoniae	1/1
H. influenzae	0.5/0.5
M. catarrhalis	1/1
Aeromonas spp.	1/1

E. coli



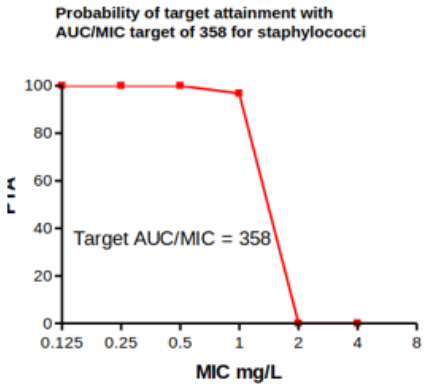
- Comment Carbapenemases
  - Some carbapenems may tests susceptible in presence of a carbapenemase
  - Some data suggests that outcome may not be favorable in such cases
  - If carbapenems are used, a high dose and combination may be necessary
  - Several new agents are recommended in clinical guidelines
  - "If a carbapenemase is detected the clinical response to treatment with carbapenems may be impaired even in the absence of clinical resistance. Other novel antimicrobials should be preferred treatment, but if unavailable, carbapenems could be considered if high exposure is used and/or in combination with a second active agent. Consider switching therapy in complicated infections."
- Daptomycin

licensed for *S. aureus* only, doses 4-6 mg/kg

	ECOFF
S. aureus	1 mg/L
CoNS	1 – 4 mg/L

breakpoints V 16.0

	S ≤	R >
S. aureus	1	1
CoNS	Note	Note



EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Guidance document on use of daptomycin to treat infections with enterococci or coagulase-negative staphylococci

Updated December 2025
(minor revision from previous version to include Coagulase-negative staphylococci treatment)

- Minor Changes
  - Staphylococci: report cefazolin, cefotaxime, ceftriaxone, cefuroxime iv, susceptible increased exposure in MSSA
  - *S. pneumoniae*
    - aminopenicillin breakpoints lowered to ECOFF for meningitis and endocarditis
    - cefazolin, cefadroxil, cefalexin no longer '-' but IE

- *Campylobacter coli:* Erythromycin disk breakpoints revised
- RAST
  - aztreonam and aztreonam-avibactam breakpoints
  - trimethoprim-sulfamethoxazole breakpoints revised for *A. baumannii*
- In the Pipeline
  - V 16.1 expected in May
    - more anaerobes
    - breakpoints carbapenems
    - breakpoints fluoroquinolones
  - Consultations
    - carbapenem breakpoints
    - fluoroquinolone breakpoints
    - EUCAST dosing tab adapted to pediatric use
    - VetCAST tables

SC DISCUSSION (MAIN POINTS)

- The question was asked if there was any updated guidance on the trimethoprim-sulfamethoxazole changes. EUCAST is working on publishing a rationale document. There was a lack of clinical data, so it was decided to lower the breakpoints to the ECV for most organisms.
- There was further discussion on the aminopenicillin breakpoints for *S. pneumoniae* being lowered for meningitis and endocarditis. The penicillin breakpoints were at the ECOFF already, so the aminopenicillin breakpoints were also set to the ECOFF as there is no clinical data to support anything aside from the ECOFF for these clinical indications.

9. **INVESTIGATIONAL BREAKPOINTS AD HOC WORKING GROUP (A. KHAN AND M. WIKLER)**

- AHWG Plans
  - CLSI definition for ”investigational agents”
  - Review agents currently labeled as investigational in M100
  - Evaluate M100 for scope of global representation
  - Develop structure for CLSI review of investigational breakpoints
- New CLSI Definition of Investigational Agents
  - Motion passed in June 2025 CLSI meeting for standardized definition between M23 and M100
  - Revise M100 definition of investigational breakpoints to ”antimicrobial agents not yet approved by any regulatory agency”
  - Vote: 13-0-0-1

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.

  - “Other” Agents

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

Designation	Definition	Test	Report[a]	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. Approved to change to "Any regulatory agency"	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.

  - AHWG Proposed Standardized Definitions

	Approved by any regulatory agency	Approved after M23 process	Commonly used in US?	Housed
Full breakpoints	Yes	Yes	Yes	M100, Table 1&2
Investigational	No	No		CLSI website
* Other	Yes	Yes	No	M100, Table 2

- Legacy agents still labeled as “INV.” in M100 that no longer fit new definition
  - Breakpoints set when agents were new but these are now approved and have not been “transitioned”
  - These agents were not reviewed per M23 process
    - Pefloxacin
    - Cefetamet

- Ceftibuten
- Fleroxacin
- Teicoplanin

- Preliminary info on legacy agents still labeled as "INV."
  - Breakpoints set when agents were new, pre-approval but were not reviewed per M23 process after approval obtained

Agent	Organism	Breakpoint use	Not approved in	Approved but no longer used in	Approved, used in
Cefetamet	Enterobacterales (except *Morganella*), *Haemophilus*	Oral	USA, Canada, Australia, NZ	Western Europe	Asia, Africa, MENA, Latin America, Eastern Europe
Ceftibuten	Enterobacterales	Oral, urine only		North Am, West Europe, Aus/ NZ	

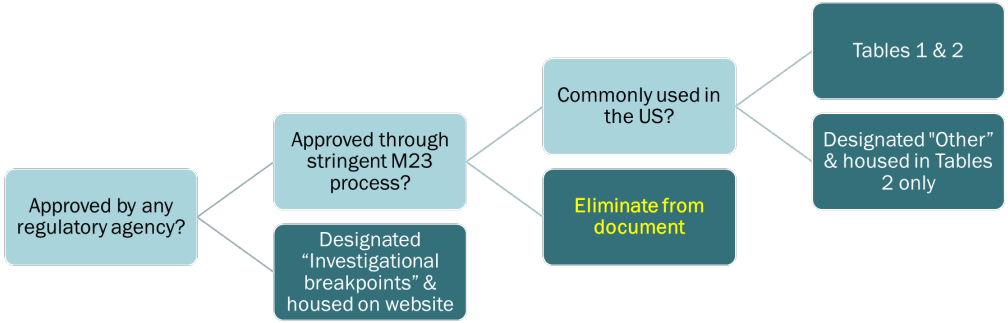
MENA= Middle East and North Africa

- Legacy agents still labeled as "INV." in M100
  - Breakpoints set when agents were new, pre-approval but were not reviewed per M23 process after approval obtained

Agent	Organism	Breakpoint use	Not approved in	Approved but no longer sold, or used in	Approved, used in
Pefloxacin	*Salmonella, Shigella*	Surrogate marker for ciprofloxacin	USA, Australia, Canada, NZ	Western Europe, Japan, South Korea	Africa, SE Asia, limited use in parts of MENA/ Latin America/ Eastern Europe
Fleroxacin	Enterobacterales, *S. aureus, Haemophilus*		USA, Canada, Aus/ NZ	Western Europe (safety issues), Japan, South Korea	Asia, parts of Africa

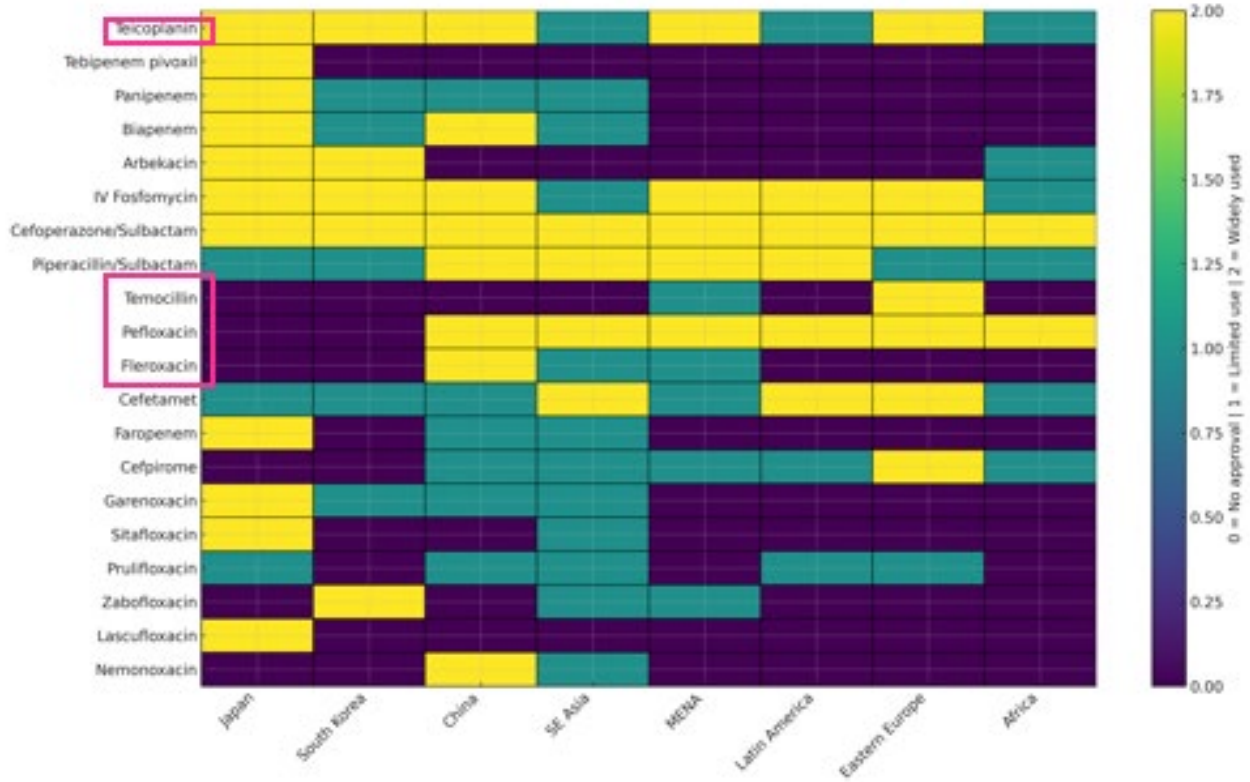
Agent	Organism	Not approved in	Approved but no longer sold, or used in	Approved, used in
Teicoplanin	*S. aureus,* Enterococci (mostly *E. faecalis*)	USA	Canada, Aus/ NZ	Europe, Asia, Latin America, MENA, Africa

- Definition Decision Tree
  - AHWG Recommendation: Eliminate agents currently designated as "investigational" in M100 (now approved, have not undergone M23 review process)



- Cefepime-zidebactam Investigational Breakpoints
  - Investigational breakpoints set but not published
  - Status of agent: Wockhardt submitted to DCGI (India) and FDA (October 2025), "fast track" status granted so approval ETA mid to end 2026
  - AHWG Recommendation: To create investigational breakpoints page on the CLSI website and add cefepime-zidebactam as INV agent
- Evaluate global, non-US gap agents
  - Approved agents not used in the US but still used in other regions globally and absent from M100
  - CLSI aims to serve laboratories worldwide but how are we doing in practice?
  - Does CLSI M100 adequately represent and include breakpoints for agents that are NOT used in the United States but widely used in other regions globally?
  - Global non-US gap agents
    - Type 1: Non-FDA approved agents that are approved by another regulatory agency

In M100 as "INV"



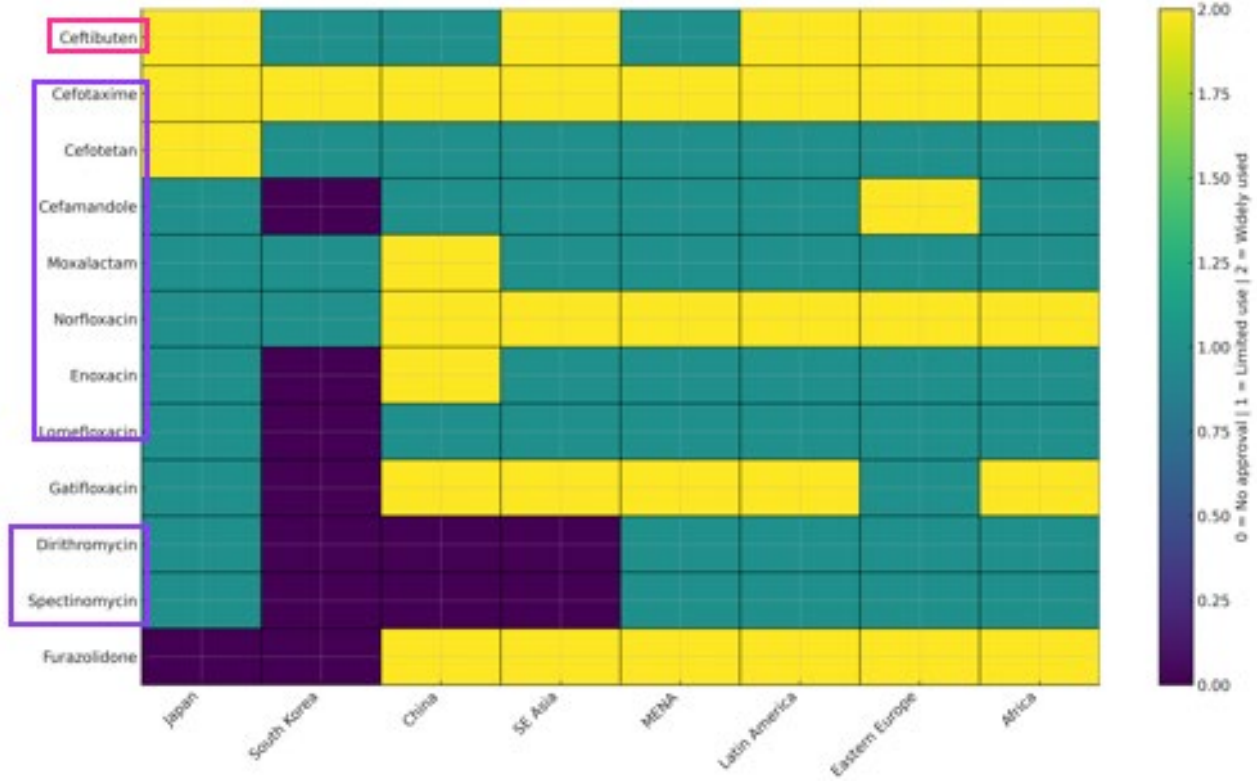
Agent	Organism	Regulatory/ use status
Tebipenem (new oral carbapenem)	*Haemophilus, Moraxella, S. pneumoniae*, Some Enterobacterales (*E. coli, K. pneumoniae, P. mirabilis*)	• Approved in Japan **only** • Pending FDA approval, received fast track designation (Oct 2025)
Panipenem (old IV carbapenem)	*Enterobacterales, Haemophilus, Moraxella*, Streptococci, *S. aureus*, some anaerobes	• Approved & in limited use in SE Asia (Japan, South Korea mainly)

- Type 2: FDA-approved agents that are no longer sold/ used in the US but are still used in other regions globally

In M100 as "INV"

In M100 as "OTHER"

*Many of these have not been updated recently



Agent	Organism	Regulatory/ use status
Fosfomycin IV	*S. aureus,* CoNS, *E. faecalis,* Enterobacterales, some anaerobes, etc	• Not FDA approved • Approved, in use in EU, Asia, MENA
Arbekacin	*S. aureus*	• Not FDA approved • Approved in Asia Pacific, anti-MRSA use in Japan
Cefoperazone -sulbactam	*Acinetobacter,* Enterobacterales, *Haemophilus, Neisseria,* anaerobes	• Not FDA approved • Approved & in use in Asia, MENA, Eastern Europe, Latin America (highly used across global south)
Piperacillin-sulbactam	Enterobacterales, anaerobes, *Acinetobacter,*	• Not FDA approved • Approved, in use in Asia (esp China/ India), MENA, Latin America– used due to affordability vs pip-tazo

- AHWG Recommendation: Review the “Global non-US gap agents” and create priority list of agents missing from M100 that are potentially worthy of breakpoints review

- Develop structure for CLSI review of breakpoints
  - Need for more structured guidance for companies to approach CLSI to set breakpoints (timeline, how to initiate, what about if etc)
    - This applies to any breakpoints including investigational
    - M23 has recommendations but no clear timeline/ procedural info on stages of review
    - AHWG will also discuss procedure to transition agents out of “investigational” status once they are approved ▫ full M23 review required for breakpoints
  - Current breakpoints process relies on companies approaching CLSI
    - This can lead to inequity for drugs used in the global south
    - AHWG Recommendation: CLSI should do proactive outreach for valuable agents that need breakpoints (all agents, including investigational)

SC DISCUSSION (MAIN POINTS)

- The AHWG is encouraged to revisit the changes to Tables 1 and the labelling of agents as “Inv” and “Other” that occurred over the last couple years to ensure consistency and reasoning.
- Discussion ensued on how to handle agents that were designated as “Inv” agents that are now approved outside of the US but have not undergone a full M23 review. The AHWG proposed removing them to a website until reviewed. However, there was concern about the resources needed for a M23 review and the lower priority compared to other ongoing items.
- One suggestion was not to go backwards but to define a process moving forward.
- Another suggestion was to evaluate what other regulatory or standards development organizations have established for breakpoints with these agents and compare them to what is currently in the M100.
- Clear guidance is needed on how to use “Inv” moving forward and publishing investigational breakpoints. Currently they are being housed on the MicroFree website on the right hand side away for the documents (eg, cefepime-zidebactam investigational breakpoints).
- There is a possibility to place “Investigational” breakpoints on the CLSI website moving forward in the “Resource Files”.
- AHWG was asked to revisit the inclusion of pefloxacin, cefetamet, ceftibuten, fleroxacin, teicoplanin and how to proceed with these agents and present their recommendations at the June meeting. The working group feedback was that these agents are unique (eg, pefloxacin is a surrogate) and need to reviewed individually.

10.	**ANAEROBES WORKING GROUP (D. CARPENTER AND S. COPSEY-MAWER)**
	
	ANAEROBE ANTIBIOGRAM
	• M100- Appendix D- in 2026 publication
	• Publications in preparation (Gram Positive and Gram Negative)
	  ○ Tables completed
	  ○ Working on draft manuscript
	
	EUCAST UPDATE
	• EUCAST version v16.0 published on January 1, 2026
	• No anaerobe updates – v16.1 to come out May/June 2026
	• Discussed EUCAST – ATU (Area of Technical Uncertainty)
	
	OTHER BUSINESS
	• Intrinsic resistant – Expected Clinical Failure
	  ○ How to apply to anaerobes – using antibiogram data
	  ○ Look at data before June meeting
	• CA-SFM Antibiogram committee of French Society of Microbiology
	  ○ Adopted EUCAST guidelines in 2014
	  ○ CA-SFM created a rationale document that is available on the CA-SFM website
	  ○ The purpose of publication and reaching out was to provide awareness and "uncritical" acceptance EUCAST breakpoints
	• Aerotolerant gram-positive (eg, *Actinomyces*) were excluded from the antibiogram. Gap – need data to establish reference method.
	
	NEXT STEPS
	• Finish manuscripts
	• QC and breakpoints for disk diffusion – Monthly meetings between now and June so data is ready to present to AST Subcommittee
	  ○ Clindamycin
	  ○ Metronidazole
	  ○ Meropenem
	• Work with Intrinsic Resistance AHWG – how to apply to anaerobes
	
	SC DISCUSSION (MAIN POINTS)
	• Discussion ensued about where the anaerobes breakpoints should live. Options included a supplement to M11 or within M45.

11. **TEXT AND TABLES WORKING GROUP (A. BOBENCHIK)**

TERMINOLOGY – "ORGANISM" OR "MICROORGANISM"

- Does TTWG prefer to use one or the other?
- "Organism" used 277 times and "microorganism" used 4 times.
- Update to use "organism" throughout for consistency.

DOSAGE FOR TRIMETHOPRIM/SULFAMETHOXAZOLE AND Β-HEMOLYTIC STREPTOCOCCI

- New SXT breakpoints approved for M100-36th Edition, but no dosage comment
- Discussed with Breakpoints Working Group Chairholders and members of the AHWG on proposal of comment option despite the lack of a standard dose used for the breakpoint
  - Different from other dosages in Tables 2, the SXT breakpoint was based on clinical trial dosing
  - Concern that lower doses than what was used in clinical trial might be used
  - Introduction to Table 2 Dosages states "CLSI susceptible or susceptible-dose dependent breakpoints added or revised since 2010 have been based on specific dosage regimen(s); these dosage regimens are listed in the table below."
  - How to address new or revised breakpoints that do not follow the typical standard/specific dosages?
  - How best to convey information and where (eg, footnote in M100 or supplemental material)?
- Examples of a proposed comment/placement in Table 2 Dosages

Antimicrobial Agent	Dosage Regimen Used to Establish S or SDD Breakpoint
Table 2H-1. *Streptococcus* spp. β-Hemolytic Group	
Ceftaroline	600 mg IV q 12 h
Dalbavancin (*S. pyogenes*, *S. agalactiae*, and *S. dysgalactiae* only)	1500 mg IV once or 1000 mg IV once followed 1 wk later by 500 mg IV once
Oritavancin	1200 mg IV once
Tedizolid (*S. pyogenes* and *S. agalactiae* only)	200 mg IV/PO q 24 h
Telavancin	10 mg/kg IV q 24 h
Trimethoprim-sulfamethoxazole	No specific dosage regimen was used due to limited PK/PD data

Antimicrobial Agent	Dosage Regimen Used to Establish S or SDD Breakpoint
Table 2H-1. *Streptococcus* spp. β-Hemolytic Group[1]	
Ceftaroline	600 mg IV q 12 h
Dalbavancin (*S. pyogenes*, *S. agalactiae*, and *S. dysgalactiae* only)	1500 mg IV once or 1000 mg IV once followed 1 wk later by 500 mg IV once
Oritavancin	1200 mg IV once
Tedizolid (*S. pyogenes* and *S. agalactiae* only)	200 mg IV/PO q 24 h
Telavancin	10 mg/kg IV q 24 h

[1]No specific dosage regimen was used for trimethoprim-sulfamethoxazole due to limited PK/PD data

SC DISCUSSION (MAIN POINTS)

- Majority favored placing a comment in line with the agent and add a comment similar to "No specific dosage regimen was used".

TOPICS DISCUSSED WITH METHODS WORKING GROUP

- Appendix A: ß-lactamase Inhibitors (BLI)/Metallo-ß-lactamases (MBL)
  - Conveying that certain BLIs are not appropriate for MBLs
  - Does this information belong in M100? If yes, where? Appendix A, Appendix B, Tables 1, etc?
  - Text and Tables Working Group felt having this information consolidated in M100 would be very helpful for users
- Appendix G – Using Molecular Assays for Resistance Detection
  - Do the tables need to be reviewed/updated?
  - Table G3 – Referral to use of "reference method"
    - For example, expand to say "performed using a reference or standard method…"
    - Is the intent that a reference/standard method is needed or could any other method be used as the arbiter?

TOPICS DISCUSSED WITH QUALITY CONTROL WORKING GROUP

- Appendix C – Quality Control Strains for Antimicrobial Susceptibility Tests
  - With new Appendix I and recommendations for selection of QC, are there any updates to this Appendix of QC strains to help with that information or to complement it?
  - User friendliness: areas for improving how this table is used or how to find relevant information?
  - Thorough review of included information for all QC strains – is it totally inclusive or are updates needed?
  - QC Working Group will also be working on adding colony count information

MECILLINAM METHOD IN TABLE 2A-1

- Table 5A-1 (QC table), footnote v states that mecillinam should be performed by agar dilution, but this is not mentioned in Table 2A-1.
- See fosfomycin as an example

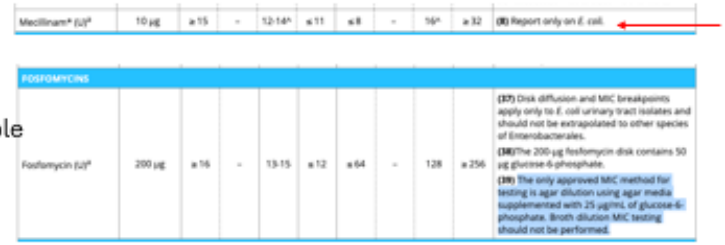
Table 5A-1

Mecillinam	0.03-0.25[v]

v. This test should be performed by agar dilution only.

Table 2A-1

Example



Add new comment?
"The only approved MIC method for testing is agar dilution."

SC DISCUSSION (MAIN POINTS)

- There was discussion about evaluating the fosfomycin comments as well due to new methods being FDA approved.
- A review of all method comments should be undertaken to ensure consistently.

A motion to accept the proposed comment "Only approved reference MIC method for testing is agar dilution." or a similar comment for mecillinam in Table 2A-1. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

TABLES 2C AND 2D TESTING CONDITIONS BOX UPDATES FOR DALBAVANCIN

- Agar dilution is NOT recommended for daptomycin and dalbavancin but no comment included for dalbavancin
  - 2012 poster of broth microdilution vs agar dilution for dalbavancin (from L. Koeth)
  - During meeting, oritavancin was also mentioned as not working by agar dilution (2017 Journal of Global Antimicrobial Resistance paper)
- Amend statement in Testing Conditions box to include dalbavancin and oritavancin

Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.

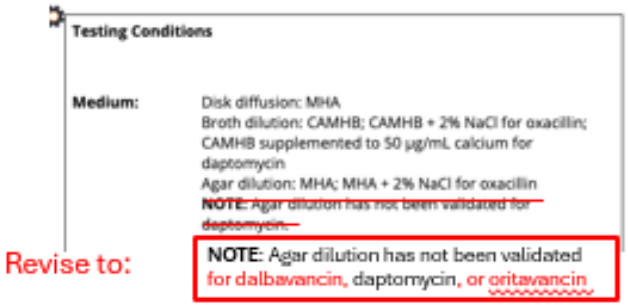
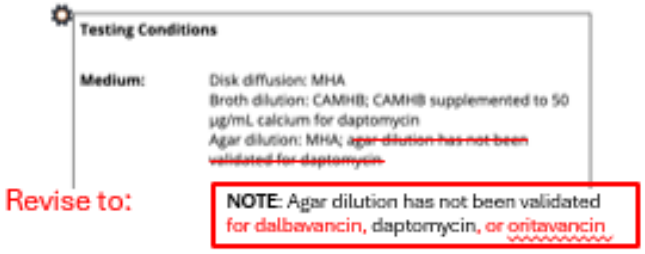


Table 2D. Zone Diameter and MIC Breakpoints for *Enterococcus* spp.



SC DISCUSSION (MAIN POINTS)

- Discussion ensued on how to best handle these types of scenarios where there is data to support the use of a method or discourage a method without any previous mention in M100.
- It might be difficult to address all agents for which agar dilution was evaluated instead of adding to the testing conditions. There was discussion on how much data would be required to add the recommendations.
- There was a suggestion to add a comment to see the QC recommendations and say it implies to isolate testing in addition to QC organisms.

- Action Item: Text and Tables Working Group to look at the specific drugs in these tables and evidence across the classes.

TABLE 2B-5. *BURKHOLDERIA CEPACIA* COMPLEX COMMENT UPDATE

- Should specific drugs be added to the comment or add a more concise comment?
- It was suggested to change the current comment in CLSI M100 36th Edition, Table 2B-5 to: "Antimicrobial susceptibility testing is not performed for organisms of the *B. cepacia* complex due to issues with method accuracy and limited clinical outcome data." However, the feedback was to keep the specifics to the comment because it is valuable data for the laboratories to understand the reason why breakpoints were removed.

Current comment in CLSI M100-Ed35, Table 2B-5:
"(3) Laboratories can consider adding the following comment to the laboratory report: "Antimicrobial susceptibility testing is not routinely performed for *B. cepacia* complex due to the lack of accurate test methods. MICs for ceftazidime, levofloxacin, meropenem, minocycline, or trimethoprim-sulfamethoxazole with wild-type isolates are high and might be above the MICs typically achievable by routine antimicrobial dosing."

Proposed comment amendment as published in CLSI AST News Update Volume 11 Issue 1, November 2025:
"(3) Laboratories can consider adding the following comment to the laboratory report: "Antimicrobial susceptibility testing is not performed for organisms of the *B. cepacia* complex due to issues with method accuracy and limited clinical outcome data. Consultation with an Infectious Diseases specialist is highly recommended."

Table 2B-3. MIC Breakpoints for *Burkholderia cepacia* Complex

Testing Conditions		QC Recommendations
Medium:	Broth dilution: CAMHB	Refer to the following: • Table 5A-1 that lists acceptable QC ranges • Appendix I to develop a QC plan
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard	
Incubation:	35°C ± 2°C; ambient air; 20–24 hours	

General Comments

(1) Minimal inhibitory concentration (MIC) and disk diffusion breakpoints for *B. cepacia* complex organisms were removed based on data showing that two CLSI reference antimicrobial susceptibility testing (AST) methods, broth microdilution (BMD) and agar dilution, do not correlate. These findings are supported by additional studies conducted by European Committee on Antimicrobial Susceptibility Testing (EUCAST) and a Brazilian study demonstrating problems with *B. cepacia* complex AST.[1,2]

(2) Epidemiological cutoff values (ECVs) are available in Appendix F, which are for epidemiological use only. In several cases, ECVs are above MICs typically achievable by routine antimicrobial dosing for similar organisms.

(3) Laboratories can consider adding the following comment to the laboratory report: "Antimicrobial susceptibility testing is not routinely performed for *B. cepacia* complex due to the lack of accurate test methods. MICs for ceftazidime, levofloxacin, meropenem, minocycline, or trimethoprim-sulfamethoxazole with wild-type isolates are high and might be above the MICs typically achievable by routine antimicrobial dosing."

(4) If testing is performed, reference BMD (frozen) is the only reproducible method and laboratories might consider including the comment, "correlation of MIC values with clinical outcome is not known."

SC DISCUSSION (MAIN POINTS)

- Text and Tables Working Group suggested mocking up and modifying the language for the suggested comment but to keep the specifics about "MICs for ceftazidime, levofloxacin, meropenem, minocycline or trimethoprim-sulfamethoxazole with wild-type isolates are high and might lie above the MICs typically achievable by routine antimicrobial dosing" within the same section of M100.
- Action Item: Text and Tables Working Group and Dr. Mathers to revise comment 3 in the *B. cepacia* complex table.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

HARMONIZE TABLE 2 GENERAL COMMENT REFERENCES TO CLSI M02 QUICK GUIDE

2A-1

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see CLSI M02[2]). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see CLSI M02-Ed14-QG[3]). Hold the petri dish a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., ignore the thin veil of swarming growth in an otherwise obvious zone of growth inhibition. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

Also in: 2B-1, 2B-2, 2B-4, 2C, 2G, 2H-1
(N/A for 2B-3 & 2J)

2D

(4) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the petri dish a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

Add at end of 2nd sentence

2E

(4) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the petri dish a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

Add at end of 2nd sentence

2F

(2) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. For some agents, eg, fluoroquinolones or cephalosporins, only 2 to 3 disks may be tested per plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the petri dish a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

Add at end of 3rd sentence

2H-2

(2) For disk diffusion, measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not measure the zone of inhibition of hemolysis. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

Add at end of 1st sentence

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

TIER 2 QC

DEBIO-1452

- Background

Drug Name:	Debio 1452 (0.1 µg disks)			Votes						
QC Strain	**Range**	**% In**	**Median**	**Mm**	**Disk**	**Media**	**Labs**	**Gavan**	**Range Finder**	**Comments**
S. aureus ATCC 29213 – Initial	20-26	97.5%	23	7	22, 24	3@23	1@21, 1@22, 4@23, 3@24	20-26, 7, 97.5%	19-27, 9, 100%	Additional data requested due to 2 mm difference in disk
S. aureus ATCC 29213 – New lot disks	17-23	96.8%	20	7	20, 21	19, 20, 2@21, 22	19, 2@20, 2@21	17-23, 7, 96.8%	NA	Only 5 labs. Median is lower with new data.
S. aureus ATCC 29213 – Combined	19-25	94.9%	22	7	22, 23	2@21, 22, 2@23	NA	19-25, 7, 94.9%	NA	Alternative range 19-26, 8mm, 98.0% Add footnote indicating supplemental QC with approved range.

June 2025
Approved range for *S. aureus* ATCC 25923. Summary provide on subsequent slides. For informational purposes only.
Decision: Since Tier 2 data was available, pursue *S. aureus* ATCC 29213 as option for supplemental QC for troubleshooting (tighter range and easier to read) .

Media: Original Tier 2 - BBL, Bio-Rad, Hardy, New disk lot testing – BBL, Bio-Rad, Hardy, Oxoid, Remel
Disks: Mast, Oxoid (same for original Tier 2 and new disk lot study)

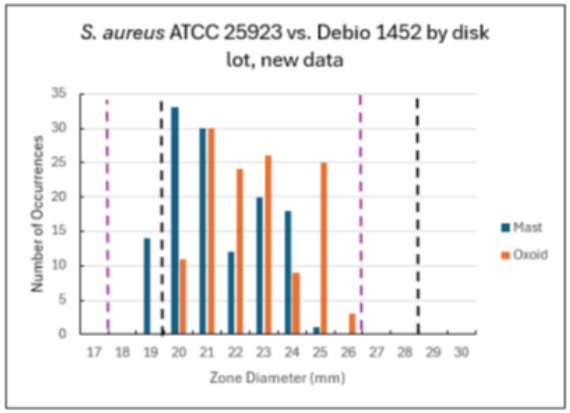
- Proposed Disk Diffusion QC Ranges

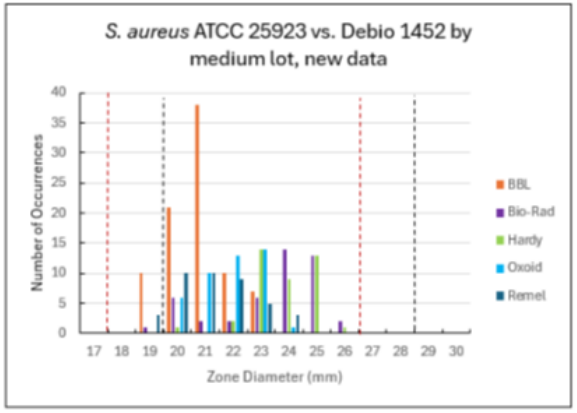
Drug Name:	Debio 1452 (0.1 µg disks)	Votes	June 2025 11/0/1/2 No range change (20-27). Add to Tier 3. Pursue *S. aureus* ATCC 29213

QC Strain	Range	% In	Median	Mm	Disk	Media	Labs	Gavan	Range Finder	Comments
S. aureus **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%, 9mm	Variability: Disk 2mm, Media 1mm, Lab 5mm Media mfg lot 2 mode 23mm
S. aureus **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.
S. aureus **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	
S. aureus **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. With no change made to disk manufacturing median ranged from 23, 25, 21, 22).
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.
Decision: Leave range as 20-27 (still >95% in range). Add to Tier 3 to monitor. Pursue *S. aureus* ATCC 29213 as option for supplemental QC for troubleshooting (tighter range and easier to read, data is available).

- Re-test New Disk Analysis – June 2025

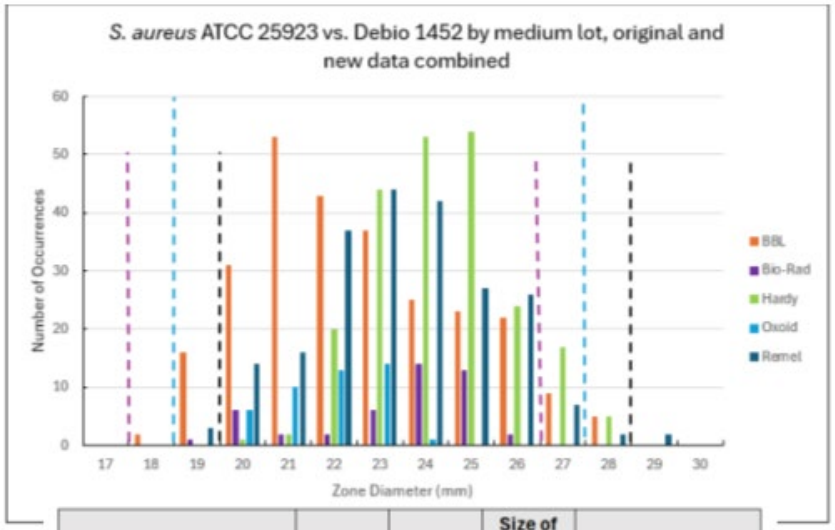




Refer to page 7:
Disk manufacturers vary by 1 mm.
Media manufacturers: Original Tier 2 only varied by 1 mm, Re-test varied by 4 mm with BBL and Remel at low end, Hardy and Bio-Rad at high end

QCWG 2025 June 6

- Original and Re-test Combined – June 2025



Summary	Range	Range	Size of range	% in range	
Gavan Statistic	Initial	20-28	9	98.1%	530/540
Rangefinder	Initial	20-28	9	98.1%	530/540
Gavan Statistic	New	18-26	9	100%	256/256
Gavan Statistic	Combined	19-27	9	98.0%	780/796

DEBIO-1453

- Background

Drug: Debio 1453	**Abbreviation (Glossary II & III):** TBD	**Previous ID:** DPM016544
Solvent (Table 6A): DMSO	**Diluent (Table 6A):** DMSO	**Preparation (Table 6C combination agents):** ??
Route of administration (Glossary II): IM and PO	**Class (Glossary I & II):** TBD	**Subclass (Glossary I & II):** TBD
Study Report by: Micriobiologics	**Pharma Co:** Debiopharm	**Control Drugs:** Ciprofloxacin

Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, etc): No significant impacts seen • **ISO/TS 16782 assessment of Tier 2 study materials**: ??
Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC)**: ??
Discussion	Tier 1 Agar Dilution Summary: Media from Difco and HiMedia yielded the same distribution for 19 isolates although Difco resulted in more robust growth and easier reads 3-fold higher or lower than standard inocula yielded similar results Shift in agar pH from standard at 7.1 to either 6.8 or 7.7 had little to no impact on the observed MIC Tier 1 range for ATCC 49226 = 0.03-0.12 µg/mL Tier 2 Study: GC Agar media manufacturers: Neogen, Remel, Hardy. Isovitale X: 2 lots from BD Colony counts (n= 65): mean 1.2 x 10^8 CFU/mL ± 9.66 x 10^7 CFU/mL; range 5.00 x 10^6 – 3.55 x 10^8 Ciprofloxacin control – 100% in range

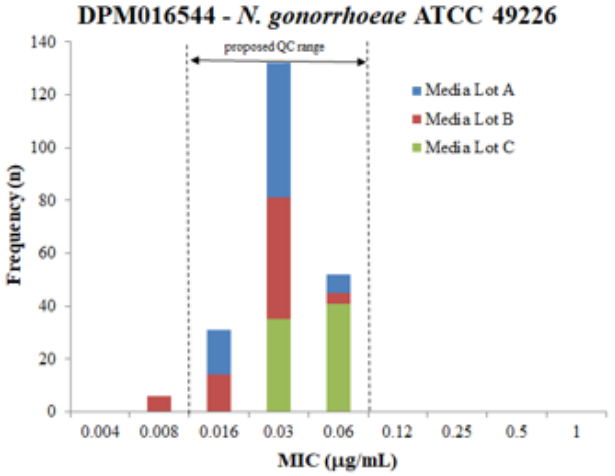
- Proposed MIC QC Ranges

Drug Name:	Debio 1453	Votes:	8/0/0/1 For/Against/Absent/Abstain

QC Strain	Range	% In	Mode	Dil	Shoulder	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
N. gonorrhoeae ATCC 49226	0.016-0.06	97.3%	0.03	3	39% @ 0.06	2@0.03, 0.06	7@0.03, 0.06	0.016-0.06	0.016-0.06	Some media variability

- Lab 3: 6 replicates on Lot B not reported due to no growth
- For lab 6, 4 replicates of data (n=12) were removed due to technical issue (10-fold under inoculation based on smaller volume delivered with site 6 pins) and 1 replicate of lot A (n=1) was removed due to no growth
- Some media variability (lot C had slightly higher MICs); overall the data was still tight distributing across 3 dilutions for nearly all replicates with a strong mode at 0.03 µg/mL

MIC (µg/mL)	Lot A	Lot B	Lot C	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Total
0.004												
0.008		6							1	5		6
0.016	17	14				9	5	5	1		10	30
0.03	51	46	35	11	20	10	25	20	12	15	20	133
0.06	7	4	41	19	10	5		5	3	10		52
0.12												
0.25												
0.5												
1												
Total	75	70	76	30	30	24	30	30	17	30	30	221
GEOMEAN	0.028	0.025	0.044	0.047	0.038	0.027	0.027	0.030	0.030	0.030	0.024	0.031
MODE	0.03	0.03	0.06	0.06	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
MIN	0.016	0.008	0.03	0.03	0.03	0.016	0.016	0.016	0.008	0.008	0.016	0.008
MAX	0.06	0.06	0.06	0.06	0.06	0.06	0.03	0.06	0.06	0.06	0.03	0.06
RANGE	3	4	2	2	2	3	2	3	3	4	2	4



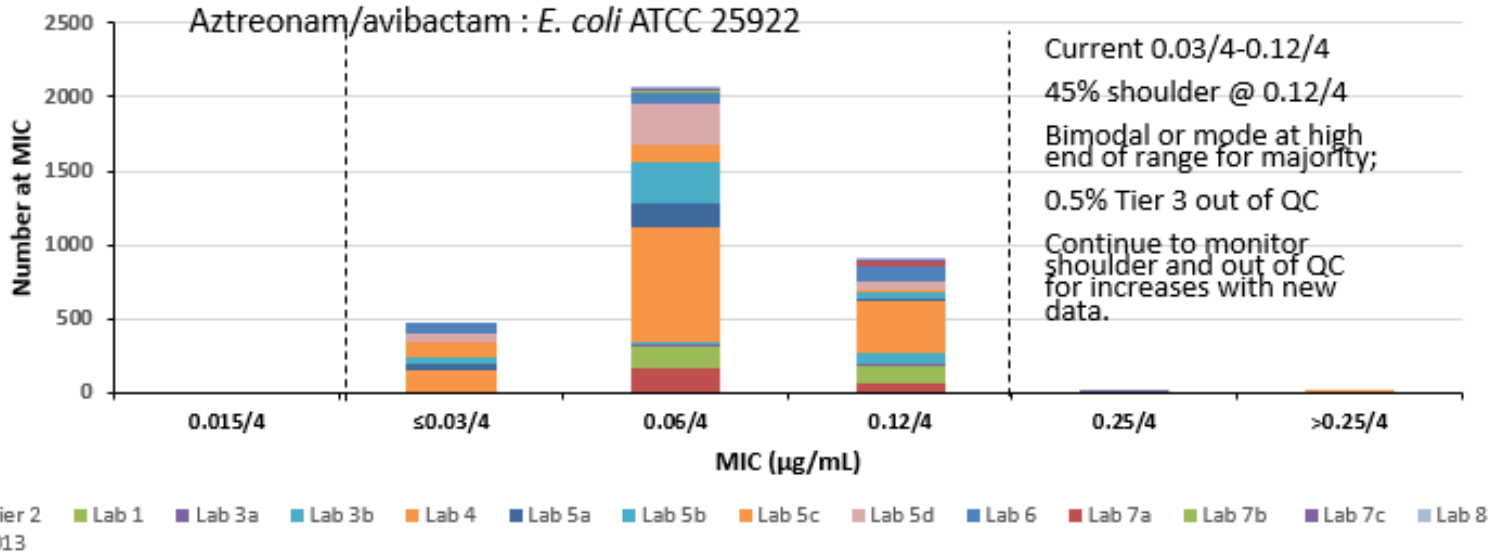
QCWG 2026 Jan

A motion to accept the Debio 1453 MIC QC range for *Neisseria gonorrhoeae* ATCC 49226 (0.016-0.06 µg/mL). Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

TIER 3 MIC QC (PRESENTED BY E. GARRETT)

QC Strain	Antimicrobial	Current Range	Action Recommended	Concern/Analysis	Reported
E. coli ATCC 25922	Aztreonam/ avibactam	0.03/4-0.12/4	With new data, shoulder at 45% and <1% out of QC. No change recommended. 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. <1% out of QC. Jan 2026: Added 391 results from one lab; no change from prior analysis, shoulder at 0.12/4 now at 45% and <1% out of QC.	21-Jun

QC Strain	Antimicrobial	Current Range	Action Recommended	Concern/Analysis	Reported
K. pneumoniae ATCC BAA-1705	Meropenem	8-64	Signal not observed at two other labs, need more data, continue to monitor.	This is for QC integrity check. Signal from single study with three sites having 23/80 (28%) of results out of QC low with MIC values (≤4). Jun 2025: 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. Jan 2026: An additional 125 new results were provided by one of the labs already included, 100% of these results were >4.	25-Jun
E. coli NCTC 13353	Ceftibuten	16-64	No new data provided for review, continue to monitor.	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
S. pneumoniae ATCC 49619	Doxycycline	0.016-0.12	Insufficient data for review, need more data, continue to monitor.	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. Jan 2026: data provided for one lab with only 9 replicates, 8 of 9 results were in the middle of the range	23-Jun



Antimicrobial: Aztreonam-Avibactam
QC Strain: E. coli 25922 Range 0.03/4-0.12/4

	Orig Tier 2 Jan 2013	Lab 1		Lab 3a	Lab 3b	Lab 4	Lab 5a	Lab 5b	Lab 5c	Lab 5d	Lab 6	Lab 7a	Lab 7b	Lab 7c	Lab 8	Combined	% by MIC	Tier 3 only total	Shoulder % -Tier 3
0.015/4																0	0%	0	
≤0.03/4	10	3		1		143	43	43	106	52	69					470	14%	460	
0.06/4	165	146		16	23	766	164	282	112	275	78	5	13	10	9	2064	60%	1899	
0.12/4	62	124		16	67	350	11	55	13	63	100	18	13	11	2	905	26%	843	44%
0.25/4		7				4				1	1		1	1		15	0%	15	
>0.25/4						1										1	0%	1	
Total	237	280		33	90	1264	218	380	231	391	248	23	27	22	11	3455		3218	

Antimicrobial: Meropenem
QC Strain: K. pneumo BAA_1705 Range 8-64

	Orig Tier 2	Lab 1a	Lab 1b	Lab 1c	Lab 2	Lab 3a	Lab 3b		Combined	% by MIC	Tier 3 only total	Shoulder % -Tier 3
≤4		12	9	2		1			24	6%		
>4						47			47	12%		
8		3	12	19		40	57		131	34%		
>8						24			24	6%		
16		3		19	2	15	55		94	25%		
>16						14						
32				1	30	4	12		47	12%		
64							1		1	0%		
128									0	0%		
Total	0	18	21	41	32	145	125		382		0	

Antimicrobial: Doxycycline
QC Strain: S. pneumo 49619 Range 0.016-0.12

	Orig Tier 2	Lab 1								Combined	% by MIC	Shoulder % - Orig M23
0.008										0	0	
0.016										0	0%	
0.03		2								2	22%	
0.06		6								6	67%	
0.12		1								1	11%	
0.25										0	0%	
0.5										0	0%	
1										0	0%	
Total	0	9	0	0	0	0	0	0	0	9		

TIER 3 DISK DIFFUSION QC

- Antimicrobic/organism combinations monitoring/compiling data to re-evaluate the current QC range or have no QC ranges.
- CLSI M23 Tier 3 requirements: 3 labs, 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.

QC Strain (ATCC)	Antimicrobial	Current Range	Date Reported	Concern	Update		Action Recommended
E. coli NCTC 13353	Ceftibuten 30 µg	15-23	Jan 2024	Zone diameters in the lower part of range and out of range	Jun 2025 Jan 2026	No additional data. No additional data.	Request additional data. Continue to monitor until January 2027.
S. aureus ATCC 25923	Debio 1452	20-27	Jan 2026	Differences in performance by disk and media manufacturer	Jan 2026	No additional data requested; the disks are not commercially available	Request additional data when available

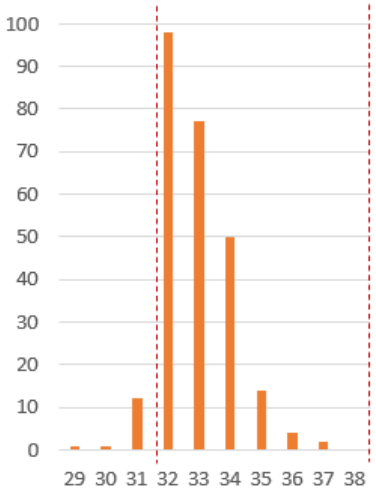
QC Strain (ATCC)	Antimicrobial	Current Range	Date Reported	Concern	Update		Action Recommended
E. coli ATCC 25922	Aztreonam-avibactam 30-20 µg	32-38	Jan 2026	Zone diameters in the lower part of range and out of range	Jan 2026	Data received from 2 labs with 4 disk mfg and 3 media mfg, total 259 results.	Data from both labs has mode 32. EUCAST is considered revising range. No action taken at this meeting. Will discuss Jun 2026; request additional data, analyze by combining with Tier 2 data, and discuss adjusting range in concordance with EUCAST
P. aeruginosa ATCC 27853	Meropenem-vaborbactam 20-10 µg	29-35	Jan 2026	Differences in performance by disk manufacturer	Jan 2026	Data received from 2 labs with 3 disk mfg and 2 media mfg, total results 149	Difference in media for 2 disks (MAST, Oxoid) from 1 lab but still within QC range Will discuss Jun 2026; request additional data

AZTREONAM-AVIBACTAM

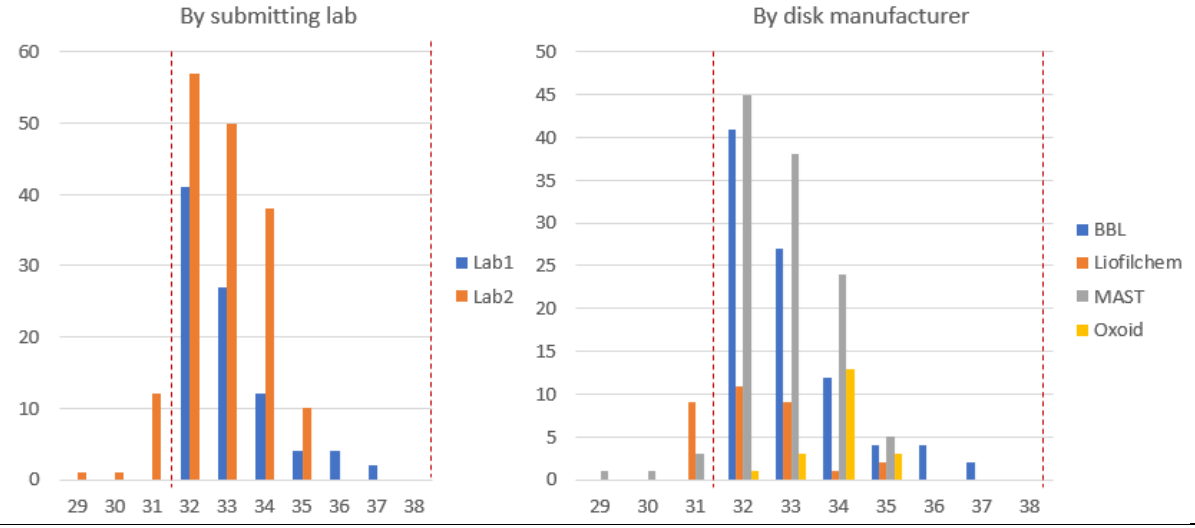
- *E. coli* ATCC 25922
- Quality Control Working Group Discussion and Recommendation
    - No vote taken
    - Recommend collecting more data and analyzing by combining Tier 2 with Tier 3
    - EUCAST is considering revising QC range down (currently 32-38 mm)

Aztreonam-avibactam
E. coli ATCC 25922

QC range: 32-38		Submitter		Disk				Media		
Diameter	total	BD	EUCAST	BBL	Liofilchem	MAST	Oxoid	BBL	Bio-Rad	Oxoid
29	1		1			1			1	
30	1		1			1				1
31	12		12		9	3		1	1	10
32	98	41	57	41	11	45	1	58	11	29
33	77	27	50	27	9	38	3	43	10	24
34	50	12	38	12	1	24	13	35	2	13
35	14	4	10	4	2	5	3	12	2	
36	4	4		4				4		
37	2	2		2				2		
38	0									
total	259	90	169	90	32	117	20	155	27	77
Mean	32.88	32.99	32.82	32.99	32.25	32.79	33.90	33.12	32.59	32.49
SD	1.15	1.24	1.10	1.24	1.11	1.02	0.72	1.17	1.19	0.97
+2SD	35.18	35.47	35.02	35.47	34.46	34.84	35.34	35.46	34.96	34.43
-2SD	30.58	30.51	30.63	30.51	30.04	30.75	32.46	30.78	30.22	30.56
Median	33	33	33	33	32	33	34	33	33	32



Aztreonam-avibactam Tier 3
E. coli ATCC 25922
QC range: 32-38



Aztreonam-avibactam Tier 3
E. coli ATCC 25922
QC range: 32-38

		Submitter		Disk				Media		
Diameter	total	BD	EUCAST	BBL	Liofilchem	MAST	Oxoid	BBL	Bio-Rad	Oxoid
29	1		1			1			1	
30	1		1			1				1
31	12		12		9	3		1	1	10
32	98	41	57	41	11	45	1	58	11	29
33	77	27	50	27	9	38	3	43	10	24
34	50	12	38	12	1	24	13	35	2	13
35	14	4	10	4	2	5	3	12	2	
36	4	4		4				4		
37	2	2		2				2		
38	0									
total	259	90	169	90	32	117	20	155	27	77
Mean	32.88	32.99	32.82	32.99	32.25	32.79	33.90	33.12	32.59	32.49
SD	1.15	1.24	1.10	1.24	1.11	1.02	0.72	1.17	1.19	0.97
+2SD	35.18	35.47	35.02	35.47	34.46	34.84	35.34	35.46	34.96	34.43
-2SD	30.58	30.51	30.63	30.51	30.04	30.75	32.46	30.78	30.22	30.56
Median	33	33	33	33	32	33	34	33	33	32

Calculated QC Range
30 to 36
Range
7
% Obs. Captured
98.10%
Prob'ty Outside Range
0.015

Median	33
0.5 Median range	3.25 (4)
Gavan	29-37

Aztreonam-Avibactam: CLSI QCWG Tier 2 June 2015

QC Strain (ATCC)	QC Range Approved mm or dil	WG Vote: Y/N/A/NP	% in Range	Mode/ Median	# mm or dilutions	Shoulder %	Footnote	Variability/Comments
E. coli 25922	32-38	9/0/2/2	97.5	35	7	NA		Proposed 32-38 (97.5%, 7mm) or 31-38 (99.4%, 8mm) RF: 30-38mm (9mm)

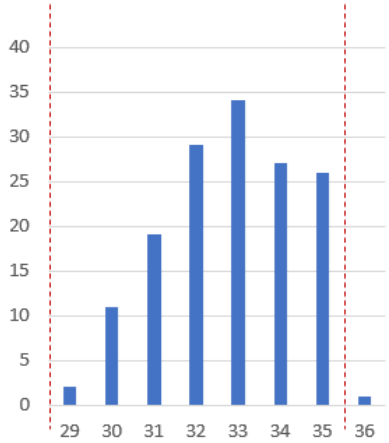
- Study conducted by CMI for AstraZeneca Pharmaceuticals (Report ATM-AVI-M2-026)
- No significant variability observed in media (BD, Remel, Hardy) or disk (BD, MAST) .Some lab to lab variability.
- 91/480 (18.9%) of data pts were in 37-38 range
- 9/480 (1.8%) were in 30-31 range

MEROPENEM-VABORBACTAM

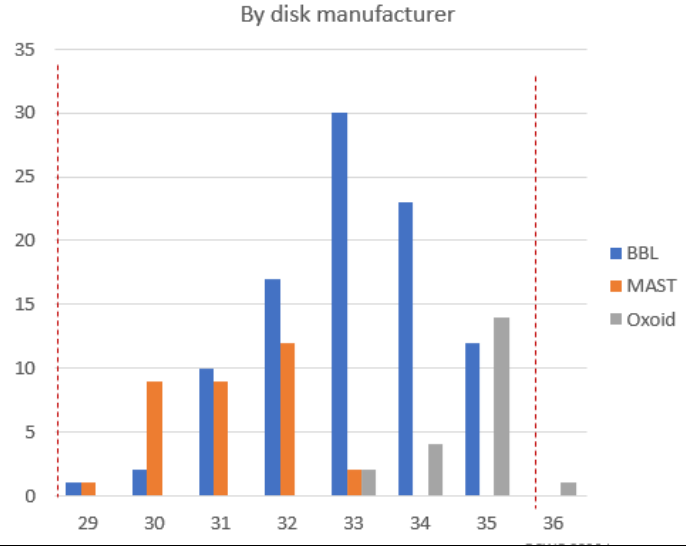
- *P. aeruginosa* ATCC 27853
- Quality Control Working Group Discussion and Recommendation
  - No vote taken
  - Recommend collecting more data

Meropenem-vaborbactam Tier 3
P. aeruginosa ATCC 27853

QC range: 29-35		Submitter		Disk			Media	
Diameter	total	BD	EUCAST	BBL	MAST	Oxoid	BBL	Oxoid
29	2	1	1	1	1		2	
30	11	2	9	2	9		9	2
31	19	10	9	10	9		15	4
32	29	17	12	17	12		21	8
33	34	30	4	30	2	2	31	3
34	27	23	4	23		4	25	2
35	26	12	14	12		14	20	6
36	1		1			1		1
total	149	95	54	95	33	21	123	26
Mean	32.83	33.00	32.52	33.00	31.15	34.67	32.83	32.81
SD	1.58	1.31	1.95	1.31	1.00	0.73	1.56	1.74
+2SD	35.99	35.63	36.42	35.63	33.16	36.13	35.94	36.30
-2SD	29.66	30.37	28.62	30.37	29.14	33.21	29.72	29.32
Median	33.00	33.00	32.00	33.00	31.00	35.00	33.00	32.00



Meropenem-vaborbactam Tier 3
P. aeruginosa ATCC 27853
QC range: 29-35



Disk

Diameter	total	BBL	MAST	Oxoid
29	2	1	1	
30	11	2	9	
31	19	10	9	
32	29	17	12	
33	34	30	2	2
34	27	23		4
35	26	12		14
36	1			1
total	149	95	33	21
Mean	32.83	33.00	31.15	34.67
SD	1.58	1.31	1.00	0.73
+2SD	35.99	35.63	33.16	36.13
-2SD	29.66	30.37	29.14	33.21
Median	33.00	33.00	31.00	35.00

MAST and Oxoid testing performed in parallel by 1 lab

Meropenem-Vaborbactam – 2016 June CLSI Tier 2 Study

QC Strain (ATCC)	QC Range Approved mm or dil	WG Vote: Y/N/A/NP	% in Range	Mode/ Median	# mm or dilutions	Shoulder %	Footnote to add with drug/range	Variability/Comments
P. aeruginosa ATCC 27853	29 - 35	9/0/1/1	99.3%	32	9		See footnote – routine QC.	Confirmed by Range Finder Lab median 31-33

- Materials used for Media: Hardy, Remel, BBL; for disks Mast, Bio Rad
  - No media or disk effects

COLONY COUNTS AND INOCULUM PREPARATION

- Tier 2 QC studies
  - Practices differ by lab when preparing inoculum (if/when to adjust).
  - Standards/Guidance differ regarding "range" and need for adjustment (CLSI, EUCAST, ISO 20776-1)
- Should inoculum be adjusted for other QC strains to meet the colony count range for E. coli ATCC 25922?
  - No adjustments at all
  - Stay within the "range" published for turbidity measuring devices
  - Make bigger adjustments as needed
- Does difference in CFU/mL impact zones/MICs?
  - Within same log, >1 log or when colony counts are outside target range for E. coli 25922
  - What info is available from Tier 1 or other studies?
- Should we establish colony count ranges for each QC strain OR publish data from Tier 2 QC Studies for informational use?
- Should CLSI recommendations be revised (CLSI M02, M07, M100, M23)
  - When performing CLSI studies?
  - When testing in user QC and/or clinical isolates in routine clinical labs?
  - Note: M2/M7 due for reassessment in 2027
- Current Standards

Document	Strain	CFU/mL	When to do colony counts? / Comments
CLSI M02 DD, 3.4	E. coli ATCC 25922	Suspension: 1-2 x 10^8	"Laboratories are encouraged, but not required to perform CC on inoculum suspensions at least quarterly".
CLSI M07 BMD. 3.8, App. B2	E. coli ATCC 25922 Use CC to guide CFU/ml for Neisseria meningitidis Comments on colony counts for anaerobe strains.	**Well**: 2-10 x 10^5 **most** 3-4 x 10^5	"Laboratories are encouraged but not required to perform CC on inoculum suspensions at least quarterly".
M100, Table 5G	Any	**Approximately** 5 x 10^5 CFU/ml **(see M07)**	Troubleshooting – "Perform colony count of GC well immediately after inoculation and before incubation (**E. coli ATCC 25922 closely approximates 5 x 10^5 CFU/mL** – see M07"
CLSI M23 Tier 2 QC studies, 3.2	Any		"Each laboratory should determine the colony counts to document the inoculum for at least one QC strain on each day of testing – a minimum of 5 colony counts should be presented for each QC strain" Colony count "average" and "min/max" included in Tier 2 report but only reviewed if troubleshooting
ISO 20776-1, 4.4 BMD	E. coli mentioned without detail **Different dilutions of suspension of McFarland 0.5 may be necessary as determined by colony counts in preliminary tests**	Suspension (DD): 1-2 x 10^8 Well (BMD): **Target** 5 x 10^5 **range** 2-8 x 10^5	
FDA Guidance (BMD) VIII. D	See column 4	**Approximately** 5 x 10^5	"Ideally this should include all QC isolates daily, isolates for reproducibility testing and 10% of fresh isolates (see reference method in CLSI M07)"

- Tier 2 QC Studies – Colony Count Data Preliminary Assessment
  - Tier 2 colony counts vary by lab and by study
    - Report average, min and max. Studies rarely rejected since average within range.
    - Detailed data review or additional statistics not done routinely
    - Occasionally troubleshoot if large lab variability, Range finder excludes lab if outlier for 2 central tendencies.
  - All QC strains average colony count generally within range for *E. coli* ATCC 25922
    - Median and average similar (average, min/max are routinely included in Tier 2 reports)
  - Multiple studies < 90% within *E. coli* ATCC 25922 range and/or within same log for all QC strains.
  - *E. coli* ATCC 25922 less variable and more frequently within range than other strains
  - *S. pneumoniae* 49619 colony count consistently lower than *E. coli* ATCC 25922.
  - *H. influenzae* ATCC 49247 similar to *E. coli* 25922 (less variability than *S. pneumoniae* ATCC 49619)
  - Minimum colony counts generally out of range (for all QC strains including *E. coli* 25922).
  - Maximum colony counts rarely out of range (for all QC strains including *E. coli* 25922).
  - Note: Disk diffusion has additional potential for variability with techniques when swabbing plate
- Potential Actions/Next Steps
  - Tier 2 QC study colony count data statistics
    - Finish compiling colony count data from Tier 2 studies and analyze for each QC strain

- Assess potential impact to MIC/DD zones with colony counts (adjusted vs non adjusted, same/different log)?
  - Which antimicrobials/classes potentially impacted (e.g., beta lactams, carbapenems, Tier 1 studies)
  - Information sources (summarize Tier 1 studies, evaluate Tier 2 QC Study outliers, small study?)
- Potential options to publish information/additional guidance
  - Create white paper about colony counts and inoculum preparation – Preferred approach
  - Potentially also add to M100 Appendix C (QC Strains for AST tests) - see options/mock ups
    - Average or median, min/max
    - Standard deviation, range +/- 1 or range +/- 2 standard deviation,
    - Indicate if adjustments were used/are needed or not
- M100 troubleshooting guide
  - Include antimicrobials/classes where MIC/zones potentially impacted by inoculum,
  - Indicate if adjustment is needed or not for the QC strain
- Tier 2 QC Summary Reports/CLSI Minutes
  - Start including colony counts in QC Summary (note: CLSI includes in KM100 dosages to set BP)
- Suggest additions to CLSI M02, M07 for next publication (due to reassess 2027)

SC DISCUSSION (MAIN POINTS)

- There was discussion on what organisms are evaluated during Tier 1 studies. Do the studies include organisms with specific antimicrobial resistance mechanisms as toto the ability to detect resistance due to some mechanisms may be impacted by the inoculum effect.
- It could be helpful information to the laboratory to understand when the QC is out of range and the impact of the inoculum effect.
- There were questions on the need to add the inoculum colony count information to the standard QC tables. Is it too much information to be added to M100? If included, need to provide guidance on how laboratories need to use the information.
- The colony count can be very helpful for troubleshooting with commercial manufacturers.
- There was discussion about how the colony count generally does not impact whether QC organisms are in range.
- Colony counts will not be helpful for disk diffusion due to so many other variables contributing.
- The colony count is important for method development as well.
- Majority agreed it is important but unlikely to be placed in the CLSI M100 and more likely to be published as a white paper.

MISCELLANEOUS/FUTURE MEETINGS

- 80% Read - Review QC Tables to determine if footnote needed
- BL-BLI Combination QC: Compile list of discrepancies between CLSI and EUCAST for potential harmonization
- Potentially publish mode/median
  - In process of compiling historical summary of Tier 2 QC Studies with mode/median by media, disk, overall results
  - Reassess after historical summary is available (publish in CLSI rationale/historical summary, add to M100 tables, and/or address in white paper)
- Guidance for study design/data requirements to develop QC ranges for
  - Qualitative (eg, screening, specialty tests)
  - Supplemental use only
  - When changing solvents

13.	**ADJOURNMENT** Dr. Mathers thanked the participants for their attention. The meeting was adjourned at 12:00 PM Mountain Standard (US) time.

2026 JANUARY AST MEETING SUMMARY MINUTES PLENARY 2: Monday, 26 January 2026 1:00 PM – 5:30 PM Mountain Standard Time (US)	
#	**Description**
1.	**OPENING** Dr. Mathers opened the meeting at 1:00 PM Mountain Standard (US) time.

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

PSEUDOMONAS AERUGINOSA CARPAPENEMASE TESTING

- Objectives of the Informal AHWG
  - To review the current literature assessing laboratory strategies and clinical importance of carbapenemase detection in *P. aeruginosa*
  - To provide suggested criteria for carbapenemase testing in carbapenem-resistant *P. aeruginosa*, with the corresponding text to be discussed and considered for inclusion in the M100-37th edition 2027
  - To provide insights about accuracy of current methods to detect carbapenemases according to carbapenemase types in *P. aeruginosa*
  - To provide suggestions to be included in CLSI M100 Appendix G
- Background
  - Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) is included in the high-risk category of WHO priority pathogen list
  - Although several mechanisms can confer carbapenem resistance (eg, porin mutations, efflux pumps, PDC overexpression), carbapenemase production represents the most challenging mechanism from therapeutic and epidemiological perspectives
  - Carbapenemases in CRPA vary geographically, with some regions facing outbreaks or endemicity of CRPA producing KPCs, VIMs, NDMs, and coproduction of enzymes
  - The CLSI M100 includes methods to detect carbapenemases in Enterobacterales and *P. aeruginosa*. Although there are criteria to apply these methods and to report results for carbapenemase-producing Enterobacterales, there are no criteria for *P. aeruginosa*.
  - There is no algorithm to detect carbapenemases in *P. aeruginosa* in EUCAST guidelines
  - Unexpected AST phenotypes are also appearing in some regions, and this could lead to treatment failures. (eg, KPC-producing *P. aeruginosa* susceptible to ceftolozane/tazobactam).
- Rationale to Detect Carbapenemases in *P. aeruginosa*
  - Carbapenemase detection in *P. aeruginosa*, can be useful to guide antimicrobial therapy, as currently available BL/BLIs, target specific enzymes (eg, avibactam, relebactam) as well as upcoming compounds (eg, taniborbactam). Additionally, other agents lack activity against any carbapenemase (eg, ceftolozane/tazobactam and cefepime/enmetazobactam).
  - Ruling out a carbapenemase may help explore other mechanisms of resistance like PBP-3 mutations or PDC mutations and provide insights about treatment strategies.
- Global Epidemiology of Carbapenemases in CRPA
  - Lancet Microbe. 2023 Mar;4(3):e159-e170
- Antibiotic activity against carbapenemase-producing *Pseudomonas aeruginosa*
  - Adapted from Macesic N et. al, Lancet. 2025 Jan 18;405(10474):257-272, Gottesdiener et al. AAC. In Press
  - Although imipenem-relebactam and even ceftazidime-avibactam resistance is common among KPC-producing *P. aeruginosa*

AGENT	KPC- PRODUCER	NDM-PRODUCER	VIM-PRODUCER	GES -PRODUCER	IMP- PRODUCER	OXA-48 PRODUCER
Ceftazidime/avibactam	Active	Inactive	Inactive	Variable	Inactive	Active
Ceftolozane/tazobactam	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Cefepime/enmetazobactam	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Cefepime/taniborbactam	Active	Variable	Active	Active	Inactive	Active
Cefepime/zidebactam	Active	Active	Active	Active	Active	Active
Cefiderocol	Active	Variable	Active	Active	Active	Active
Imipenem/relebactam	Active	Inactive	Inactive	Variable	Inactive	Inactive

- CLSI Guide to detect carbapenemases in *P. aeruginosa*

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

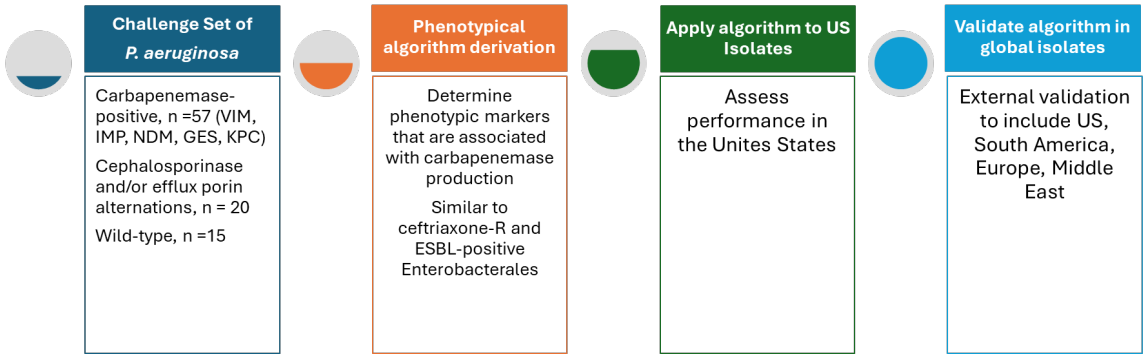
Institutional treatment guidelines, infection prevention procedures, or epidemiological investigations may necessitate identification of carbapenemase-producing Enterobacterales and *P. aeruginosa*.[1] **Tests that detect the type of carbapenemase are recommended to inform treatment decisions in carbapenem-resistant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

Table 2: Carbapenems and Enterobacterales

Isolates resistant to any carbapenem tested (eg, ertapenem, imipenem, meropenem) should be tested for a carbapenemase using phenotypic and/or molecular assays. An exception to this recommendation is *Proteus*, *Providencia*, and *Morganella* spp. that are only resistant to imipenem. These assays should identify and ideally differentiate the presence of specific carbapenemase types (eg, KPC, NDM, OXA-48, VIM, IMP).

- Criteria to apply carbapenemase testing in CRPA
  - Do we need to test all carbapenem-resistant *P. aeruginosa* for carbapenemases?



- Development and Application of a Pragmatic Algorithm to Guide Definitive Carbapenemase Testing to Identify Carbapenemase-Producing *Pseudomonas aeruginosa* (Gill et al. Antibiotics (Basel). 2020;9:738. PMID: 33120865)
- Multicenter, Prospective Validation of a Phenotypic Algorithm to Guide Carbapenemase Testing in Carbapenem-Resistant *Pseudomonas aeruginosa* Using the ERACE-PA Global Surveillance Program (Gill et al. OFID 2021. PMID: 35106312)

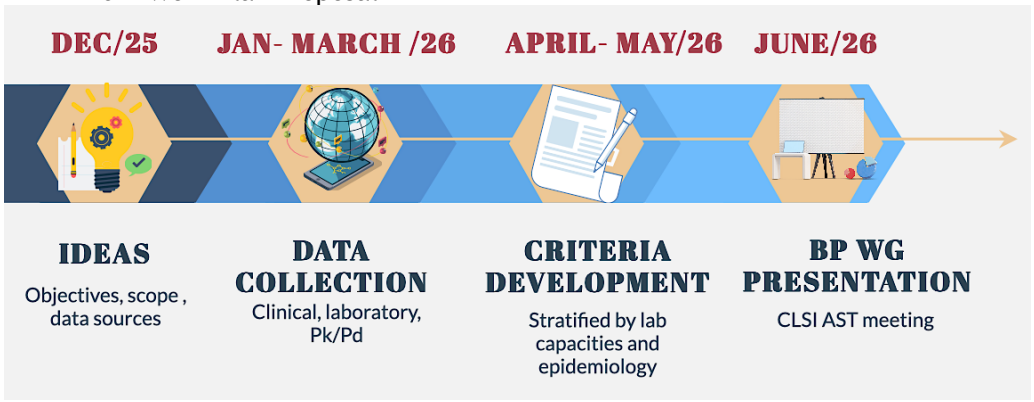
Criteria	Sensitivity	Specificity	PPV	NPV	Carbapenemases missed
Imipenem or meropenem-R **And** Cefepime and ceftazidime-NS	85 (80-89)	56 (52-60)	49	88	Isolates with genotypic carbapenemase, N = 20 -GES, n = 13 -VIM, n = 6 -KPC, n = 1 No genotypic carbapenemase, n = 20
Imipenem or meropenem-R **And** Cefepime and ceftazidime-NS **OR** ceftolozane/ tazobactam-NS	92 (88-95)	54 (50-59)	50	93	Isolates with genotypic carbapenemase, n = 4 -GES, n = 1 -VIM, n = 2 -KPC, n = 1 No genotypic carbapenemase, n = 17

Reduced testing by ~40% but not missing significant proportion of carbapenemase-producing *P. aeruginosa*

- Similar criteria performed well in other cohorts (J Clin Microbiol. 2021;59:e02874-20 and Microbiol Spectr. 2025:e0319624)

Population	Total Isolates Carbapenem-R (% Carbapenemase-positive)	Criteria	Reduction of testing using criteria	True Positive	False Negative
United States AR Lab Network	6192 (3%)	Imipenem or meropenem-R **And** Cefepime or ceftazidime-NS	50%	178	17 (VIM>KPC)
		Imipenem or meropenem-R **And** Ceftolozane/tazobactam-NS	75%	223	0
Collection from various origins	136 (52%)	Imipenem or meropenem-NS **And** Ceftolozane/tazobactam Etest >256 mg/L	60%	69	2 (KPC, GES)

- AHWG Discussions
  - A virtual meeting was held on December 1st, to define the scope and objectives of the AHWG.
    - The clinical need to detect carbapenemases in *P. aeruginosa* as well as approaches suitable for laboratories with different testing capacities was discussed.
  - The group made suggestions to gather data related to the following:
    - Carbapenemase prevalence in the US and abroad
    - The availability of indicator antibiotics (eg, imipenem, meropenem, ceftolozane/tazobactam, etc.) in commercial AST panels.
    - Published literature discussing the performance of different methodologies to detect carbapenemases in *P. aeruginosa*.
    - Data of clinical outcomes of carbapenemase-producing CRPA vs non-carbapenemase-producing CRPA
  - Work Plan Proposal



- Breakpoints Working Group Discussion and Recommendation
  - Clarification that intent to detect and differentiate carbapenemases because of the importance of the carbapenemase type in selecting therapy
  - Discussion about whether group should also assess performance of tests to detect carbapenemases

- SPM carbapenemases prevalent in *P. aeruginosa* in Brazil and Bolivia
- In Virginia, clinical labs being asked to send CRPA isolates to state public health labs -> criteria would help to decrease burden

SC DISCUSSION (MAIN POINTS)

- Suggestion to collect MIC data and other antimicrobial agent classes.
- Decision to make this informal group a formal ad hoc working group and report to the Methods Working Group.

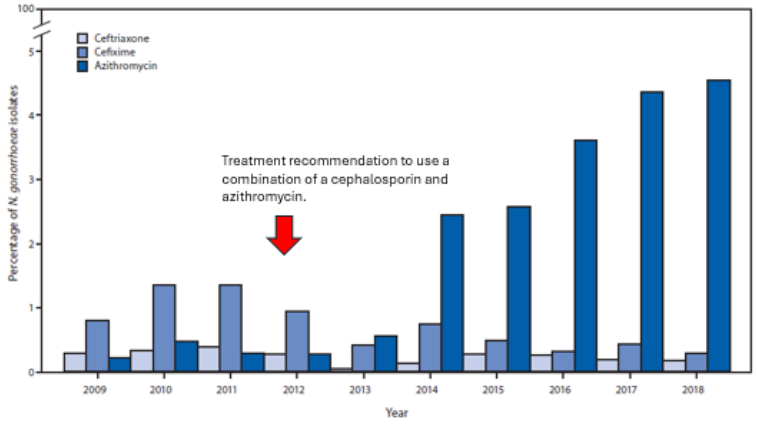
AZITHROMYCIN MIC BREAKPOINTS FOR *NEISSERIA GONORRHOEAE*

- Current Azithromycin Breakpoints

	Susceptible	Intermediate	Resistant	Comment
CLSI	≤ 1µg/mL	None	None	Breakpoint presumes that azithromycin is used in a recommended regimen that includes an additional antimicrobial agent.
	1 g IV/PO once; presumes use in an approved regimen that includes an additional agent (eg, ceftriaxone 250 mg IM once)			
FDA	None	≤ 1µg/mL	≥ 2µg/mL	FDA Rationale for Breakpoints Recognition Decision: Azithromycin and Neisseria gonorrhoeae | FDA
EUCAST	None	None	None	Azithromycin is always used in conjunction with another effective agent. For testing purposes with the aim of detecting acquired resistance mechanisms, the ECOFF is 1 mg/L.

- Increasing Azithromycin Resistance in Gonorrhea

FIGURE. Percentage of *Neisseria gonorrhoeae* isolates with elevated minimum inhibitory concentrations (MICs)* to ceftriaxone, cefixime, and azithromycin — Gonococcal Isolate Surveillance Project, United States, 2009–2018



Source: CDC. Sexually Transmitted Disease Surveillance 2018. https://www.cdc.gov/std/stats18/default.htm.
* Elevated MIC = ceftriaxone ≥0.125 µg/mL; cefixime ≥0.25 µg/mL; azithromycin ≥2.0 µg/mL.

CDC removed azithromycin from treatment guidelines in 2020

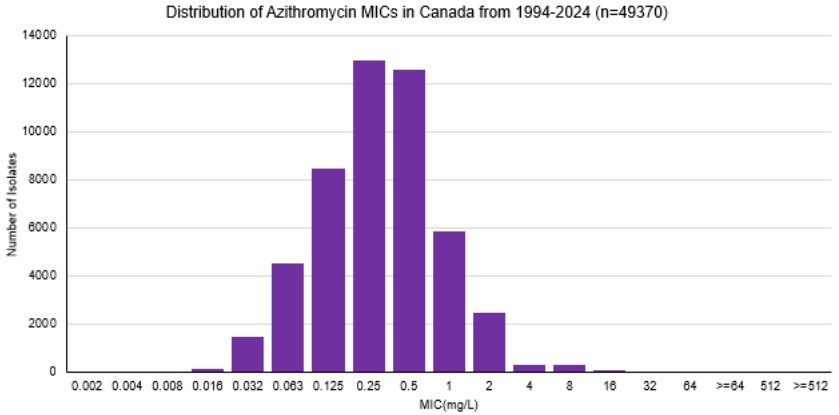
- Treatment Guidelines: Gonorrhea
  - U.S. Guideline
    - Primary: Ceftriaxone 500 mg IM in a single dose
    - Alternatives
      - Gentamicin 240mg IM + azithromycin 2g orally
      - Oral Cefixime, 800 mg
  - WHO Guideline
    - Primary: Ceftriaxone 1g IM in a single dose
    - Alternatives
      - Cefixime 800 mg orally and test of cure
      - Cefixime 800 mg orally plus azithromycin and no test of cure
      - Spectinomycin 2g IM as a single dose plus azithromycin 2g orally
      - Gentamicin 240 mg IM as a single dose plus azithromycin 2g orally
      - Note: Extended-release azithromycin is not recommended for treatment of *Neisseria gonorrhoeae* infections. It has been used in Japan.
- FDA Rationale for Breakpoints Recognition Decision: Azithromycin and *Neisseria gonorrhoeae*

"Azithromycin may be recommended by CDC consultants for the management of drug-resistant gonorrhea as part of a treatment regimen on a case by case basis. Information regarding the MIC for azithromycin of a clinical *Neisseria gonorrhoeae* isolate may be helpful in the management of drug-resistant gonorrhea and for public health surveillance purposes.

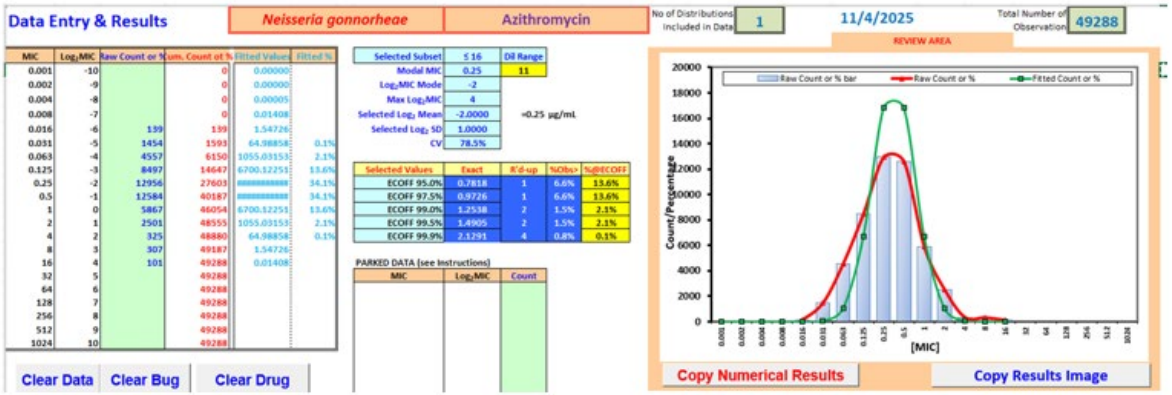
FDA agrees with CLSI's assessment of surveillance data that ≤ 1 mcg/mL is the end of the natural wild-type distribution and there are few isolates with MICs > 1 mcg/mL. FDA is not aware of pharmacokinetic/pharmacodynamic data that would inform breakpoints.

With respect to clinical outcome data, it is FDA's assessment that published reports are not supportive of establishing a susceptible breakpoint. In a series from public sexually transmitted disease clinics in the U.S. published in the early 1990s, a high treatment success rate was reported for monotherapy with a single oral 2-gram azithromycin dose. However, in a clinical trial evaluating a single 2 gram oral dose of azithromycin with extended release formulation, failures of eradication were reported for subjects with isolates with MIC as low as 0.5 mcg/mL."

- MIC Distribution Data
  - MIC Distribution Data – Canada

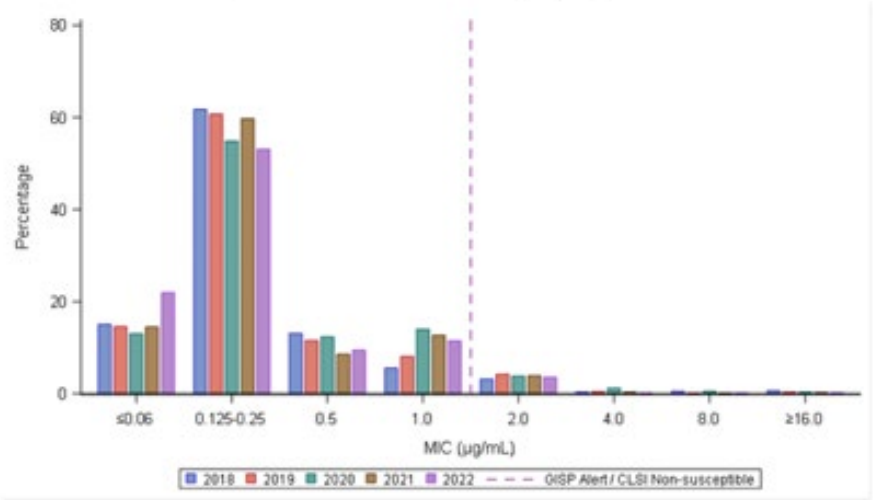


  - ECV: 1 ug/mL (ECOFF finder based on Canadian data)



  - MIC Distribution Data – US (CDC)

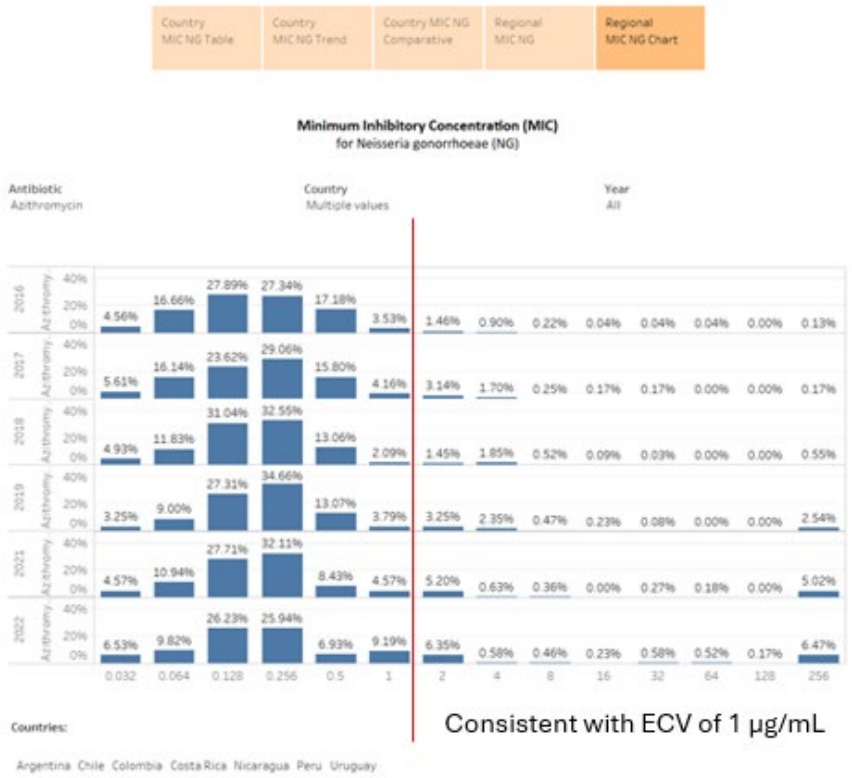
Figure 3. Distribution of Azithromycin Minimum Inhibitory Concentrations (MICs) Among *Neisseria gonorrhoeae* Isolates, Gonococcal Isolate Surveillance Project (GISP), 2018-2022



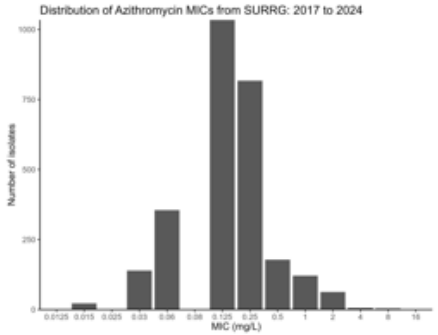
Year	≤0.06 n (%)	0.125-0.25 n (%)	0.5 n (%)	1.0 n (%)	2.0 n (%)	4.0 n (%)	8.0 n (%)	≥16.0 n (%)	Total
2018	777 (15.1)	3186 (61.7)	675 (13.1)	287 (5.6)	163 (3.2)	16 (0.3)	25 (0.5)	31 (0.6)	5160
2019	799 (14.6)	3326 (60.7)	632 (11.5)	442 (8.1)	229 (4.2)	24 (0.4)	9 (0.2)	19 (0.3)	5480
2020	486 (13.0)	2053 (54.9)	462 (12.3)	522 (14.0)	142 (3.8)	44 (1.2)	19 (0.5)	13 (0.3)	3741
2021	553 (14.5)	2284 (59.7)	327 (8.6)	483 (12.6)	150 (3.9)	12 (0.3)	4 (0.1)	10 (0.3)	3823
2022	809 (22.0)	1955 (53.1)	347 (9.4)	423 (11.5)	130 (3.5)	6 (0.2)	6 (0.2)	8 (0.2)	3684

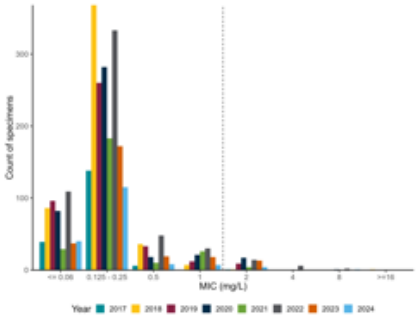
GISP Alert Value: azithromycin MIC ≥2.0 µg/mL; CLSI Non-susceptible = azithromycin MIC ≥2.0 µg/mL.
CLSI = Clinical & Laboratory Standards Institute.
Non-susceptible = Category used for isolates when only a susceptible breakpoint has been designated and the MIC is above the susceptible breakpoint.
As of the end of 2022, the CLSI has not established an azithromycin resistance breakpoint for *N. gonorrhoeae*.

- Distribution of MICs from 7 countries in Latin America 2016-2022



- Guilford County, NC (provided by Elizabeth Palavecino)





SURRG MICs for Azithromycin among GC isolates using agar dilution

Characteristic	Overall N = 2,742	<= 0.06 N = 518	0.125 - 0.25 N = 1,851	0.5 N = 178	1 N = 121	2 N = 63	4 N = 6	8 N = 4	>=16 N = 1
Year, n (%)									
2017	184 (6.7)	39 (7.5)	138 (7.5)	6 (3.4)	0 (0)	1 (1.6)	0 (0)	0 (0)	0 (0)
2018	499 (18)	86 (17)	368 (20)	36 (20)	7 (5.8)	1 (1.6)	0 (0)	0 (0)	1 (100)
2019	410 (15)	96 (19)	260 (14)	33 (19)	12 (9.9)	9 (14)	0 (0)	0 (0)	0 (0)
2020	421 (15)	82 (16)	282 (15)	18 (10)	21 (17)	17 (27)	0 (0)	1 (25)	0 (0)
2021	252 (9.2)	29 (5.6)	183 (9.9)	10 (5.6)	26 (21)	4 (6.3)	0 (0)	0 (0)	0 (0)
2022	542 (20)	109 (21)	333 (18)	48 (27)	30 (25)	14 (22)	6 (100)	2 (50)	0 (0)
2023	259 (9.4)	37 (7.1)	172 (9.3)	19 (11)	18 (15)	13 (21)	0 (0)	0 (0)	0 (0)
2024	175 (6.4)	40 (7.7)	115 (6.2)	8 (4.5)	7 (5.8)	4 (6.3)	0 (0)	1 (25)	0 (0)

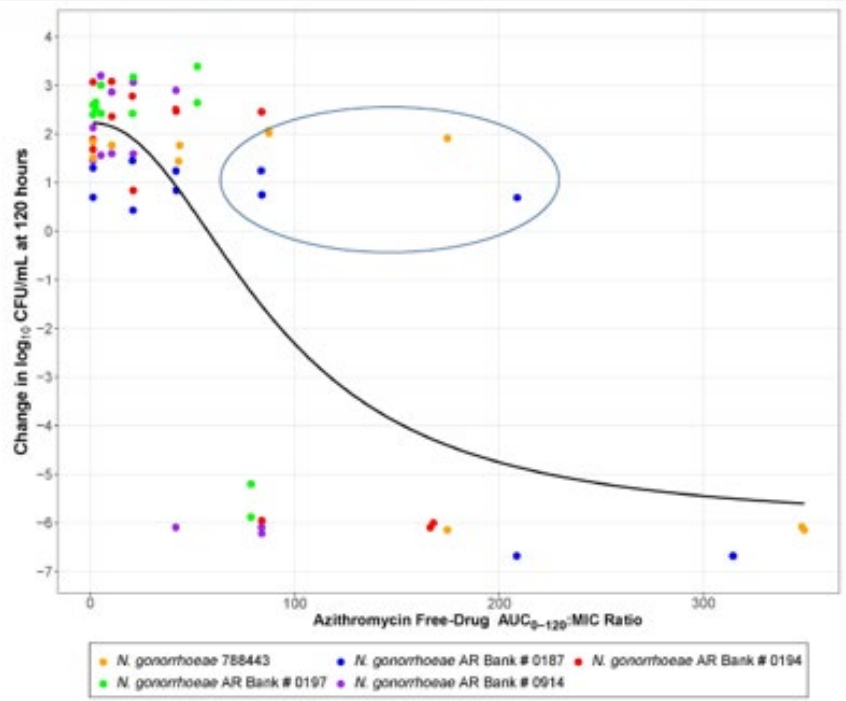
- EUCAST

MIC distributions of azithromycin and ceftriaxone tested against a pooled collection of 149 *N. gonorrhoeae* isolates

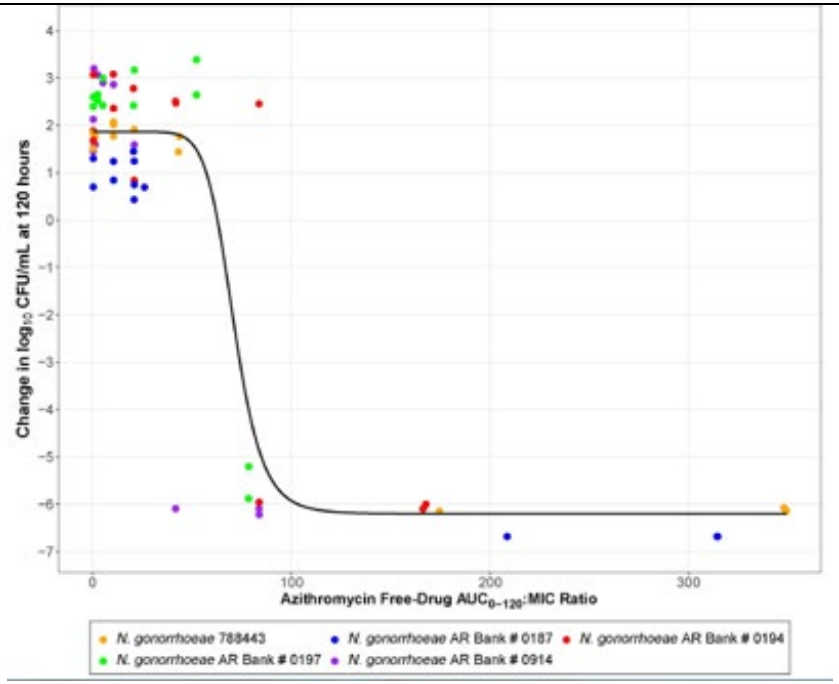
Isolate group (n)	Number of isolates (cumulative percentage) inhibited by MIC (mg/L)															
	Azithromycin															
	≤ 0.001	0.002	0.004	0.008	0.015	0.03	≤ 0.03	0.06	0.12	0.25	0.5	1	2	> 2	MIC_{50}	MIC_{90}
All isolates (n=149)							3 (2.0)	12 (10.1)	32 (31.5)	34 (54.4)	48 (86.6)	4 (89.3)	0 (89.3)	16 (100)	0.25	> 2
Clinical isolates (n=84)[a]							3 (3.6)	12 (17.9)	27 (50.0)	20 (73.8)	15 (91.7)	0 (91.7)	0 (91.7)	7 (100)	0.12	0.5
	Ceftriaxone															

- Mutations by MIC
  - Summary of Mutation Associated with Azithromycin Resistance
    - Multiple mutations are associated with azithromycin resistance
    - Some of the mutations can be found in isolates at a MIC ≤ 1µg/mL. Mutations are additive in phenotype. It is challenging to assign a direct MIC to mutation correlation.
    - Not all resistance is associated with a known mutation
    - Some mutations are associated with clades (ie, in some geographical areas, there is a higher prevalence of strains with reduced susceptibility to azithromycin and have a higher potential to become resistant to azithromycin).
  - Analysis Information
    - Gonococcal Antimicrobial Surveillance Program (GASP)-Canada Surveillance data

	▪ 8412 isolates collected between 1994-2023 tested with both agar dilution and WGS ▪ Mutations investigated: • ermB, ermC, ermF (very rare) • 23S A2059G + C2611T • mtrR promoter • mtrD R714, S821, K823 • rplD G70D ▪ Mutations not detected in our dataset • ermA, ereA/B, mefA • mtrC disrupted • rplV 90 ARAK insert • PK/PD Analysis ○ Analyses Data from the Hollow-Fiber *In vitro* Infection Model (USCAST) ▪ It was hypothesized that the poor relationship observed between the change in bacterial burden at 20 hours for *N. gonorrhoeae* and azithromycin free-drug $AUC_{0\text{-}120}$:MIC ratio was due to amplification of resistant subpopulations associated with individual treatment regimens over the study duration ▪ Unclear if these strains harbored carbapenemases



- Updated Analyses Data from the Hollow-Fiber *In vitro* Infection Model (USCAST)
  - PK/PD analysis were undertaken using MIC values considered to be representative of the bacterial populations after administration of each individual treatment regimen over 120 hours
  - The median MIC of the isolates on the drug-supplemented plates was evaluated in place of the baseline MIC if the growth was observed on the drug-supplemented agar plates at any time point throughout the study, and if the bacterial burden at 120 hours was greater or equal to that observed at baseline
  - Model more predictive of efficacy if performed MIC testing on resistant subpopulations



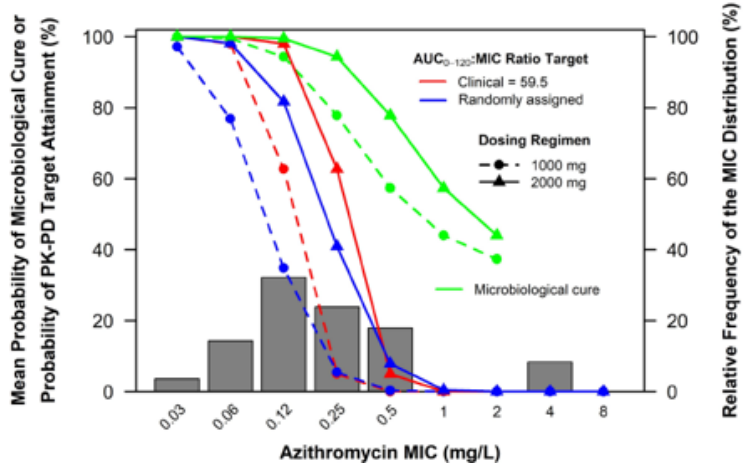
- Clinical PK/PD Relationship for Azithromycin Based on Data from Patients with Uncomplicated Gonorrhea (USCAST)
  - Among the 3 studies conducted in Japanese male patients with gonococcal urethritis who received a single 2000 mg oral dose of AZM-SR, 1 evaluated the clinical PK/PD relationship for microbiological response post-therapy using PK data collected
  - A logistic regression model was developed to evaluate the relationship between microbiological response and total-drug AUC-MIC ratio
    - The estimated linear component of the logistic function was reported to be the form: -0.798 + 0.063*AUC:MIC ratio
    - Mean model-predicted percent probabilities of microbiological cure by MIC were calculated among the simulated patients using the above-described logistic regression model
    - A total-drug plasma AUC:MIC ratio target of 59.5 was associated with 95% probability of microbiological cure. The percent probability of simulated patients achieving the 59.5 was also assessed.
- Percent Probabilities of Model-Predicted Microbiological Cure Post-Therapy and PK-PD Target Attainment by MIC (USCAST)

MIC (mg/L)	Percent probabilities of model-predicted microbiological cure post-therapy and PK-PD target attainment by azithromycin MIC, dosing regimen, and PK-PD target approach[a]					
	1000 mg PO			2000 mg PO		
	Mean model-predicted percent probabilities of microbiological cure[b]	Percent probabilities of PK-PD target attainment		Mean model-predicted percent probabilities of microbiological cure[b]	Percent probabilities of PK-PD target attainment	
		Clinical target[c]	Randomly assigned non-clinical targets[d]		Clinical target[c]	Randomly assigned non-clinical targets[d]
0.03	>99.9	100	97.1	>99.9	100	100
0.06	99.5	98.0	76.8	>99.9	100	98.2
0.12	94.3	62.7	34.8	99.5	98.0	81.7
0.25	77.8	4.96	5.46	94.3	62.7	40.9
0.5[e]	57.4	0	0.30	77.8	4.96	7.86
1	44.0	0	0.02	57.4	0	0.44
2	37.3	0	0	44.0	0	0.02
4	-[f]	0	0	-[f]	0	0
8	-[f]	0	0	-[f]	0	0
Overall[e]	79.7	38.9	27.0	89.3	65.2	55.0

a. Dark gray-shaded cells indicate mean percent probabilities of microbiological cure ≥ 95% and light gray-shaded cells indicate percent probabilities of PK-PD target attainment ≥ 90% based on exposures generated using inflated interindividual variance of the population PK model parameters.
b. Model-predicted percent probabilities of microbiological cure were calculated using the logistic regression model reported by Soda *et al.* [Antimicrob Agents Chemother 2017;62:e01409-17].
c. The clinical total-drug plasma $AUC_{0\text{-}120}$:MIC ratio target associated with 95% probability of microbiological cure was ≥ 59.5 [Soda *et al.* Antimicrob Agents Chemother 2017;62:e01409-17].
d. Selected from an estimated log normal distribution based on the non-clinical azithromycin free-drug plasma $AUC_{0\text{-}120}$:MIC ratio targets associated with a 5-$\log_{10}$ CFU/mL reduction from baseline for five *N. gonorrhoeae* isolates described in slide 10.
e. Based on data for azithromycin MIC distributions for 84 clinical *N. gonorrhoeae* isolates from the SENTRY Antimicrobial Surveillance Program shown in slide 5. The MIC_{90} value for azithromycin against *N. gonorrhoeae* was 0.5 mg/L.
f. Mean model-predicted percent probabilities of microbiological cure were not reported beyond an MIC value of 2 mg/L which was the largest observed MIC value among patients whose data was used to fit the logistic regression model reported in Soda *et al.* [Antimicrob Agents Chemother 2017;62:e01409-17].

Assessments for azithromycin based on inflated interindividual variance, a clinical PK-PD relationship, and clinical and non-clinical PK-PD targets

Based on plasma exposure, not taking into account exposure in genital tract OR high intra-cellular concentrations



- Clinical Outcomes Data
  - Multicenter Trial of Single-Dose Azithromycin vs Ceftriaxone in the Treatment of Uncomplicated Gonorrhea
    - Hansfield et al. Sex Transm Dis 1994. PMID: 9071422
    - Randomized open-label trial of azithromycin 2 g po (monotherapy) vs. ceftriaxone 250 mg IM
    - Patients with uncomplicated gonorrhea recruited from 10 US STI clinics
    - Cure: Culture negative at first follow-up visit after treatment (1 week after treatment)

        - 724 patients enrolled -> 548 (76%) evaluable at follow-up visit
        - 213 patients treated with azithromycin and culture-negative at 1 week returned for follow-up culture at 2 weeks -> all negative
        - No outcomes by MIC data
    - Clinical trial (without comparator arm) of sustained-release azithromycin for gonococcal urethritis in 130 men in Japan
        - Yasuda M, et al. J Infect Chemother 2016;69(11):3116-3118.
        - Limitations of this study include no pharmacokinetics data for the extended-release formulation (which may have insufficient tissue concentration at the infection site). Test of cure was performed by NAAT at 7 days. Therefore, failure rates may have been inflated by detection of dead organisms.
        - MIC distribution of *Neisseria gonorrhoeae* isolates was higher than in US at the time (no standardization of methods?)
    - Microbiological Response Post-Azithromycin Treatment by MIC
        - Takahasi S, et al. Antibiotics 2014;3:109-120.
        - Yasuda M, et al. J Infect Chemother 2016;22:841-843.
        - Soda M, et al. Antimicrob Agents Chemother 2017;62[e]01409-17.
        - Microbiological response by MIC in Japanese male patients with gonococcal urethritis of a 200 mg extended-release azithromycin single oral dose was assessed in 3 separate studies
        - The data demonstrated a high percentage of microbiological eradication of MIC values up to 0.25 mg/L which decreased at higher MIC values, thus supporting a susceptible breakpoint of 0.25 mg/L.
        - Based on positive NAAT in follow-up, not clinical failure or culture-based failure
- Summary of Azithromycin Data

Category	Conclusion	Data Source	Comments
ECV from MIC Distribution	1 µg/mL	See slide 9-24	Established by CLSI SC in 2019 and reported in a published rationale document. Multiple mutations associated with azithromycin have been identified. Some isolates with a MIC of 1µg/mL can harbor one or more of these mutations and some isolates at this MIC do not have any known mutations.
PK/PD Breakpoint	0.12 µg/mL	See slide 25-34	Four isolates evaluated in a hollow fiber model. PK-PD analysis was based upon MIC values expected after treatment with azithromycin after 120h.
Clinical Outcome	0.25-0.5 µg/mL	See slide 35-38	Treatment outcomes decline when the MIC is 1ug/mL. These data are limited because extended-release azithromycin (2g) was used and this formulation may not achieve the levels of immediate release azithromycin.

- GC AHWG Discussion and Recommendation

Option	Susceptible	Intermediate	Resistant	Pros	Cons	Votes from AHWG
1	≤ 0.5 µg/mL	1µg/mL	≥ 2µg/mL	Azithromycin has activity for treatment of gonorrheae infections. A comment should be added that it is not intended to be used as monotherapy or as primary therapy.	The susceptible BP is below the ECV. However, isolates at the ECV are a mixture of isolates with mutations associated with azithromycin resistance. The susceptible designation supports use of azithromycin as alternative therapy.	3
2	≤ 1µg/mL	None	None	The susceptible MIC is at the ECV. A comment should be added that it is not intended to be used as monotherapy or as primary therapy.	The "susceptible" interpretation is not consistent with the current treatment guidelines.	1
3	None	None	None	Harmonize with EUCAST. Include the EUCAST comment: "Azithromycin is always used in conjunction with another effective agent. For testing purposes with the aim of detecting acquired resistance mechanisms, the ECOFF is 1 mg/L."	This communication plan has not been previously used by CLSI. Education is needed and the subcommittee may need to re-evaluate the colistin breakpoint.	1
4	None	≤ 1µg/mL	≥ 2µg/mL	Harmonize with FDA and EUCAST (note, this decision is harmonized with EUCAST in principle, but it is communicated differently). This approach clearly communicates that azithromycin is efficacy is limited even if used in combination with another drug.	This approach may inadvertently communicate that azithromycin should not be used for treatment under any circumstances.	5

- Breakpoints Working Group Discussion and Recommendation
  - Clinical gonorrhea experts on AHWG wanted to have a susceptible breakpoint and preferred not to have an I/R category
    - Clinicians may think azithromycin not useful if only reported as "I"
  - CLSI precedence for I/R breakpoint are with polymyxins: is this colistin?
    - This drug has a randomized trial that shows it works
  - Desire to harmonize with FDA at least on breakpoint of ≤ 1 µg/mL and EUCAST ECV (instead of decreasing to ≤ 0.5 µg/mL)
  - Motion to accept azithromycin MIC breakpoints for *N. gonorrhoeae* current susceptible MIC breakpoint (≤ 1 µg/mL) and the proposed resistant MIC breakpoint (≥ 2 µg/mL). WG Vote: 8-2-0-3. Motion passed.
  - Reasons for rejection: "a lot to digest", meets susceptible criteria? Resistance mutations at 1 µg/mL
  - Retain the current comment: "Breakpoint presumes that azithromycin is used in an approved regimen that includes an additional antimicrobial agent."
- What about the azithromycin dosage?

Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints (Continued)

Antimicrobial Agent	Dosage Regimen Used to Establish S or SDD Breakpoint
Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)	
Lefamulin (*H. influenzae* only)	150 mg IV or 600 mg PO q 12 h
Table 2F. *Neisseria gonorrhoeae*	
Azithromycin	1 g IV/PO once; presumes use in an approved regimen that includes an additional agent (eg, ceftriaxone 250 mg IM once)

SC DISCUSSION (MAIN POINTS)

- Using molecular testing for eradication was a concern but is the standard used in the GC community to evaluate eradication.
- The CDC guidelines reflect that a NAAT positive on test of cure assessment should be confirmed by culture due to the possibility of persistent nucleic acid present and does not reflect organism viability.
- Discussions ensued about what the recommended dose would be. The current CLSI azithromycin recommended dose in the M100 is 1g IV/PO but the data from the 1994 trial presented used 2g PO. Also, current US and WHO guidelines recommend 2g PO in combination with another agent.

A motion to accept the current azithromycin susceptible MIC breakpoint (≤ 1 µg/mL) and the proposed resistant MIC breakpoint (≥ 2 µg/mL) based on a dosage of 2g PO for *Neisseria gonorrhoeae*. Vote: 13 for, 1 against, 0 abstain, 0 absent (Pass)

Against Vote Reasoning:

- Data does not support combination therapy as proposed in the vote.

AZITHROMYCIN DISK DIFFUSION BREAKPOINTS FOR *NEISSERIA GONORRHOEAE*

- Azithromycin Disk Breakpoint

	Susceptible	Intermediate	Resistant	Comment
CLSI current MIC	≤ 1µg/mL	None	None	Breakpoint presumes that azithromycin is used in a recommended regimen that includes an additional antimicrobial agent.
CLSI Disk Diffusion (current)	≥ 30mm	None	None	
Revised Disk Diffusion	≥ 30mm	27-29mm	≤ 26 mm	Note: M23 allows the disk test to have an intermediate category even if there is no intermediate category for the MIC test.

Data sources: CDC, Argentina National Lab surveillance

- Azithromycin Disk Correlates

	Susceptible	Intermediate	Resistant
Revised Disk Diffusion (option 1)	≥ 30mm	27-29mm	≤ 26 mm

Count of MIC	Column Labels																																			
Row Labels	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	50	Grand Total
16	2	3	2	2	2	1			1																											13
8		1	1	3	4	6	3	4	1		1	1																								25
4						3	21	20	15	10	7	4	1	1	1																					83
2										1	7	8	16	11	1	2	1																			47
1													5	8	10	14	7	4	5	2		1	1													57
0.5														1	1	6	6	6	4	9	7															40
0.25																		1	3	5	8	8	9	13	2	2	1	3		2						57
0.125																			4	2	7	8	10	12	7	5	3	6	1	1	2	1				69
0.06																					4	3	4	1	7	5	1	3	4	5	4		1	1		43
0.03																									2	3	4	4	2	4	5	2	1	2	1	30
0.016																											1	1	4	2	2	3	3	1		17
Grand Total	2	4	3	5	6	10	24	24	17	11	15	13	22	21	13	22	14	11	16	18	26	20	24	26	18	15	10	17	11	14	13	6	5	4	1	481

MIC Range	Discrepancy Rates		
	Very Major, %	Major, %	Minor, %
≥ R + 1	0	N/A	0.8
R + S	1	4.8	44.2
≤ S - 1	N/A	0	3

Table 10. Guideline for Acceptable Discrepancy Rates (Without Intermediate Range)

MIC Range	Discrepancy Rates		
No Intermediate Range	Very Major, %	Major, %	Minor, %
≥ R + 1	< 2	N/A	< 5
R + S	< 10	< 10	< 40
≤ S − 1	N/A	< 2	< 5

Abbreviations: MIC, minimal inhibitory concentration; N/A, not applicable; R, resistant; S, susceptible.

- Breakpoints Working Group Discussion and Recommendation
  - Motion to accept the azithromycin disk diffusion breakpoints (S ≥ 30 mm, I 27-29 mm, R ≤ 26 mm) for *Neisseria gonorrhoeae*. WG Vote: 10-0-0-3. Motion passed.

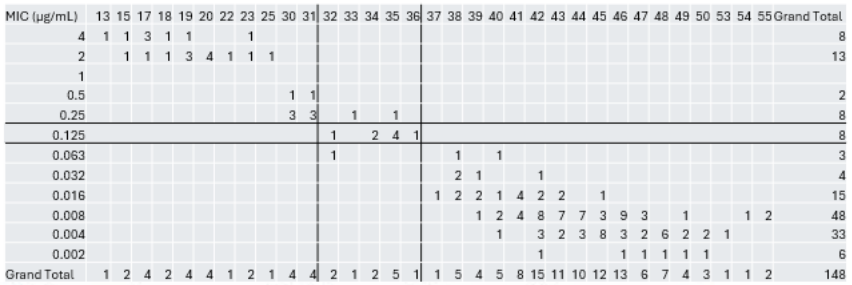
A motion to accept the azithromycin disk diffusion breakpoints (S ≥ 30 mm, I 27-29 mm, R ≤ 26 mm) for *Neisseria gonorrhoeae*. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

CEFIXIME DISK DIFFUSION BREAKPOINTS FOR *NEISSERIA GONORRHOEAE*

- Current CLSI Breakpoints

Drug	MIC (µg/mL)			Disk (mm)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Ceftriaxone (30µg disk)	≤0.125	0.25	≥0.5	None	None	None
Cefixime (5µg disk)	≤0.06	0.125	≥0.25	None	None	None

- Cefixime Aggregated Disk to MIC Correlate Data



MIC (µg/mL)	13	15	17	18	19	20	22	23	25	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	53	54	55	Grand Total
4	1	1	3	1	1			1																										8
2		1	1	1	3	4	1	1	1																									13
1																																		
0.5										1	1																							2
0.25										3	3		1		1																			8
0.125												1		2	4	1																		8
0.063												1						1		1														3
0.032																		2	1			1												4
0.016																	1	2	2	1	4	2	2		1									15
0.008																			1	2	4	8	7	7	3	9	3		1			1	2	48
0.004																				1		3	2	3	8	3	2	6	2	2	1			33
0.002																						1				1	1	1	1	1				6
Grand Total	1	2	4	2	4	4	1	2	1	4	4	2	1	2	5	1	1	5	4	5	8	15	11	10	12	13	6	7	4	3	1	1	2	148

S: ≥37 mm
I: 32-36 mm
R: ≤31 mm

1 dil Intermediate		VME	Major	Minor
≥ I+2	≥0.5	0%	N/A	0%
I+1 to I-1	0.063 to 0.25	0%	0%	16%
≤I-2	≤0.032	N/A	0%	0%

Table 10. Guideline for Acceptable Discrepancy Rates (Without Intermediate Range)

MIC Range	Discrepancy Rates		
No Intermediate Range	Very Major, %	Major, %	Minor, %
≥R+1	<2	N/A	<5
R+S	<10	<10	<40
≤S−1	N/A	<2	<5

Abbreviations: MIC, minimal inhibitory concentration; N/A, not applicable; R, resistant; S, susceptible.

- Breakpoints Working Group Discussion and Recommendation
  - Motion to accept the cefixime disk diffusion (5 µg disk) breakpoints (S ≥ 37 mm, I 32-36 mm, R ≤ 31 mm) for *Neisseria gonorrhoeae*. WG Vote: 10-0-0-3. Motion passed.

A motion to accept the cefixime disk diffusion (5 µg disk) breakpoints (S ≥ 37 mm, I 32-36 mm, R ≤ 31 mm) for *Neisseria gonorrhoeae*. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

CEFTRIAXONE DISK DIFFUSION BREAKPOINTS FOR *NEISSERIA GONORRHOEAE*

- Current CLSI Breakpoints

Drug	MIC (µg/mL)			Disk (mm)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Ceftriaxone (30µg disk)	≤0.125	0.25	≥0.5	None	None	None
Cefixime (5µg disk)	≤0.06	0.125	≥0.25	None	None	None

- Ceftriaxone 30 µg Disk
  - There is a high rate of major and minor errors. To accurately detect resistance, labs may decide to test all isolates with a disk diffusion resistant or intermediate result with an MIC method.
  - Identifying *N. gonorrhoeae* isolates as ceftriaxone-not susceptible is a public health emergency

MIC (µg/mL)	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	54	55	56	57	58	59	60	Grand Total
4			1																											1
2		1		1	1	1																								4
1	1		2	1	1	4																								9
0.5					1	2	1					1																		5
0.25									1			1																		2
0.125								1		1	1	3	3	1		1	1													12
0.063								1	1					3		1	3	1	1		1	1								13
0.032							1	1	1		3	2	2	1	5	4	3	3	6	2	1	1		2						38
0.016							1	1	1	1	3	4	4	5	9	17	8	16	7	11	5	13	8	2	1	2				119
0.008			1					1		3	2	5	5	6	2	12	6	13	10	12	9	10	4	2	3	1	1		1	109
0.004										2		1	1	3	3	7	9	11	19	8	9	11	6	6	9	4	5	2	1	117
0.002										1						1	2	2	2	2	5	3	4	2	2	3	1	1	3	34
Grand Total	1	1	4	2	3	7	3	5	4	8	9	17	15	19	19	43	32	46	45	35	30	39	22	14	15	10	7	3	5	463

1 dil Intermediate		VME	Major	Minor
≥ I+2	≥1	0%	N/A	0%
I+1 to I-1	0.125 to 0.5	0%	0	32%
≤I-2	≤0.063	N/A	5%	22%

Table 9. Guideline for Acceptable Discrepancy Rates (With Intermediate Ranges)*

MIC Range		Discrepancy Rates		
1-Dilution Intermediate Range	2-Dilution Intermediate Range	Very Major, %	Major, %	Minor, %
≥I+2	$\geq I_{upp}+2$	<2	N/A	<5
I+1 to I−1	$I_{upp}+1$ to $I_{low}-1$	<10	<10	<40
≤I−2	$\leq I_{low}-2$	N/A	<2	<5

Abbreviations: I, intermediate; I_{upp}, higher MIC in a two-dilution intermediate range; I_{low}, lower MIC in a two-dilution intermediate range; MIC, minimal inhibitory concentration; N/A, not applicable.
*See example in Appendix H.

- Would a 5 µg Ceftriaxone Disk Work Better?
  - Data from Erika Matuschek's EUCAST lab

N. gonorrhoeae
70 isolates
Disk diffusion on EUCAST experimental medium (agar from two manufacturers tested in parallel)
MIC determination with gradient test according to the manufacturer's instructions

CRO 5 µg	≤0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2
6											
7											
8											
9											
10											
11											
12											
13											
14											1
15											
16											
17											
18											
19											
20											
21											
22											
23											1
24									1		2
25											
26											
27									3		
28									2		
29					1						
30					1	1	3				
31				2	1		1				
32			1	1	4	1	2	1			
33				2	2		3	1			
34		1	5	5	5		2				
35	1	3	6	2	1						
36	1	1	6	1	4		3				
37		3	5	1	1		2				
38	2	4	5	1							
39		5	3								
40	3	2	1	1							
41	2	3									
42	1	1									
43	1	1									
44	2										
45	2										
46	2										
47	1										
48											
49											
50											

    - Cefixime has a 5-µg disk and works great
    - Uses different media than CLSI
    - This disk would more accurately detect resistance, but not intermediate.
    - Could be confusing to have 2 disk masses
    - Discussed in Methods Working Group
    - But this will take years
- Ceftriaxone Disks: What if set a resistant-only disk diffusion breakpoint?
    - Short-term solution: If zone ≤ 35 mm, then disk test could screen for resistance

MIC (µg/mL)	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	54	55	56	57	58	59	60	Grand Total
4			1																											1
2		1		1	1	1																								4
1	1		2	1	1	4																								9
0.5					1	2	1					1																		5
0.25									1			1																		2
0.125								1		1	1	3	3	1		1	1													12
0.063								1	1					3		1	3	1	1		1	1								13
0.032							1	1	1		3	2	2	1	5	4	3	3	6	2	1	1		2						38
0.016							1	1	1	1	3	4	4	5	9	17	8	16	7	11	5	13	8	2	1	2				119
0.008			1					1		3	2	5	5	6	2	12	6	13	10	12	9	10	4	2	3	1	1		1	109
0.004										2		1	1	3	3	7	9	11	19	8	9	11	6	6	9	4	5	2	1	117
0.002										1						1	2	2	2	2	5	3	4	2	2	3	1	1	3	34
Grand Total	1	1	4	2	3	7	3	5	4	8	9	17	15	19	19	43	32	46	45	35	30	39	22	14	15	10	7	3	5	463

- Breakpoints Working Group Discussion and Recommendation

Count of 0.008	Column Labels																															
Row Labels	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	Grand Total
4			1																														1
2				1		1	1	1																									4
1			1		2	1	1	4																									9
0.5						1	2	1					1																				5
0.25										1			1																				2
0.125									1		1	1	3	3	1		1	1															12
0.063									1	1					3		1	3	1	1		1	1										13
0.032								1	1	1		3	2	2	1	5	4	3	3	6	2	1	1				2						38
0.03																							3										3
0.016								1	1	1	1	3	4	4	5	9	17	8	16	7	11	5	13			8	2	1	2					119
0.015																								6	7	6							19
0.008									1		3	2	5	5	6	2	12	6	13	10	12	9	10	5	11	9	4	2	3	1	1		1	133
0.004											2		1	1	3	3	7	9	11	19	8	9	11	9	7	6	6	6	9	4	5	2	1	139
0.002											1						1	2	2	2	2	5	3	3	3	1	4	2	2	3	1	1	3	41
Grand Total			1	1	3	2	3	7	3	5	4	8	9	17	15	19	19	43	32	46	45	35	30	39	26	28	22	22	14	15	10	7	3	5	538

	Very Major	Major	minor
≥I + 2	0	NA	0
I + 1 and I-1	5.30%	0	10.5
≤I -2	NA	0.41%	0

Table 9. Guideline for Acceptable Discrepancy Rates (With Intermediate Range)[a]

MIC Range		Discrepancy Rates		
1-Dilution Intermediate Range	2-Dilution Intermediate Range	Very Major, %	Major, %	Minor, %
≥I + 2	≥I_{upr} + 2	< 2	N/A	< 5
I + 1 to I − 1	I_{upr} + 1 to I_{lwr} − 1	< 10	< 10	< 40
≤I − 2	≤I_{lwr} − 2	N/A	< 2	< 5

Abbreviations: I, intermediate; I_{upr}, higher MIC in a two-dilution intermediate range; I_{lwr}, lower MIC in a two-dilution intermediate range; MIC, minimal inhibitory concentration; N/A, not applicable.
[a]See example in Appendix H.

  - Intermediate category for MIC but not for disk. An unusual scenario, but errors are limited.
  - Motion to accept the ceftriaxone disk diffusion (30 µg disk) resistant only breakpoint (R ≤ 35 mm) for *Neisseria gonorrhoeae*. If a zone of ≥ 36 mm, MIC test is necessary for breakpoint determination. WG Vote: 10-0-0-3. Motion passed.

SC DISCUSSION (MAIN POINTS)

- There was uncertainty with moving forward with the 30 ug disk recommendation.
- Decision to revisit the 30 µg ceftriaxone disk breakpoints at the June 2026 meeting. Review with the GC AHWG 5 µg ceftriaxone disk data.

A motion to approve the ceftriaxone disk diffusion (5 µg disk) and QC study for *Neisseria gonorrhoeae* using CLSI M23 criteria. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

CEFOTAXIME AND CEFTRIAXONE BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Current CLSI Breakpoints and Correlates

M100 S35 (2025)

Table 2B-2. *Acinetobacter* spp. (Continued)

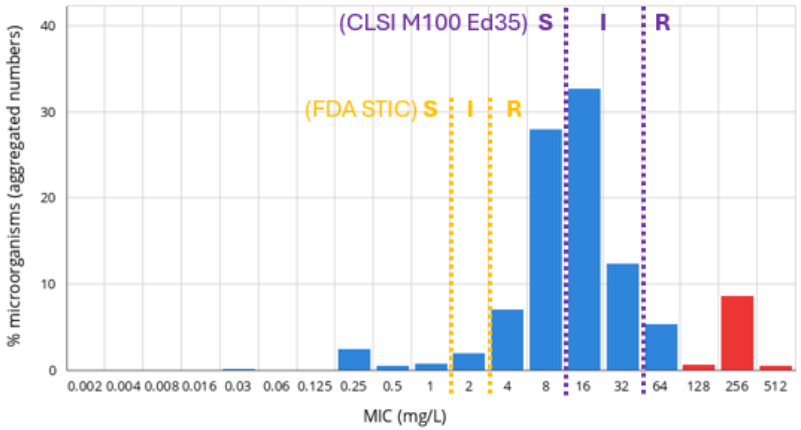
Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL		
		S	I	R	S	I	R
Ceftazidime	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32
Cefepime	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32
Cefotaxime	30 µg	≥ 23	15–22	≤ 14	≤ 8	16–32	≥ 64
Ceftriaxone	30 µg	≥ 21	14–20	≤ 13	≤ 8	16–32	≥ 64

- None of these cephalosporins have EUCAST breakpoints
- Current State of Breakpoints: FDA vs CLSI

Drug	S (CLSI)	I (CLSI)	R (CLSI)	S (FDA)	I (FDA)	R (FDA)
Ceftazidime	<=8	16	>=32	CLSI	CLSI	CLSI
Ceftriaxone	<=8	16-32	>=64	NR	NR	NR
Cefotaxime	<=8	16-32	>=64	<=1	2	>=4

  - Ceftazidime: FDA recognizes MIC breakpoints but not disk correlates
  - Ceftriaxone: Neither MIC or disk breakpoints recognized
  - Cefotaxime: Different MIC breakpoints
- Microbiology Data
  - Key mechanisms of resistance to cephalosporins in *Acinetobacter baumannii* complex
    - Outer membrane is intrinsically less permeable to cephalosporins (2- to 7-fold) compared to *P. aeruginosa*
      - Smaller number of outer membrane porins (OMPs)
      - OMPs are smaller than those in other gram-negative organisms
    - Chromosomal blaADC (class C; Acinetobacter-derived cephalosporinase)
      - Not inducible
      - Overexpression occurs in the presence of certain upstream insertion sequences (eg, ISAba1-blaADC-30 increases ceftazidime MIC 4-fold in an isogenic background)
      - Substitutions within blaADC can also increase the hydrolysis of ceftazidime or broaden its hydrolytic spectrum (eg, to include cefepime)
    - Wide variety of other ß-lactamases, including blaOXA (which vary in their hydrolysis of cephalosporins) and, less commonly, other carbapenemases (including metallo-ß-lactamases)
    - Efflux pump overexpression
  - Data sources: *Acinetobacter baumannii* complex reference BMD **cefotaxime** MIC distributions
    - EUCAST MIC distribution website: 1,036 A. baumannii isolates; accessed 10/1/2025
    - JMI/Element SENTRY Microbiology Visualization Platform: no cefotaxime data for *Acinetobacter* spp.
    - ATLAS (Pfizer): no cefotaxime data for *Acinetobacter* spp.
    - Shionogi 5-year SIDERO-WT surveillance: no cefotaxime data

- Cefotaxime MIC distribution: *A. baumannii*



EUCAST
A. baumannii
Ceftriaxone MIC distribution

1,036 isolates

EUCAST has set an ECOFF of 64 mg/L (confidence interval 16-512 mg/L)

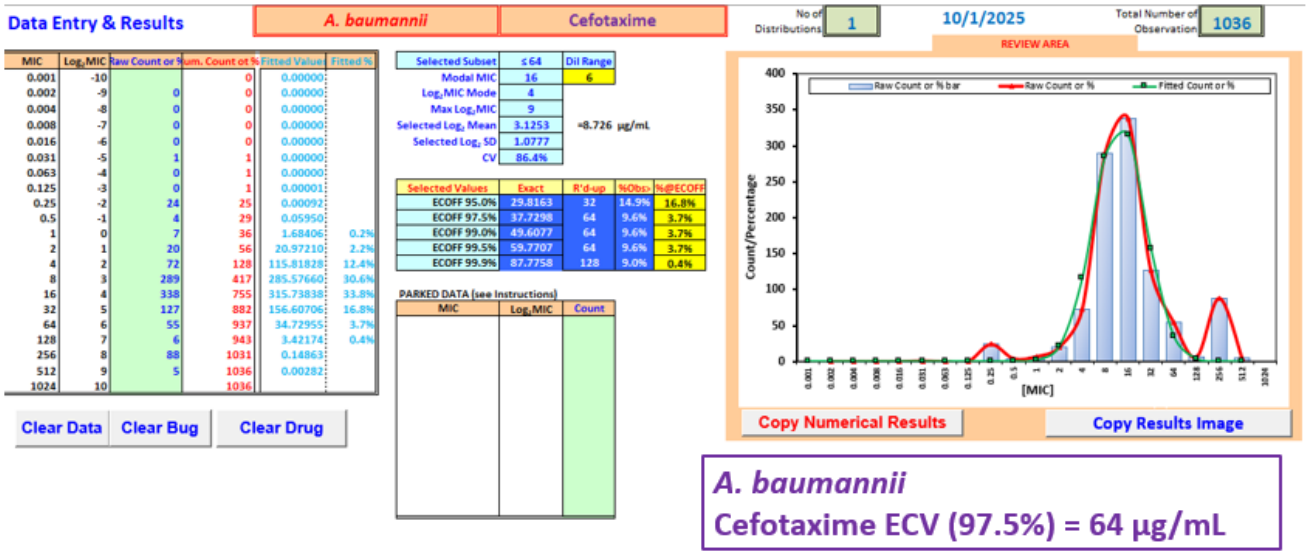
"Dash in breakpoint tables indicates that the agent is unsuitable for treatment of infections caused by the organism or group of organisms and that testing and clinical use should be avoided. If included, report resistant without prior testing."

The current CLSI susceptible breakpoint is below the modal wild-type MIC (16 µg/mL) in this distribution

CLSI M100 Ed35 breakpoints shown

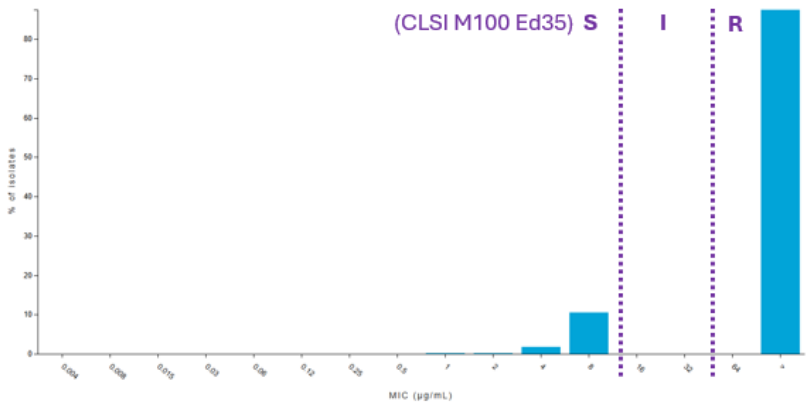
Cefotaxime MIC, µg/mL	0.002	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Isolates (cumulative %)	0 (0)	0 (0)	0 (0)	0 (0)	1 (<0.1)	0 (<0.1)	0 (<0.1)	24 (2.4)	4 (2.8)	7 (3.5)	20 (5.4)	72 (12.4)	289 (40.3)	338 (72.9)	127 (85.1)	55 (90.4)	6 (91.0)	88 (99.5)	5

- ECOFF finder for cefotaxime "ECV" using EUCAST dataset



A. baumannii
Cefotaxime ECV (97.5%) = 64 µg/mL

- *A. baumannii* cefotaxime ECV summary
  - Limited cefotaxime MIC distribution data were available for review.
  - Based on the EUCAST *A. baumannii* cefotaxime MIC distribution, the ad hoc working group recommends using an ECV of 64 µg/mL for the purpose of breakpoint setting.
  - The existing CLSI susceptible breakpoint (≤ 8 µg/mL) cuts into the wild-type cefotaxime MIC distribution for *A. baumannii* and is below the wild-type mode of the EUCAST MIC distribution.
- What about *Acinetobacter* spp. other than those in the *A. baumannii* complex?
  - ECVs are set for species, not for organism groups
    - Exception: closely related species that can't be readily separated by MALDI-TOF MS can have ECVs established (*A. baumannii* complex falls into this category)
  - Cefotaxime ECVs for other *Acinetobacter* spp. may be different, but none of the accessed data sources have cefotaxime MIC distribution data to evaluate this
  - Laboratories using MALDI-TOF MS for isolate identification may be able to identify many *Acinetobacter* spp. outside of the *A. baumannii* complex to the species level
    - But not all clinical laboratories have MALDI-TOF MS capabilities, and automated biochemical identification systems cannot reliably identify many *Acinetobacter* spp. isolates to the species level
    - Having a cefotaxime breakpoint that applies only to certain, relatively uncommonly isolated *Acinetobacter* spp. may be complicated for laboratories to implement – unclear that this would be worthwhile to pursue
- Data sources: *Acinetobacter baumannii* complex reference BMD ceftriaxone MIC distributions
  - JMI/Element SENTRY Microbiology Visualization Platform: 9,260 *A. baumannii* complex isolates; accessed 9/30/2025
  - EUCAST MIC distribution website: 3,054 *A. baumannii* isolates; accessed 9/30/2025
  - Mayo agar dilution data courtesy of Nicolynn Cole and Audrey Schuetz: 1,122 *A. baumannii* complex isolates (2020-2025)
  - ATLAS (Pfizer): no ceftriaxone data for *Acinetobacter* spp.
  - Shionogi 5-year SIDERO-WT surveillance: no ceftriaxone data
- Ceftriaxone MIC distribution: *A. baumannii* complex



JMI/Element SENTRY Public
***A. baumannii* complex**
Ceftriaxone MIC distribution

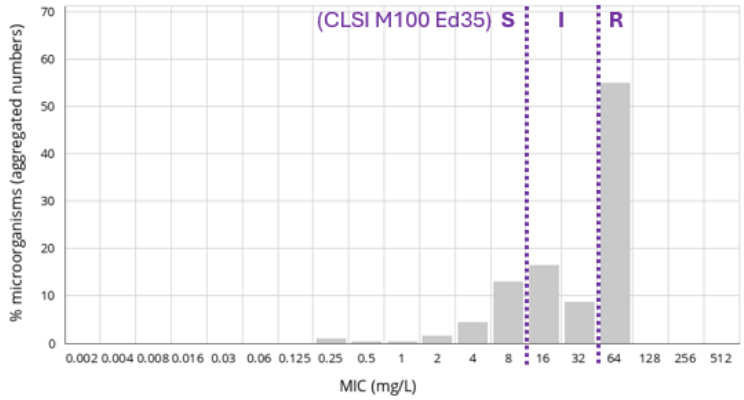
9,260 isolates

Wild-type MIC distribution data are truncated on the high end

CLSI M100 Ed35 breakpoints shown

Ceftazidime MIC, µg/mL	0.06	0.12	0.25	0.5	1	2	4	8	16	32	> 32
Isolates (cumulative %)	1 (< 0.1)	0 (< 0.1)	0 (< 0.1)	1 (< 0.1)	5 (0.1)	12 (0.2)	163 (2.0)	979 (12.5)	8099		

- Ceftriaxone MIC distribution: *A. baumannii*



EUCAST
A. baumannii
Ceftriaxone MIC distribution

3,054 isolates
Based on a **single distribution**

EUCAST has not set an ECOFF

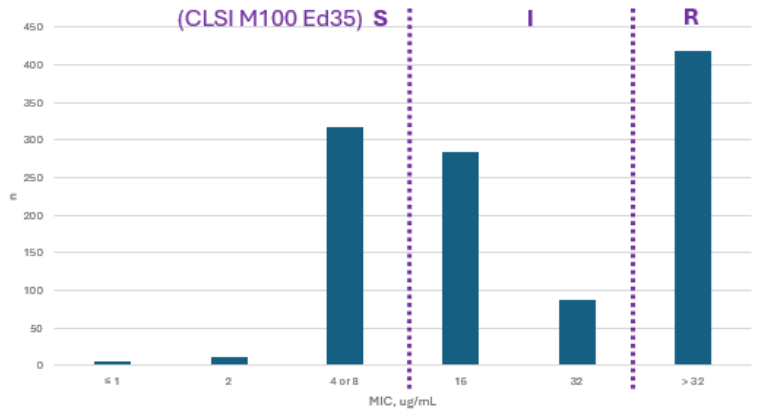
"Dash in breakpoint tables indicates that the agent is unsuitable for treatment of infections caused by the organism or group of organisms and that testing and clinical use should be avoided. If included, report resistant without prior testing."

Data are truncated on the high end, but the current CLSI susceptible breakpoint is below the modal wild-type MIC (16 µg/mL) in this distribution

CLSI M100 Ed35 breakpoints shown

Ceftazidime MIC, µg/mL	0.002	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	> 32
Isolates (cumulative %)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	25 (0.8)	7 (1.0)	7 (1.3)	46 (2.8)	134 (7.2)	395 (20.1)	502 (36.5)	264 (45.2)	1674

- Ceftriaxone MIC distribution: *A. baumannii* complex



Mayo
***A. baumannii* complex**
Ceftriaxone MIC distribution

1,122 isolates (2020-2025)

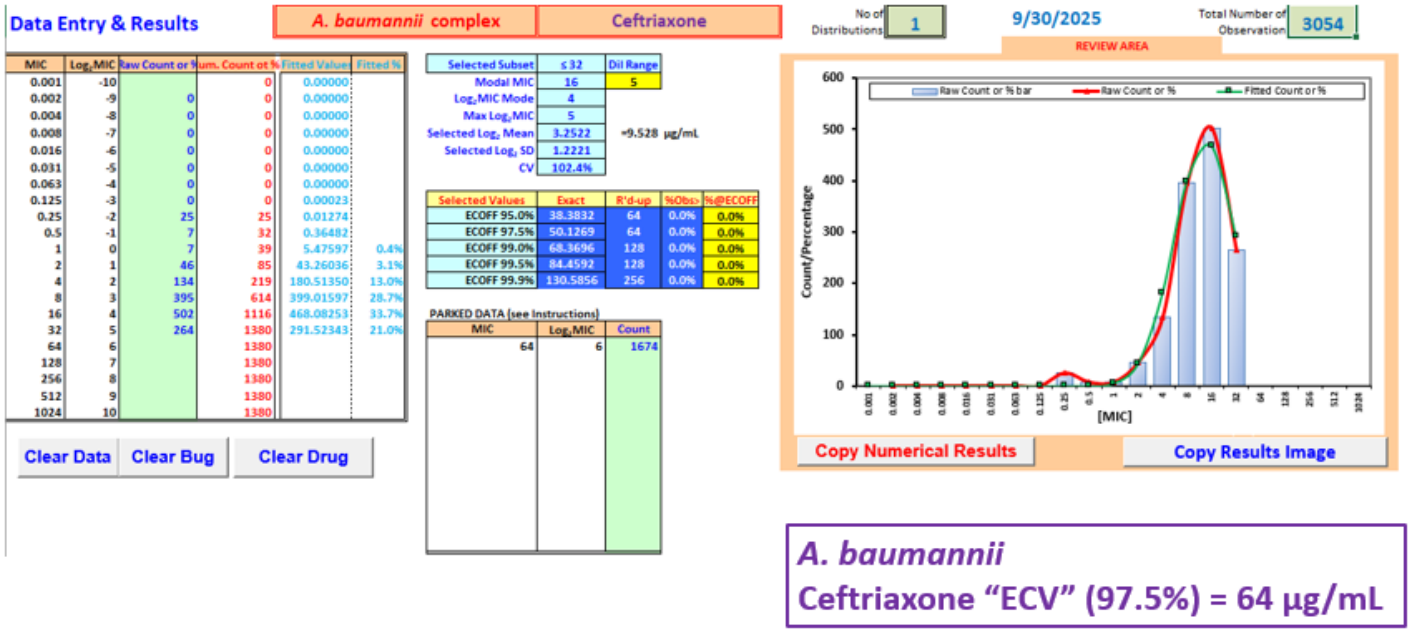
Tested by **agar dilution** (1, 2, 8, 16, and 32 µg/mL)

Isolates for which MICs are 4 or 8 µg/mL are not separated by testing scheme, so modal wild-type MIC is undetermined, but could be as high as 16 µg/mL (as in the EUCAST data)

CLSI M100 Ed35 breakpoints shown

Ceftriaxone MIC, µg/mL	≤ 1	2	4 or 8	16	32	> 32
Isolates (cumulative %)	5 (<0.1)	11 (1.4)	317 (29.7)	284 (55.0)	87 (62.7)	418

- Approach to ECOFF Finder for ceftriaxone and *A. baumannii*
  - Used the EUCAST dataset only
    - Excluded the JMI data because of the degree of truncation on the high end
    - Excluded the Mayo data because of the binning of isolates within the wild-type
    - EUCAST data represent a single distribution, so it is not possible to set a formal ECV
    - The fact that the highest possible "call" in the EUCAST data was only 2 dilutions above the wild-type mode also limits our ability to confidently ascertain the EC
  - Placed MICs in the bins in which they are listed; where off-scale on the high end, placed values in the next bin (eg, placed > 32 µg/mL data points in the 64 µg/mL bin
  - Put data from the second mode at the higher end of the distribution (higher than the mode in the putative wild type) into the "Parked Data" area to avoid analysis being performed in the wrong place



- *A. baumannii* complex ceftriaxone ECV summary
  - Limited ceftriaxone MIC distribution data were available for review.
  - Based on the EUCAST *A. baumannii* ceftriaxone MIC distribution, the ad hoc working group recommends using an "ECV" of 64 µg/mL for the purpose of breakpoint setting.
  - The existing susceptible breakpoint (≤ 8 µg/mL) cuts into the wild-type ceftriaxone MIC distribution for *A. baumannii* and is below the wild-type mode of the EUCAST MIC distribution.
- Other *Acinetobacter* species: Modal ceftriaxone MIC within the putative wild-type (examples)

Organism	EUCAST modal MIC, µg/mL	Mayo modal MIC, µg/mL	JMI modal MIC, µg/mL
Acinetobacter baumannii complex	16	As high as 16	Insufficient data
Acinetobacter ursingii	---	As high as 16	8 or higher
Acinetobacter radioresistens	---	4 or 8	8
Acinetobacter bereziniae	---	4 or 8	8
Acinetobacter guillouiae	---	4 or 8	8
Acinetobacter junnii	---	2	4
Acinetobacter haemolyticus	---	Insufficient data	2
Acinetobacter lwoffii	---	2	2
Acinetobacter variabilis	---	2	2
Acinetobacter johnsonnii	---	As high as 2	2

Unable to calculate ECVs for these species

Additional ceftriaxone MIC distribution data for various *Acinetobacter* spp. from Mayo (thank you to Nicolynn Cole and Audrey Schuetz)

- PK/PD Data
  - DeRyke CA, Kuti JL, Nicolau DP. Pharmacotherapy 2007.
  - Lack of any PK-PD data for ceftriaxone or cefotaxime and *Acinetobacter* spp.
    - Other investigators into ceftriaxone PK-PD have also omitted *A. baumannii* from their analyses, treating *A. baumannii* like *Pseudomonas aeruginosa*
    - Frei CR, Wiederhold NP, Burgess DS. JAC 2008.
    - Koomanachai P, Nicolau DP et al. Clin Ther 2010.
- Clinical Data
  - Overview: data are sparse.
  - Available clinical data are usually reported as 'cephalosporin' therapy
  - Subgroup of studies describing treatment of nosocomial infections
  - No outcomes by MIC
  - Cephalosporin monotherapy for *A. baumannii*
    - Suphansatit et al, Infection and Drug Resistance 2020;13:4495-4500
    - N=32; 63.6 year old average; LRTI 28.1%; 8 in ICU
    - 59.4% achieved clinical cure
    - All patients who received 'cephalosporin' monotherapy survive with 100% clinical cure
    - Conclusion/limitations:
      - Too few patients
      - No specific cephalosporins mentioned
      - Retrospective; no outcome by MIC
  - Ceftriaxone or Ceftazidime with Tigecycline
    - Lee YT et al. Eur J Clin Microbiol Infect Dis. 2013;32(9):1211-1220

- Multi-drug resistant *A. baumannii*
- Clinical outcomes of patients of nosocomial multi-drug resistant *A. baumannii*
- Imipenem and sulbactam, and tigecycline alone or in combination with other antibiotics
- Baseline: Non-tigecycline group sicker
- Conclusion: cannot be inferred due to limitations

- Proposed ceftriaxone/cefotaxime breakpoints using M23 criteria for *A. baumannii*

	Data available?	Proposed breakpoint
ECV	±	64 µg/mL
Nonclinical PK/PD cutoff	✕	N/A
Clinical exposure response (CER) cutoff	✕	N/A
Clinical cutoff	±/✕	N/A

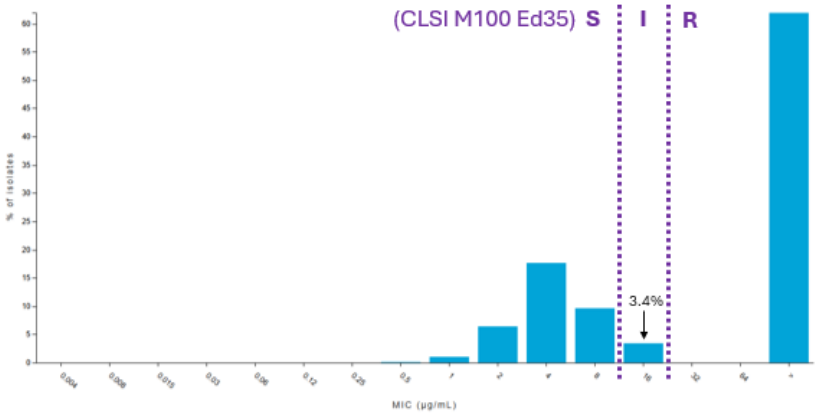
- *Acinetobacter* Ad Hoc Working Group Discussion and Recommendation
  - Option 1: Remove the ceftriaxone and cefotaxime breakpoints. AHWG Vote: 5-0-0-2
  - Option 2: Add a comment that ceftriaxone may have some activity against non-*baumannii* species. AHWG Vote: 0-4-3-0. Rejection reasoning: Too difficult to operationalize; confusing
- Breakpoints Working Group Discussion and Recommendation
  - Should *Acinetobacter* spp. be changed to *Acinetobacter baumannii* in M100 Table 2 given that all the data used for breakpoints are for *A. baumannii* complex?
  - This would be similar to separating out *Pseudomonas aeruginosa* from other *Pseudomonas* species
  - Could not find an expert in the working group meeting room who used either drug for *Acinetobacter*
  - Motion to remove cefotaxime and ceftriaxone breakpoints for *Acinetobacter* spp. in Table 2. WG Vote: 10-0-0-3. Motion passed.

A motion to remove the ceftriaxone and cefotaxime breakpoints for *Acinetobacter* spp. in Tables 1 and 2. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

CEFTAZIDIME MIC BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Microbiology Data
  - Data sources: *Acinetobacter baumannii* complex reference BMD ceftazidime MIC distributions
    - JMI/Element SENTRY Microbiology Visualization Platform: 10,751 *A. baumannii* complex isolates; accessed 9/26/2025
    - Lepak AJ et al. Antimicrob Agents Chemother 2023; 67(4): e01452-22.
      - 755 *A. baumannii* complex isolates (2018-2020, U.S.)

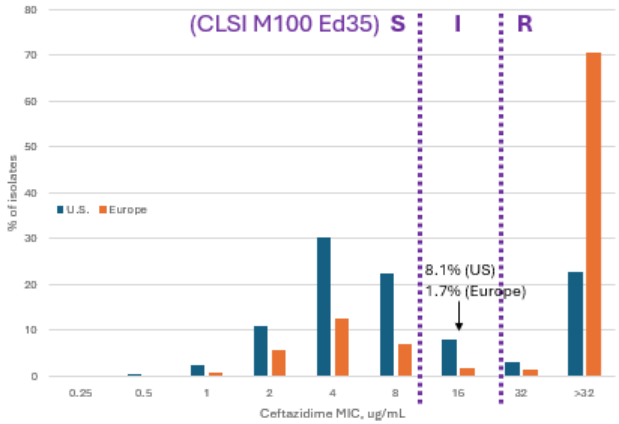
- 892 *A. baumannii* complex isolates (2018-2020, Europe)
  - ATLAS (Pfizer) Antibacterial Resistance Surveillance: 18,746 *A. baumannii* complex isolates (2019-2023, all regions); accessed 9/26/2025
  - EUCAST MIC distribution website: 3,644 *A. baumannii* isolates; accessed 9/26/2025
  - Shionogi 5-year SIDERO-WT surveillance: no ceftazidime data
- Ceftazidime MIC distribution: *A. baumannii* complex



CLSI M100 Ed35 breakpoints shown

Ceftazidime MIC, µg/mL	0.12	0.25	0.5	1	2	4	8	16	>16
Isolates (cumulative %)	0 (0)	2 (<0.1)	7 (0.1)	104 (1.1)	687 (7.4)	1894 (25.1)	1037 (34.7)	361 (38.1)	6659

- Ceftazidime MIC distribution: *A. baumannii* complex



Lepak AAC 2023
***A. baumannii* complex**
Ceftazidime MIC distribution

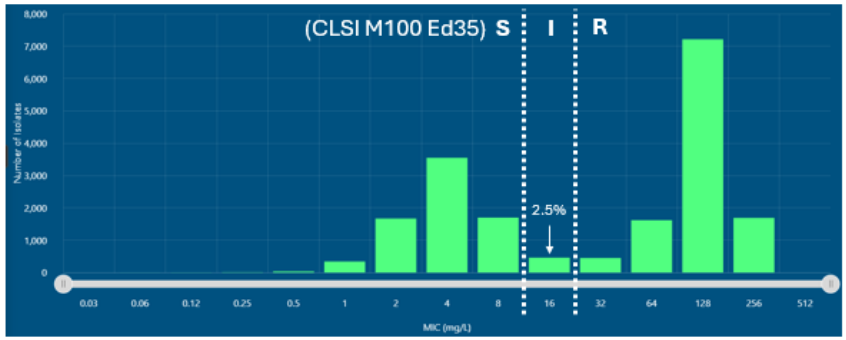
755 isolates from the U.S., 2018-2020
892 isolates from Europe, 2018-2020

Authors calculated an epidemiological cutoff value (ECV) of 16 µg/mL based on these data from the SENTRY Antimicrobial Surveillance Program

CLSI M100 Ed35 breakpoints shown

Ceftazidime MIC, µg/mL	0.25	0.5	1	2	4	8	16	32	> 32
U.S. Isolates (cumulative %)	0 (0)	1 (0.1)	17 (2.4)	82 (13.2)	229 (43.6)	169 (66.0)	61 (74.0)	23 (77.1)	173
Europe isolates (cumulative %)	0 (0)	0 (0)	8 (0.9)	52 (6.7)	112 (19.3)	62 (26.2)	15 (27.9)	12 (29.3)	631

- Ceftazidime MIC distribution: *A. baumannii* complex



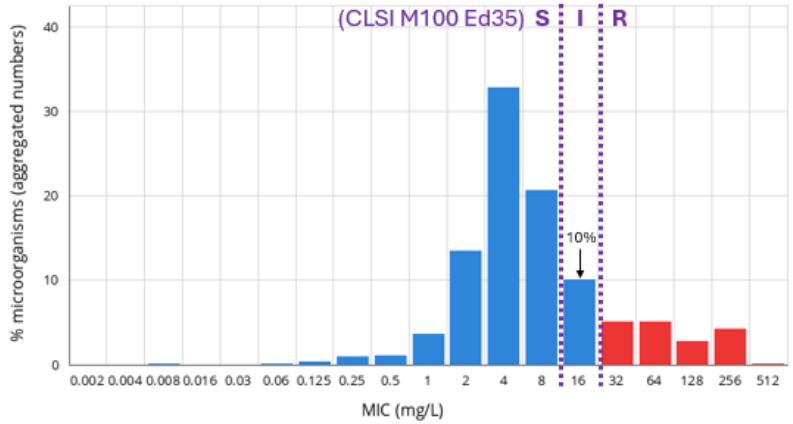
ATLAS (Pfizer)
***A. baumannii* complex**
Ceftazidime MIC distribution

18,746 isolates from all regions, 2019-2023

CLSI M100 Ed35 breakpoints shown

Ceftazidime MIC, µg/mL	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	> 128
Isolates (cumulative %)	3 (<0.1)	6 (<0.1)	11 (0.11)	34 (0.29)	343 (2.12)	1671 (11.03)	3548 (29.96)	1698 (39.02)	460 (41.47)	450 (43.87)	1620 (52.51)	7213 (90.99)	1689

- Ceftazidime MIC distribution: *A. baumannii*



EUCAST
A. baumannii
Ceftazidime MIC distribution

3,644 isolates

EUCAST has set an ECOFF of 16 mg/L (confidence interval 8-32 mg/L)

"Dash in breakpoint tables indicates that the agent is unsuitable for treatment of infections caused by the organism or group of organisms and that testing and clinical use should be avoided. If included, report resistant without prior testing."

CLSI M100 Ed35 breakpoints shown

Ceftazidime MIC, µg/mL	0.002	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Isolates (cumulative %)	0 (0)	0 (0)	1 (<0.1)	0 (<0.1)	0 (<0.1)	1 (<0.1)	9 (0.3)	34 (1.2)	37 (2.3)	133 (5.9)	490 (19.3)	1195 (52.1)	752 (72.8)	366 (82.8)	186 (87.9)	183 (92.9)	100 (95.7)	155 (99.9)	2

- Approach to ECOFF Finder for ceftazidime
  - Used the JMI SENTRY Public, ATLAS, and EUCAST datasets
    - Assigned each of these datasets as one MIC distribution
    - Did not include distributions from Lepak AAC 2023, presuming overlap with JMI SENTRY Public dataset
  - Included all species within the *A. baumannii* complex under the assumption that clinical laboratories may have difficulty distinguishing between them
  - Placed MICs in the bins in which they are listed; where off-scale on the high end, placed values in the next bin (eg, placed >128 µg/mL data points in the 256 µg/mL bin)
  - Put data from the second mode at the higher end of the distribution (higher than the mode in the putative wild type) and higher into the "Parked Data" area to avoid analysis being performed in the wrong place

Data Entry & Results | *A. baumannii* complex | Ceftazidime

No of Distributions: 3 | 9/26/2025 | Total Number of Observation: 33141

REVIEW AREA

MIC	Log_2MIC	Raw Count or %	Num. Count ot %	Fitted Values	Fitted %
0.001	-10		0	0.00000	
0.002	-9	0	0	0.00000	
0.004	-8	0	0	0.00000	
0.008	-7	1	1	0.00000	
0.016	-6	0	1	0.00000	
0.031	-5	0	1	0.00000	
0.063	-4	4	5	0.00010	
0.125	-3	15	20	0.05171	
0.25	-2	47	67	6.98549	
0.5	-1	78	145	252.48442	1.5%
1	0	580	725	##########	14.7%
2	1	2848	3573	##########	41.4%
4	2	6637	10210	##########	33.9%
8	3	3487	13697	##########	8.0%
16	4	1187	14884	90.38959	0.5%
32	5		14884		
64	6		14884		
128	7		14884		
256	8		14884		
512	9		14884		
1024	10		14884		

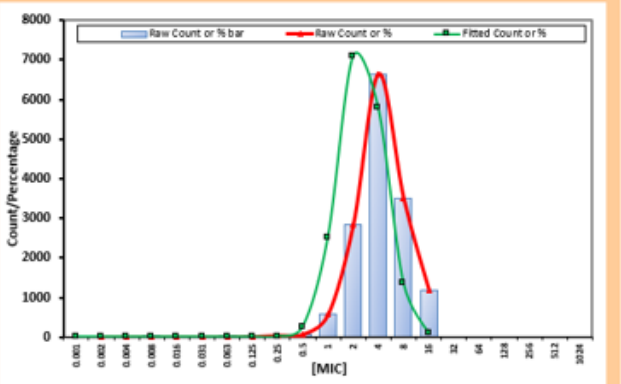
Clear Data | Clear Bug | Clear Drug

Selected Subset	≤ 16	Dil Range
Modal MIC	4	5
Log_2MIC Mode	2	
Max Log_2MIC	4	
Selected Log_2 Mean	0.8369	=1.786 µg/mL
Selected Log_2 SD	0.8480	
CV	64.2%	

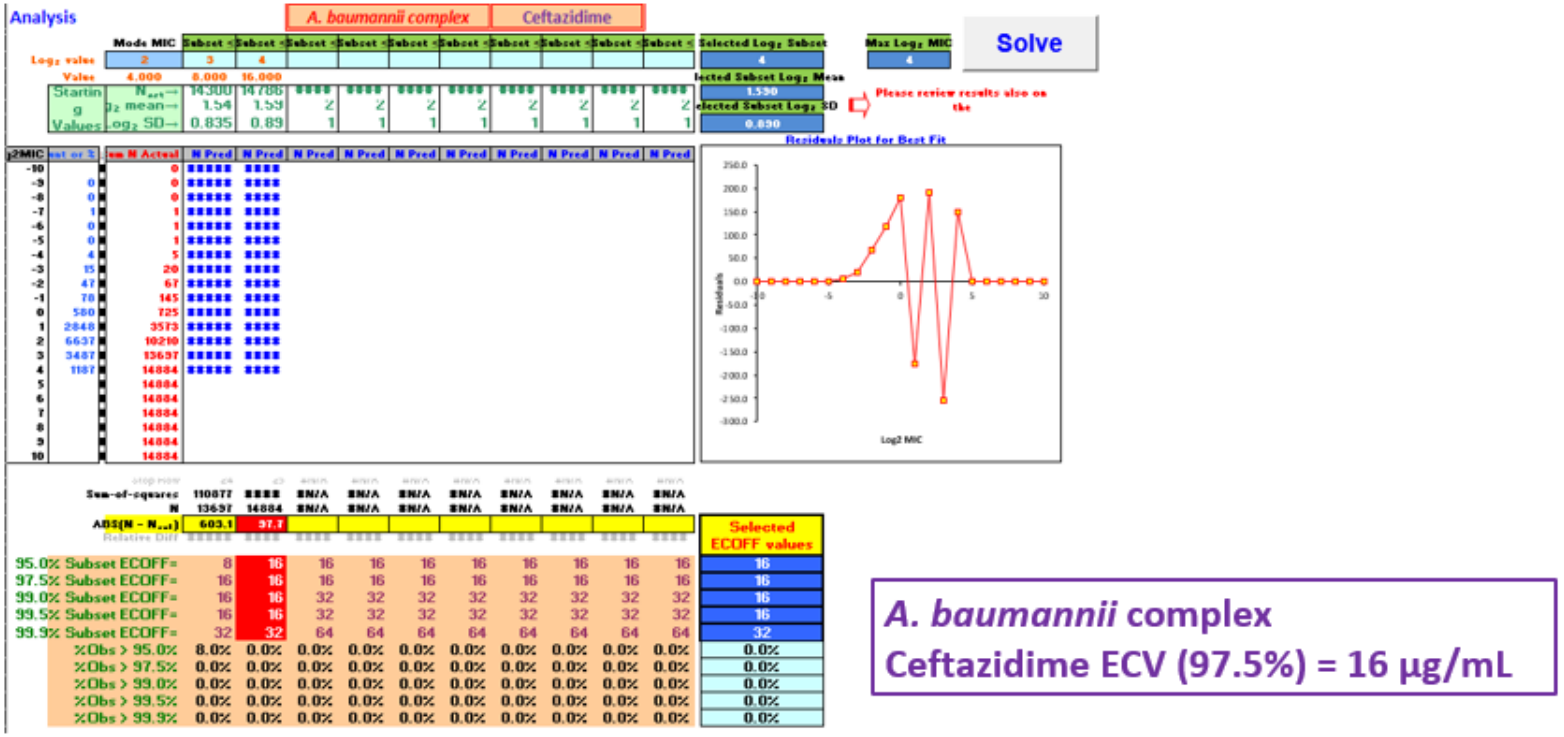
Selected Values	Exact	R'd-up	%Obs>	%@ECOFF
ECOFF 95.0%	4.6968	8	8.0%	8.0%
ECOFF 97.5%	5.6525	8	8.0%	8.0%
ECOFF 99.0%	7.0108	8	8.0%	8.0%
ECOFF 99.5%	8.1181	16	0.0%	0.5%
ECOFF 99.9%	10.9842	16	0.0%	0.5%

PARKED DATA (see Instructions)

MIC	Log_2MIC	Count
32	5	7295
64	6	1803
128	7	7313
256	8	1844
512	9	2



Copy Numerical Results | Copy Results Image

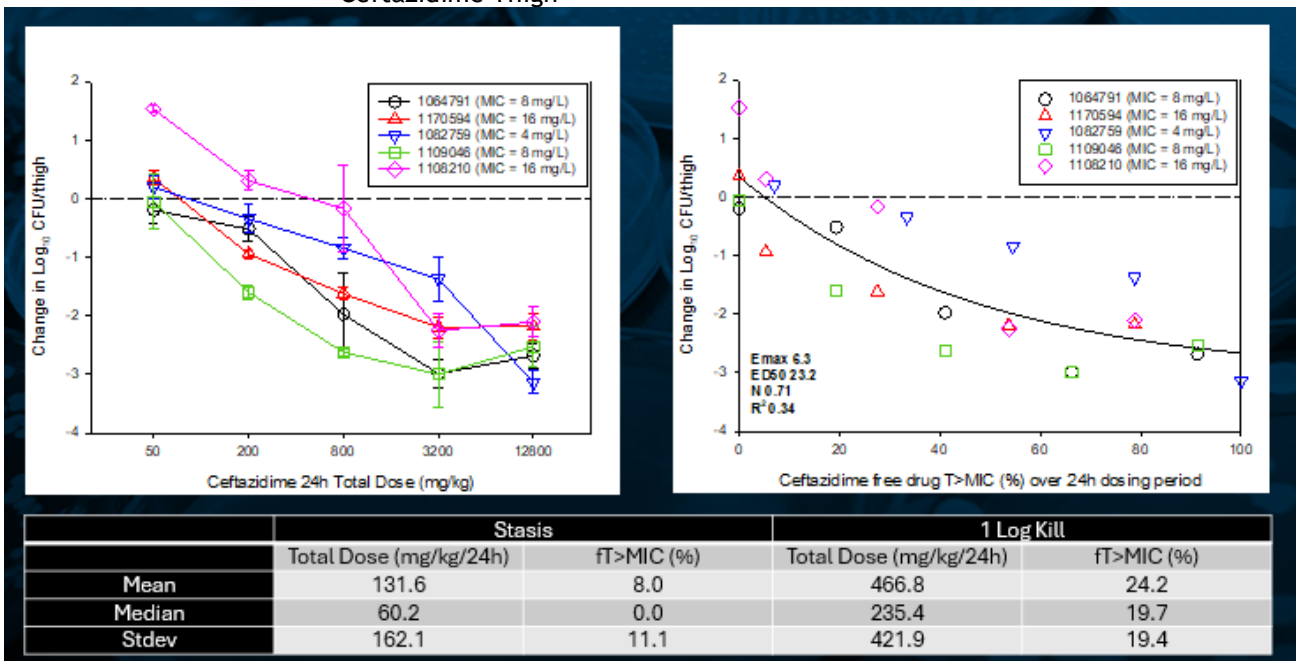


- *A. baumannii* complex ceftazidime ECV summary
  - Following review of *A. baumannii* complex ceftazidime MIC distributions from multiple data sources (JMI/Element, ATLAS, and EUCAST), the ad hoc working group suggests using an ECV of 16 µg/mL for the purpose of breakpoint setting. This matches the EUCAST ECOFF.
  - The existing susceptible breakpoint (≤ 8 µg/mL) cuts into the wild-type MIC distribution by one dilution.
    - In the various MIC distributions reviewed, the percentage of *A. baumannii* complex isolates for which ceftazidime MICs were 16 µg/mL (ie, within the wild-type distribution but above the susceptible breakpoint) ranged from 1.7% to 10%.
  - Discrepancy between ECOFF finder (official CLSI tool) and M23 re: use of species complex for ECV

- PK/PD Data
  - Lepak AJ et al. Antimicrob Agents Chemother 2023; 67(4): e01452-22.
    - 19 strains – untreated growth 24 hours
    - Inoculum:
      - Thigh 7.31 +/- 0.26
      - Lung 7.78 +/- 0.25
    - > 1 log growth
      - 13 thigh (68%)

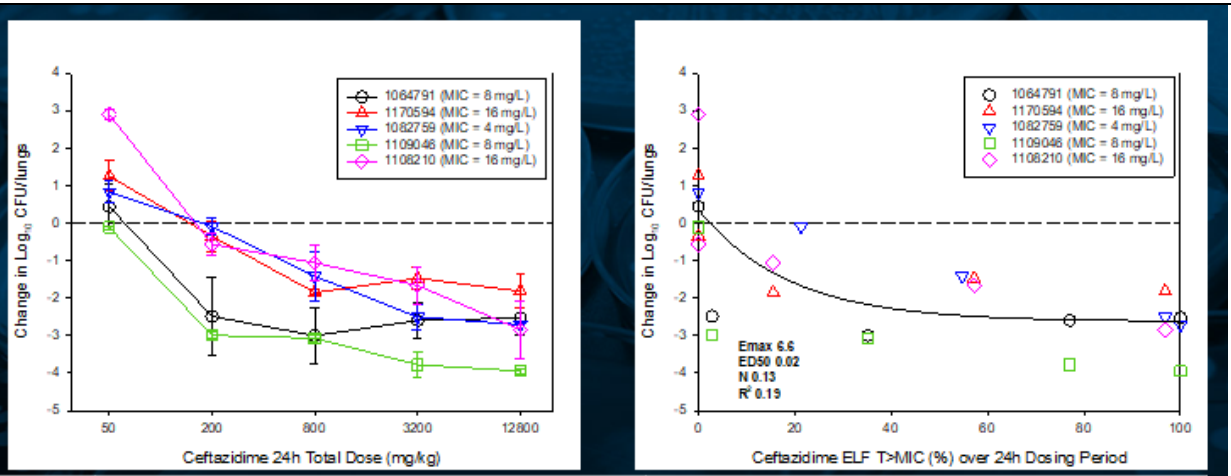
- 8 lung (42%)

▪ Thigh and Lung Treatment Studies
- 4 drugs X 5 *Acinetobacter baumannii* strains
- Neutropenic mouse lung and thigh
- Emax – stasis and 1 log kill vs plasma and ELF PK/PD drivers
- Ceftazidime Thigh



	Stasis		1 Log Kill	
	Total Dose (mg/kg/24h)	fT>MIC (%)	Total Dose (mg/kg/24h)	fT>MIC (%)
Mean	131.6	8.0	466.8	24.2
Median	60.2	0.0	235.4	19.7
Stdev	162.1	11.1	421.9	19.4

- Ceftazidime Lung



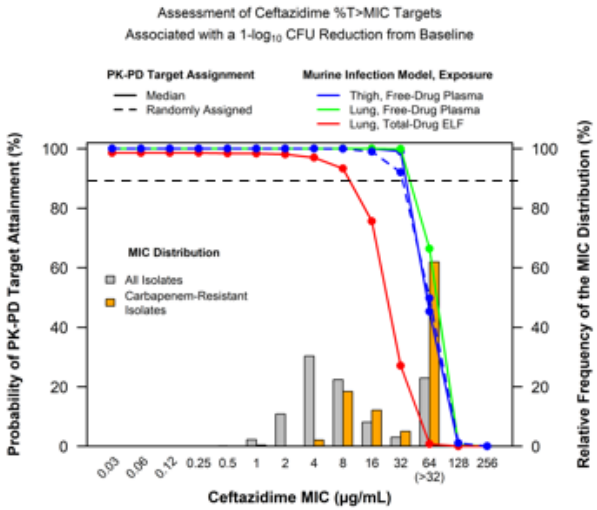
	Stasis			1 Log Kill		
	Total Dose (mg/kg/24h)	ELF T>MIC (%)	Plasma fT>MIC (%)	Total Dose (mg/kg/24h)	ELF T>MIC (%)	Plasma fT>MIC (%)
Mean	141.6	2.3	8.1	345.4	11.0	17.7
Median	132.4	0.0	0.0	330.7	0.0	13.0
Stdev	106.7	5.2	12.4	292.1	17.7	19.4

- 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
- Summary of ELF penetration ratio by agent
  - 15 patients in ICU receiving continuous infusion (4 g/day) ceftazidime for nosocomial pneumonia
  - Compared steady state plasma to ELF concentrations
  - Boselli et al. Intensive Care Medicine 2004. PMID: 14985960

Drug	Mean (%CV) ELF penetration ratio[a]	Reference
Amikacin	0.588 (39.8)	19[d]
Ceftazidime	0.228 (43.2)	20
Ciprofloxacin	1.17 (29.3[b])	21, 22
Minocycline	1.00[c] (28.3)	23

a. Represents the ratio of total-drug ELF exposure to free-drug plasma exposure based on protein binding estimates utilized for analysis.
b. Variability derived from bootstrap procedure of PK data.
c. Based on penetration ratios for tigecycline and omadacycline, a conservative ratio of 1.00 was used.
d. Using data from reference 19, the time-course of tobramycin in serum and ELF at steady-state in patients with pneumonia were characterized. Using AUC values based on these concentration-time profiles, the ELF penetration for tobramycin was determined and assumed to be equivalent for amikacin (Data on file, ICPD).

- Percent Probabilities of PK-PD Target Attainment for Ceftazidime 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
Ceftazidime	≤ 32	-	≥ 64
Ceftazidime-pneumonia	≤ 8	-	≥ 16
Ciprofloxacin	≤ 1	-	≥ 2
Minocycline	≤ 0.5/ ≤ 1[e]	-	≥ 1/≥ 2

  - Berkhout J et al. AAC 2016.
    - Against *P. aeruginosa*, 9 of 13 experiments for ceftazidime alone in thigh and lung had net stasis at 0% T>MIC (sub-MIC inhibitory effects)
    - Findings concordant with USCAST data for *A. baumannii*
- Clinical Data
  - Randomized trial of ceftazidime vs. imipenem for patients with pneumonia, septicemia, and UTI
    - Norrby et al. J Antimicrob Chemother. 1993;31(6):927-937
    - Multicenter comparative trial, Europe, 1990
    - Randomized to ceftazidime 2 g bid or imipenem-cilastatin 500mg qid given for ≥ 5 days

        - n=393
        - 9 patients with *Acinetobacter* spp.
    - Inferring data on ceftazidime efficacy for *A. baumannii* from and randomized control trial of ceftobiprole vs. ceftazidime and linezolid for healthcare-associated pneumonia
        - Awad et al. CID, 2014;59(1):51-61 DOI: 10.1093/cid/ciu219
        - Multicenter, double-blind RCT – 2005 to 2007; 795 patients
        - 157 sites: Europe, North and South America, Asia-Pacific
            - Age ≥ 18 year olds who met at least 2 clinical criteria of HAP/VAP, fever or WBC, and APACHE II ≥ 8
            - Sick population: overall mortality ~19%
            - Excluded patients with ceftazidime or ceftobiprole resistant pathogens; renal or hepatic failure; clinical conditions that interfered with efficacy assessment such sustained shock, TB etc.
        - Participants must not have had systemic antibiotic treatment for >24 hours in the 48 hours before enrollment
        - 1:1 to ceftobiprole 500 mg q8h 2 hr infusion (+placebo), or ceftazidime 2g q8h 2h infusion and linezolid 600 mg q12h IV (7-14 day courses) - additional open label Rx with fluoroquinolones or aminoglycosides allowed for patients at risk of *Pseudomonas* infection
    - Relationship of ceftazidime exposure and outcome in patients with nosocomial pneumonia using data from a randomized, double-blind Phase 3 clinical trial
        - Muller et al. J Antimicrob Chemother. 2013;68(4):900-906
        - For all Gram-negative pathogens, not just *Acinetobacter*
        - The results clearly show that the effect of treatment is not 100%. Even at very low exposures almost half the patient population is microbiologically cured.
        - MIC alone did not predict outcome as well as PK/PD indices did, including exposure and the variation therein per patient
- Proposed ceftazidime breakpoints using M23 criteria for *A. baumannii*

	Data available?	Proposed breakpoint
ECV	☑	16 µg/mL
Nonclinical PK/PD cutoff	☑ (Mouse thigh and lung infection models)	≤32 µg/mL (≤ 8 µg/mL pneumonia)
Clinical exposure response (CER) cutoff	☑ MCS	≤32 µg/mL (≤ 8 µg/mL pneumonia)
Clinical cutoff	±/ ✕	N/A

- *Acinetobacter* Ad Hoc Working Group Discussion and Recommendation
    - Option 1: Follow USCAST guidance with separate breakpoints for pneumonia and systemic infections. AHWG Vote: 0-7-0-0. Rejection reasoning: main rationale: not enough data to do this for this particular combination
    - Option 2: Maintain current ceftazidime breakpoints of ≤ 8 S, 16 I, ≥ 32 R. Add comment to clarify the breakpoint is based on dose of 2g IV q8 hours. AHWG Vote: 7-0-0-0.

- Option 3: Maintain susceptible breakpoint of ≤ 8 but expand intermediate breakpoint to 16-32 with ≥ 64 R with comment about differences between pneumonia and systemic infections. AHWG Vote: 0-7-0-0.

- Breakpoints Working Group Discussion and Recommendation
  - Concern about poor correlation between fT>MIC and efficacy and low PK-PD targets identified in the mouse thigh and lung infection model
  - If used a more conventional cephalosporin target fT>MIC of 50%, how much lower would the probabilities of target attainment be? -> An argument for being conservative.
  - Questions about the role of modeling total ELF based on mouse data
  - Ceftazidime is used by some clinicians for *Acinetobacter*, particularly in Japan (per communication with Yohei Doi)
  - AHWG only briefly discussed removing the breakpoint
  - Concern of setting breakpoint 1 dilution below ECV -> disk diffusion breakpoints do not look good
  - Motion to maintain the current ceftazidime MIC breakpoints (S ≤ 8, I 16, R ≥ 32 µg/mL) for *Acinetobacter baumannii* based on a dosage of 2g IV q8h. WG Vote: 10-0-0-3. Motion passed.

SC DISCUSSION (MAIN POINTS)

- There were concerns on continuing to include *Acinetobacter* species in the tables despite the data being mostly generated from *A. baumannii* complex.
- If limited to *A. baumannii* laboratories may use the non-Enterobacterales ceftazidime breakpoint which has the same breakpoint as being proposed.
- A discussion ensued on whether to use the species complex data in ECOFF finder. Matt Wikler volunteered to review and bring forward proposed updates to CLSI M23 as needed, regarding if species complex can or cannot be included in setting of ECV (or criteria when species complex is acceptable for use in ECV setting).

A motion to maintain the current ceftazidime MIC breakpoints (S ≤ 8, I 16, R ≥ 32 µg/mL) for *Acinetobacter baumannii* complex based on a dosage of 2g IV q8h. Vote: 3 for, 11 against, 0 abstain, 0 absent (Fail)

Against Vote Reasoning:

- Preferred *Acinetobacter* spp.

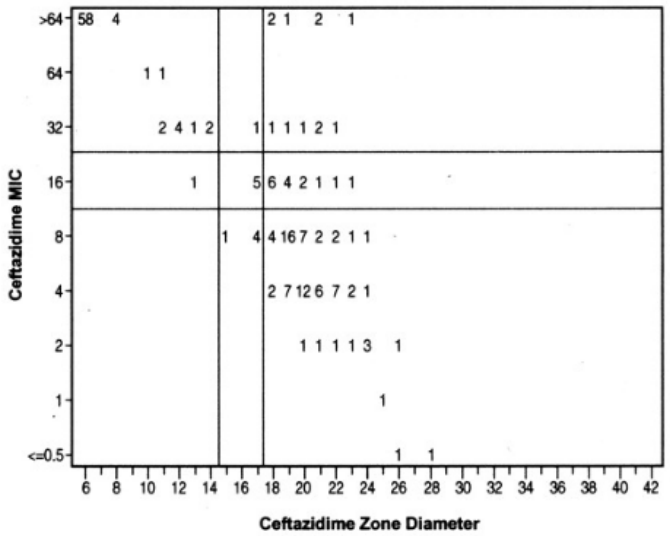
A motion to maintain the current ceftazidime MIC breakpoints (S ≤ 8, I 16, R ≥ 32 µg/mL) for *Acinetobacter* spp. based on a dosage of 2g IV q8h. Vote: 12 for, 2 against, 0 abstain, 0 absent (Pass)

Against Vote Reasoning:

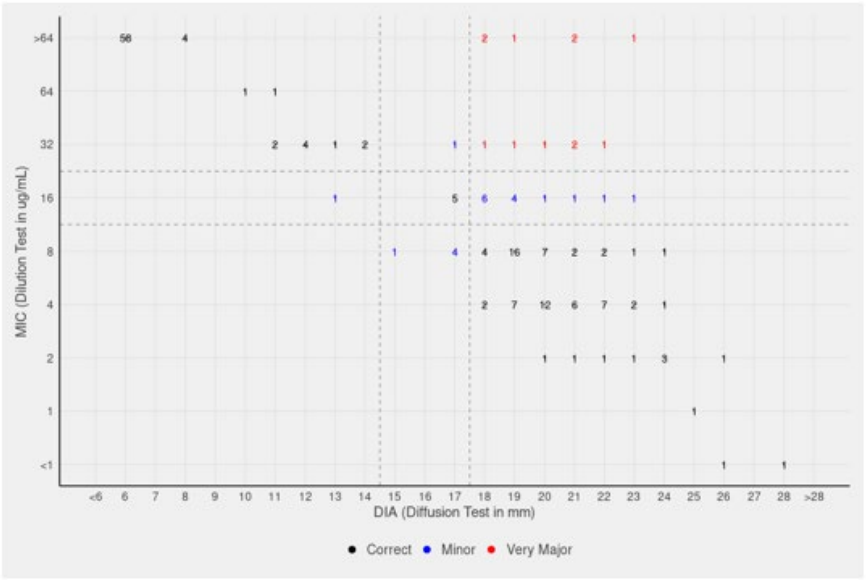
- Preferred *Acinetobacter baumannii* complex.

CEFTAZIDIME DISK DIFFUSION BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Current disk correlates in M100:
  - S: ≥ 18 mm
  - I: 15-17 mm
  - R: ≤ 14 mm
- This is the only disk correlates data set the AHWG as able to find
  - Swenson JM, Killgore GE, Tenover FC. J Clin Microbiol 2004; 42(11): 5102-8.



- Current Disk Correlates (≤ 14 R; 15-17 I; ≥ 18 S)

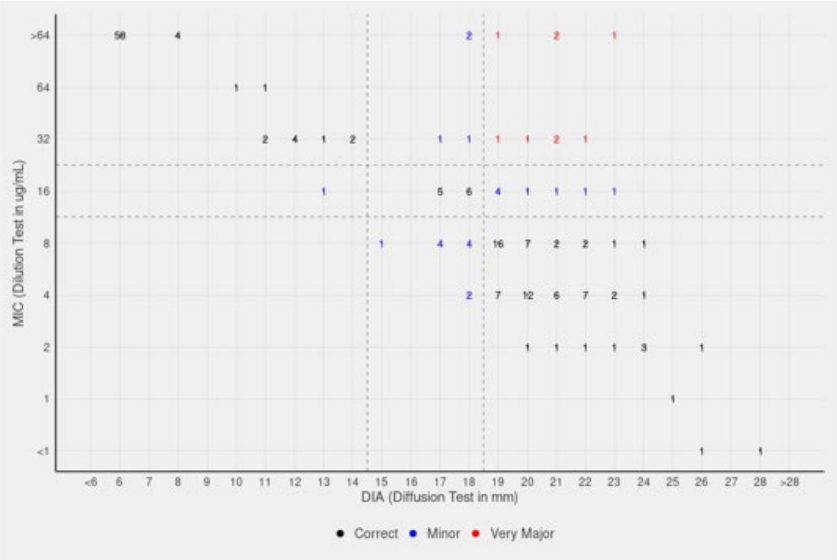


	VME	ME	mE
> I + 2	8.6%	n/a	0.0%
I +/- 1	8.1%	0.0%	28.4%
< I - 2	n/a	0.0%	0.0%

M23 Goal

	VME	ME	mE
> I + 2	< 2%	n/a	< 5%
I +/- 1	< 10%	< 10%	< 40%
< I - 2	n/a	< 2%	< 5%

- dBets Recommended (≤ 14 R; 15-18 I; ≥ 19 S)

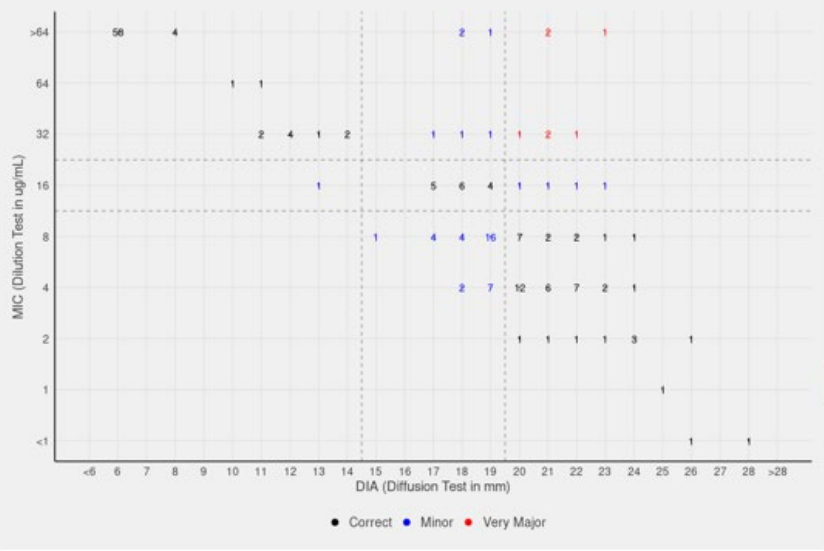


	VME	ME	mE
> I + 2	5.7%	n/a	2.9%
I +/- 1	6.8%	0.0%	27.0%
< I - 2	n/a	0.0%	4.2%

M23 Goal

	VME	ME	mE
> I + 2	< 2%	n/a	< 5%
I +/- 1	< 10%	< 10%	< 40%
< I - 2	n/a	< 2%	< 5%

- Additional Option (≤ 14 R; 15-19 I; ≥ 20 S)

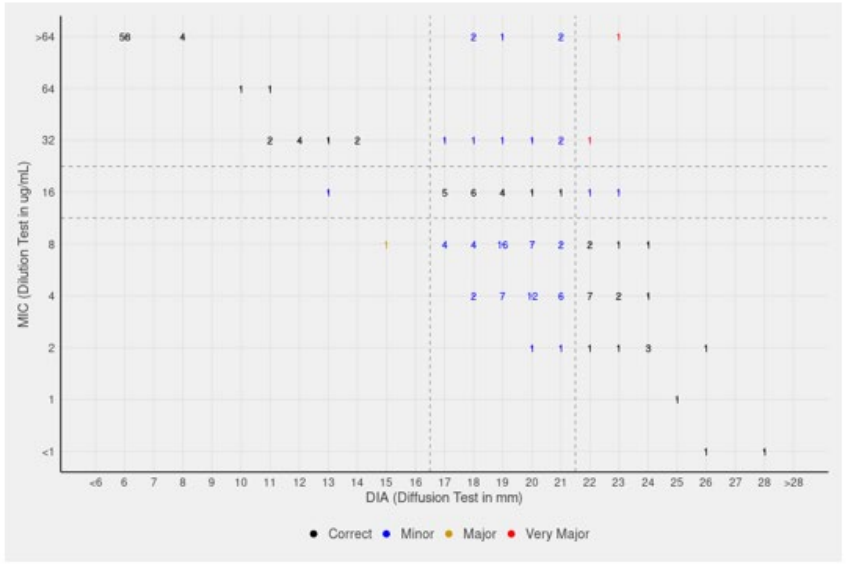


	VME	ME	mE
> I + 2	4.3%	n/a	4.3%
I +/- 1	5.4%	0.0%	44.6%
< I - 2	n/a	0.0%	18.8%

M23 Goal

	VME	ME	mE
> I + 2	< 2%	n/a	< 5%
I +/- 1	< 10%	< 10%	< 40%
< I - 2	n/a	< 2%	< 5%

- Additional Option (≤ 16 R; 17-21 I; ≥ 22 S)



	VME	ME	mE
> I + 2	1.4%	n/a	7.1%
I +/- 1	1.4%	1.4%	56.8%
< I - 2	n/a	0.0%	60.4%

M23 Goal

	VME	ME	mE
> I + 2	< 2%	n/a	< 5%
I +/- 1	< 10%	< 10%	< 40%
< I - 2	n/a	< 2%	< 5%

- Breakpoints Working Group Discussion and Recommendation
  - Decided to get more data and re-assess
  - Ayesha Khan is working on a study of MIC to disk correlates for JMI isolates
  - Question of whether related to breakpoint being set 1 dilution lower than ECV?

SC DISCUSSION (MAIN POINTS)

- The disk correlates do not work.
- A request was made to record inner and outer zones, pinpoint colonies and any trailing by BMD when performing the study.

ORAL CEPHALOSPORINS BREAKKPOINTS AD HOC WORKING GROUP REPORT

- Problems with Oral Cephalosporin Breakpoints for Enterobacterales (by Paul Edelstein)
  - Many legacy breakpoints without rationales
  - No modern population distributions for most
  - Some breakpoints in wild type distributions
  - Listing of drugs implies that they can be used for systemic infections
    - No substantiating clinical data for most
    - Breakpoints exceed systemic exposures for some/most
  - Missing a potentially useful drug (cefadroxil)
- Talk by Jim Jorgensen
  - Growing need for clinical data but recognized the weaknesses of that data quality and utility for breakpoints
  - Incorporation of PK/PD data (Bill Craig) in the 1990s

- Other Breakpoint Organizations
  - FDA

Drug (Year)	FDA indications for Enterobacterales + specified adult dosing	CLSI MIC BP	Cefazolin Surrogate	FDA STIC
Cefuroxime (1987)	**uUTI**: *E. coli, K. pneumoniae* 250 mg q12h for 7-10 d	≤4/8–16^/≥32	✓	**≤4/8–16^/≥32** (2002 label)
Loracarbef (1991)	**uUTI and uncomplicated pyelonephritis**: *E. coli* 200 mg daily – 400 mg q12h x 7 – 14 d?	≤8/16^/≥32	✓	**None** / CLSI BP not recognized (label unavailable)
Cefaclor (1979)	**UTI**, including pyelonephritis: *E. coli, P. mirabilis, Klebsiella spp.* 250 mg q8h, duration unspecified	≤8/16^/≥32	✓	**None** / CLSI BP not recognized (2004 label ≤8/16/≥32)
Cefdinir (1997)	**None** *in vitro* ONLY: *E. coli, K. pneumoniae, P. mirabilis*	≤1/2^/≥4	✓	**None** / CLSI BP not recognized (1999 label ≤1/2^/≥4)
Cefixime (1989)	**uUTI**: *E. coli, P. mirabilis* 400 mg daily, duration unspecified	≤1/2^/≥4	---	**≤1/2^/≥4** (2003 label)
Cefpodoxime (1992)	**uUTI**: *E. coli, K. pneumoniae, P. mirabilis* 100 mg q12h x 7 d	≤2/4^/≥8	✓	**≤2/4^/≥8** (2004 label)
Cefprozil (1991)	**None** *in vitro* ONLY: *E. coli, K. pneumoniae, P. mirabilis*	≤8/16^/≥32	✓	**None** / CLSI BP not recognized (2004 label ≤8/16/≥32)
Cephalexin (1971)	**Bone** infections: *P. mirabilis* **Genitourinary** tract infections, including acute prostatitis: *E. coli, P. mirabilis, K. pneumoniae* 250 mg q6h – 500 mg q12h x 7 – 14 d; max 4 g daily	**None**	✓	**None** (2004 label references "class" cephalothin MIC ≤8/16/≥32)
Cefadroxil (1980)	**UTI**: *E. coli, P. mirabilis, Klebsiella spp.* 1 g daily or q12h; max 2 g daily	**None**	---	**None** (2002 label references "class" cephalothin MIC ≤8/16/≥32)

  - EUCAST Oral Cephalosporin MIC Breakpoints for Enterobacterales

Cephalosporins[1]	MIC breakpoints (mg/L)		
	S ≤	R >	ATU
Cefaclor (uncomplicated UTI only)	IE	IE	
Cefadroxil (uncomplicated UTI only)	16	16	
Cefalexin (uncomplicated UTI only)	16	16	
Cefazolin (infections originating from the urinary tract), *E. coli* and *Klebsiella* spp. (except *K. aerogenes*)	0.001[2]	4[2]	
Cefixime (uncomplicated UTI only)	1	1	
Cefpodoxime (uncomplicated UTI only)	1	1	
Ceftibuten (infections originating from the urinary tract)	1	1	
Cefuroxime oral (uncomplicated UTI only), *E. coli*, *Klebsiella* spp. (except *K. aerogenes*), *Raoultella* spp. and *P. mirabilis*	8	8	

Cephalosporins	Uncomplicated UTI
Cefadroxil	0.5-1 g x 2 oral
Cefalexin	0.25-1 g x 2-3 oral
Cefixime	0.2-0.4 g x 2 oral
Cefpodoxime	0.1-0.2 g x 2 oral
Ceftibuten	
Cefuroxime oral	0.25 g x 2 oral

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

PK and PD data for oral cephalosporins

Agent [Ref]	Common Dosage (g)	C_{max} (mg/L)	Protein binding (%)	t½ (h)	ECOFF *E. coli* (mg/L)	Approx. % *f*T>ECOFF
Cefaclor [1]	0.5 x 3	15.9 ± 5.7	50	0.69 ± .17	4	15
Cefadroxil [2]	0.5 x 2	17.9 ± 1.0	18-20	1.0-1.9	16	0
Cefalexin [3]	0.5 x 2	18.1 ± 4.3	10-20	1.5 ± 0.6	16	0
Cefixime [1,4]	0.4 x 1	2.5-6.1	60-70	2.7-4.6	2	10
Cefpodoxime [5]	0.2 x 2	1.8-2.6	22-33	3.3-4.2	2	0
Ceftibuten [1,6]	0.4 x 1	15.0± 3.3	63	2.5 ± 0.2	1	37
Cefuroxime-axetil [1]	0.5 x 2	5.2 ± 1.0	30-50	1.1-1.4	8	0

    - USCAST Oral Cephalosporin STIC for Enterobacterales
        - Cephalexin: Not established/not supported by available evidence
        - Cefuroxime (oral): Not established/not supported by available evidence
        - Cefpodoxime: S ≤1 / R ≥ 2 µg/mL: Based on high dose (400 mg q12h) and a net bacterial stasis endpoint and intended for application to non-severe, uncomplicated infections
- Questions
    - Do sufficient data exist to support retaining/updating/adding breakpoints for any oral cephalosporins?
        - Urine only breakpoints?
        - Apply comment?
    - Are there implications for cefazolin surrogacy?
- Microbiology Data
    - JMI 2024-2025 SENTRY
    - IHMA 2018-2025 ATLAS
    - EUCAST MIC distribution data 2025
    - Limited published literature
        - Watson, 2021, DMID
        - Silver, 1977, AAC
        - Claeys, 2025, AAC
        - Blackwell, 1976, AAC
        - Bidenbach, 2016, IDT
        - Bragman, 1990, JAC
    - Summary
        - Problems with published literature on oral cephalosporins
        - Lack of standardized AST method used across papers
        - Agents covered inconsistently
        - Many papers provide MIC_{50}/MIC_{90} values but not raw data or MIC distributions

- Many papers do not provide detailed description of characteristics of isolates tested (species breakdown, genotype, phenotype etc) so cannot contextualize MIC distributions if present
- Cannot pull MIC data across papers to determine ECV/ ECOFF reliably because they may be skewed due to enrichment of resistant isolates where oral 3rd GC are known to have no utility, and labs would not consider reporting on these organisms
- Pro: Overall conclusions (eg, ceftibuten and cefpodoxime showing higher activity than others, etc) are overall consistent
- Con: Hard to make detailed comparisons across papers

- PK/PD Data
  - Noise:Signal Ratio is high
  - Have screened LOTS of PK studies for oral cephalosporins
  - Two deemed helpful so far for systemic breakpoint evaluation
  - Published literature
    - Cattrall et al. Eur J Clin Microbiol Infect Dis 2019; 38: 2311–2321
    - Yamada et al. Diagn Microbiol Infect Dis 2022; 103: 115662
  - Summary
    - So far, have not identified helpful studies for most oral cephalosporins
    - Reviewed studies of cephalexin and cefaclor are NOT supportive of systemic use for Enterobacterales.
    - Cephalexin doses of at least 1 g four times daily required for MICs ≤ 4 mcg/mL and net bacterial stasis
- Clinical Data Summary
  - Have catalogued ~ 60 clinical studies for further screening/review.
    - Of these, have screened ~ 30 and reviewed 12 in detail.
    - On initial search, prioritized studies that mentioned oral cephalosporins for systemic infections with Enterobacterales.
    - Have not catalogued every UTI study available for oral cephalosporins
  - Have not identified any studies of oral cephalosporins that focus on treatment of systemic infections with Enterobacterales without IV lead-in therapy that actually tested the oral cephalosporin
  - Have identified:
    - Few studies of lower respiratory tract infection with a minority of Enterobacterales isolates included and without detailed susceptibility or robust outcomes analyses
    - Many studies evaluating oral cephalosporins for treatment of UTI/pyelonephritis
      - Variable susceptibility testing detail
      - Many of these base susceptibility on cefazolin surrogate OR older studies occasionally use “class” cephalothin disk testing
    - Several studies that allow for oral cephalosporins as PO step-down following IV-lead in therapy for Enterobacterales bacteremia/pyelonephritis
- Oral Step-Down Therapy Application
  - Could an approach similar to amoxicillin/clavulanate and Enterobacterales be taken?
  - Several papers include oral cephalosporins as option for step-down therapy in Enterobacterales bacteremia with mixed results
  - Limitations for breakpoint evaluation:
    - Represent minority of prescribed oral antimicrobials (ie, fluoroquinolones, trimethoprim–sulfamethoxazole, and amoxicillin +/- clavulanate predominate)
    - Dosing variable and often considered “suboptimal” for systemic exposures

        - Outcomes possibly confounded by heterogeneity in duration of IV lead-in and definitive therapy
        - Susceptibility testing information not detailed
            - Not provided on an individual isolate/patient level
            - MICs not reported and/or cefazolin surrogate applied
- Preliminary Conclusions
    - So far, have not identified sufficient clinical data to support systemic breakpoints (except possibly pyelonephritis) for any oral cephalosporin
    - The microbiology and PK/PD data we have identified and reviewed are not supportive of systemic breakpoints
    - Tentative plan moving forward for Enterobacterales is to focus efforts on reviewing data for select oral cephalosporins in urinary tract infections, including pyelonephritis
- Questions for AST Subcommittee
    - What drugs to focus on for this review?
        - All Enterobacterales drugs then all *S. aureus* drugs
        - Most important Enterobacterales drugs then most important *S. aureus* drugs then leftover drugs for both Enterobacterales and *S. aureus*
    - Aim to keep systemic breakpoints based on clinical use (but mostly UTI and step-down in literature)? If not, urinary breakpoint and step-down therapy for systemic infections?
    - Please provide feedback now to guide the path forward for AHWG

SC DISCUSSION (MAIN POINTS)

- Suggestion to evaluate oral cephalosporins for *Salmonella* species.
- Internationally, oral cephalosporins are important especially cefadroxil.
- There is support for urinary only breakpoints.
- There are ongoing trials evaluating oral cephalosporin as step-down. SNAP trial: 1g q8 oral cephalexin as oral step-down for *S. aureus* bacteremia.
- Cefpodoxime and cefuroxime are most commonly requested for oral step-down therapy.
- Suggestion to focus on urinary tract and urine source to cover step-down for bacteremic UTIs.
- Suggestion to focus on *E. coli, K. pneumoniae,* and *P. mirabilis* and ignore other Enterobacterales.
- For Enterobacterales, the following oral cephalosporins are important: cefadroxil, cephalexin, cefpodoxime, and cefuroxime. Cephalexin and cefadroxil for *S. aureus*.
- Ignore agents that are rarely used.
- There is a more pressing clinical need for Enterobacterales over *S. aureus*.
- No one was aware of any dose limiting safety issues with these agents.
- IV to PO switch occurs quite quickly in pediatrics for gram-negative bacteremia, osteomyelitis, etc.
- There was a question about FDA funding for urinary tract infection models and who was awarded the funding.
- Cefazolin as a surrogate for oral cephalosporin was not accepted by the FDA.

NON-ENTEROBACTERALES TABLE 2B-5

- "Other non-Enterobacterales include *Pseudomonoas* spp. and other nonfastidious, glucose-nonfermenting, Gram-negative bacilli but exclude *P. aeruginosa*, *Acinetobacter* spp., *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia*... Recommendations for *Aeromonas* spp., *Burkholderia mallei*, *Burkholderia pseudomallei*, and *Vibrio* spp., are found in CLSI M45."
- Essentially could be any other Gram negative that grows decently well

- Not recognized by FDA
- What is recommended testing?

Table 1B-5. Other Non-Enterobacterales[a,b]

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ceftazidime	Cefepime		
	Imipenem Meropenem		
Gentamicin Tobramycin	Amikacin		
Piperacillin-tazobactam			
Trimethoprim-sulfamethoxazole			
	Aztreonam		
	Ciprofloxacin Levofloxacin		
	Minocycline		
			Cefotaxime Ceftriaxone
Urine Only			
Tetracycline			

- Where did Table 2B-5 come from?
  - M100-S1 (1986) – Table 1 separates out Enterobacteriaceae from *Pseudomonas* (footnote, *Acinetobacter)*. Table 2 all organisms with occasional footnotes for select drugs
  - M100-S3 (1991) – Table 1 changes it to *Pseudomonas aeruginosa* and other non-Enterobacteriaceae (footnote, Acinetobacter, *Xanthomonas*, *Pseudomonas* spp). Table 2 all organisms other than *Haemophilus* and *Neisseria gonorrhoeae* with more footnotes for select drugs, especially beta-lactams (including some *Pseudomonas* vs other Gram-negative organisms)
  - M100-S5 (1994) – *Streptococcus pneumoniae* gets its own Table 2C
  - M100-S6 (1995) – *Streptococcus* spp. joins *S. pneumoniae* in Table 2C
  - M100-S7 (1997) – Table 1 adds "other nonfastidious glucose nonfermenting, Gram-negative bacilli" to the non-Enterobacteriaceae footnote.
  - **M100-S8 (1998) – Introduction of Table 2B – *Pseudomonas* and Other Non-Enterobacteriaceae**
  - M100-S16 (2006) – Separate out *Acinetobacter*, *Burkholderia* and *Stenotrophomonas* MIC tables. Leave *Pseudomonas aeruginosa* and other Non-Enterobacteriaceae as table 2B-1
  - M100-S18 (2008) – Creation of table 2B-5 for non-Enterobacteriaceae
- Earliest records of the breakpoints in Table 2B-5

Drug	M100-S35	M100-S18 (2008)	M100-S16 (2006)	M100-S8 (1998)	M100-S5 (1994)	M100-S4 (1992)	M100-S1 (1986)
Ceftazidime	<=8; 16; >=32	Same	Same	Same	Same	Same	Same
Gentamicin	<=4; 8; >=16	Same	Same	Same	Same	Same	Same
Pip-tazo	<=16/4; 32/4-64/4; >=128/4	Same	Same	Same	Same	New Combo Same	Pip alone same
Trim-Sulfa	<=2; >=4	Same	Same	Same	Same	Same	Same
Cefepime	<=8; 16; >=32	Same	Same	Same	New	Not available	Not available
Imipenem	<=4; 8; >=16	Same	Same	Same	Same	Same	Same
Aztreonam	<=8; 16; >=32	Same	Same	Same	Same	Same	Same
Ciprofloxacin	<=1; 2; >=4	Same	Same	Same	Same	Same	Same
Minocycline	<=4; 8; >=16	Same	Same	Mino specific introduced	Tet at same BPs predicts	Tet at same BPs predicts	Tet at same BPs predicts

- Modifications of Table 2B-5
  - M100-S26 (2016) – Deleted carbenicillin, mezlocillin, and ticarcillin
  - M100-S27 (2017) – Deleted colistin and polymyxin B
- Gram-negative breakpoints revised in M100 since 2010
  - Enterobacterales: Aminoglycosides, Cephalosporins, Fluoroquinolones, Carbapenems, Piperacillin-tazobactam
  - *Pseudomonas aeruginosa:* Aminoglycosides, Fluoroquinolones, Carbapenems, Piperacillin-tazobactam
  - *Acinetobacter:* Aminoglycosides, Tetracyclines
  - *Stenotrophomonas:* Minocycline
- Why act now?
  - M45 is scheduled to publish in 2027
    - Includes additional organisms like non-aeruginosa *Pseudomonas* and *Achromobacter*.
    - FDA has recognized many organisms in M45
  - Potential harms in leaving it alone
    - The current susceptible breakpoints are above many PK/PD breakpoints for other Gram-negative organisms, potentially leading to false susceptibility
    - There is a wide variety of normal distributions amongst the non-fermenters that can lead to unnecessary designation of MDROs as well as confusion about what can be used
- What is the ask of the Subcommittee?
  - Form an AHWG
  - To investigate whether or not the table should remain in M100 and put forth a recommendation to the subcommittee
  - To come up with guidance materials to be published with the decision to aid laboratories in how to use the table (or not use the table in its absence)
- Breakpoints Working Group Discussion and Recommendation

	○ Multiple asks for M45 to be more fluid (living) document but will not happen for 5-10 years ○ Ideas for how to operationalize M45 for earlier updates (eg, supplement) ○ Overall support from Breakpoints Working Group but if approved by AST Subcommittee, this would live under AST Subcommittee, not the working group ○ Request to AST Subcommittee: vote to form AHWG to evaluate where M100 Table 2B-5 should exist (move to M45 eventually?). **SC DISCUSSION (MAIN POINTS)** • M45 is scheduled to be published in 2027 and includes non-aeruginosa *Pseudomonas* species and *Achromobacter* species. • M45 will not become a living document until 5-10 years. • There were thoughts around a possible supplement to the M45. • Suggestion to put into an Appendix to be moved to M45. • Decision to leave the table as is, not form an AHWG, and to reevaluate once M45 is published.
3.	**ADJOURNMENT** Dr. Mathers thanked the participants for their attention. The meeting was adjourned at 5:30 PM Mountain Standard (US) time.

2026 JANUARY AST MEETING SUMMARY MINUTES PLENARY 3: Tuesday, 27 January 2026 7:30 AM – 12:00 PM Mountain Standard Time (US)	
#	**Description**
1.	**OPENING** Dr. Mathers opened the meeting at 7:30 AM Mountain Standard (US) time.

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

CEFIDEROCOL-XERUBORBACTAM METHOD

- Brief Overview of Cefiderocol
  - Structural characteristics: cephalosporin antibiotic structurally similar to cefepime (same C-3 side chain) and ceftazidime (same C-7 side chain) that also contains catechol siderophore moiety
  - Mechanism of action: inhibits peptidoglycan cross-linking by targeting PBP3 which leads to filamentation during cell division
  - Mechanism of penetration across outer membrane: through both general porins (similar to other beta-lactams) but also through iron transporters
  - ß-lactamase stability: enhanced stability to many ß-lactamases
- Rationale for Development of Cefiderocol-BLI Combination
  - Resistance against cefiderocol remains low, but some specific ß-lactamases have been implicated in cefiderocol resistance
  - ß-lactamase inhibitor (BLI) strategy is a proven approach to reverse ß-lactam resistance mediated by ß-lactamases
  - As both serine and metallo-ß-lactamases are implicated in cefiderocol resistance, a dual serine and metallo-ß-lactamase inhibitor is the most appropriate type of BLI to be combined with cefiderocol
  - Investigational dual serine/metallo-ß-lactamase cyclic boronic BLI xeruborbactam was chosen for combination with cefiderocol
- Xeruborbactam has a Broad Spectrum of Inhibition of Class A-D Serine and Metallo Enzymes
  - Lomovskaya et al, Antimicrobial Agents and Chemotherapy 2023 15;67(11):e0044023
- Comparison of the *In vitro* Activity of Cefiderocol Alone and in Combination with Xeruborbactam vs Susceptible and Non-Susceptible Pathogens
- Xeruborbactam Restores Cefiderocol Efficacy in Mouse Neutropenic Thing Infection Model
- Reference Broth Microdilution Method for Cefiderocol Susceptibility Testing Requires Iron-Limiting Conditions
  - Cefiderocol was designed to use iron uptake pathways to penetrate across the outer membrane of gram-negative bacteria
  - Iron-transport systems are upregulated under iron-limiting conditions as occurs *in vivo*
  - Iron limitation results in increased cefiderocol *in vitro* potency (decreased "*in vitro*" MICs) against many strains of gram-negative bacteria
  - Testing cefiderocol in standard media might result in underestimation of susceptibility *in vivo* for some strains based on *in vitro* MICs
    - Cefiderocol shows efficacy against many strains that have too high MICs to be considered susceptible based on cefiderocol exposure and pharmacodynamic principles
  - In order to mimic the *in vivo* state to accurately predict efficacy, cefiderocol susceptibility testing is performed using iron-depleted medium
- Implication of Combining Cefiderocol with Xeruborbactam for Susceptibility Testing Methodology
  - Xeruborbactam is highly effective ß-lactamase inhibitor which inhibits ß-lactamases both *in vitro* and *in vivo*
  - Addition of xeruborbactam results in a significant increase in cefiderocol potency in isolates expressing ß-lactamases
  - The inhibition of ß-lactamases by xeruborbactam may alleviate the need for enhanced cefiderocol uptake through iron uptake systems to overcome its slow hydrolysis by certain ß-lactamases
  - As a result, inhibiting ß-lactamases with xeruborbactam may alleviate the requirement for *in vitro* testing of cefiderocol-xeruborbactam in iron-depleted medium
- Reproducibility Studies (JMI Laboratory)

- Objective: To assess the reproducibility of the reference broth microdilution method when testing cefiderocol-xeruborbactam in cation-adjusted Mueller-Hinton broth (CAMHB) and iron-depleted (ID) CAMHB from different media manufacturers using 100% and significant reduction (SR) endpoints
- Experimental Set up
  - Isolates
    - 214 isolates of Enterobacterales (N=66), *A. baumannii* (N=90) and *P. aeruginosa* (N=58) tested using three MHB manufacturers: BBL (2 lots, 6 independent replicates per batch), OXOID (6 replicates) and DIFCO (6 replicates)
  - Test media
    - CAMHB from BBL (two batches), OXOID and DIFCO
    - ID-CAMHB from BBL (two batches), OXOID and DIFCO
  - Replicates
    - Six biological replicates
  - MIC endpoint
    - Complete growth inhibition
    - Significant growth reduction
- Reproducibility of MIC results for cefiderocol-xeruborbactam compared to the modal MIC for the replicates when testing all isolates
- Reproducibility of MIC results for cefiderocol-xeruborbactam compared to the modal MIC for the replicates by media manufacture when testing all isolates
- Summary of reproducibility of MIC values by organism

- Reproducibility Multi-reader Study
  - Objective: To assess the reproducibility of the reference broth microdilution method when testing cefiderocol-xeruborbactam and reading results at 100% and significant reduction (SR) endpoints by multiple readers with varying levels of experience
  - Experimental Set up
    - Isolates
      - 45 isolates of Enterobacterales (N=66), *A. baumannii* (N=90) and *P. aeruginosa* (N=58) tested using three MHB manufacturers: BBL (2 lots, 3 independent replicates per batch), OXOID (3 replicates) and DIFCO (3 replicates)
    - Test media
      - CAMHB from BBL (two batches), OXOID and DIFCO
    - Replicates
      - Three biological replicates
      - 10 readers with various level of experience
    - MIC endpoint
      - Complete growth inhibition
      - Significant growth reduction
  - Reproducibility of MIC results for cefiderocol-xeruborbactam compared to the modal MIC for the replicates
  - Reproducibility of MIC results for cefiderocol-xeruborbactam compared to the modal MIC for the replicates when testing all isolates stratified by reader experience
- Proposal for Cefiderocol-Xeruborbactam AST Methodology

- Inhibition of ß-lactamases by xeruborbactam alleviates the need to test the cefiderocol-xeruborbactam combination in ID-CAMHB to test the potency of the combination
- It is proposed to test cefiderocol-xeruborbactam in CAMHB, using clear endpoints (100% growth inhibition) to simplify testing of this combination
- The proposed methodology might lead to some underestimation of cefiderocol-xeruborbactam potency and potentially some overestimation of non-susceptibility
- At the same time, this is also the most conservative approach that ensures that potency is not overestimated, and resistance is not missed or easily selected

- Methods Working Group Discussion and Recommendation
  - Do we need to vote on the use of CLSI approved method?
    - Different method than the parent drug.
    - Is cefiderocol-xeruborbactam considered a different drug than cefiderocol alone?
  - Questions around why iron depletion is necessary for FDC.
    - Been taught that iron depletion is needed for uptake. Does the new drug get into the cell in a different method? Still uses passive diffusion.
    - Other mechanisms, this caused confusion.
    - Do other BLIs overcome the need for iron depletion? Protects the cefiderocol and increases efficacy giving a buffer to not use iron depletion. Can see this with other BLIs.
  - Can resistance to non-BL be detected in CAMHB and will it affect mutations
    - No added effect of using regular vs iron depletion media since it already has mutated uptake. In the absence of beta lactamases, uptake mutations alone are not sufficient to produce resistance.
  - If the method is different, what are the chances of having something that is FDC susceptible but combination nonsusceptible.
    - This is possible due to increased uptake with iron depletion.
  - The testing shows reproducibility and follows the CLSI reference method.
  - A method that can be placed in an automated system.
  - Motion to move forward with the CAMHB method for cefiderocol-xeruborbactam at 100% inhibition. WG Vote: 4-1-3-4. Motion failed. Reasons for rejections: due to lack of discussion around iron depletion necessity. Reasons for abstentions: conflicts of interest and due to uncertainty and lack of discussion.

SC DISCUSSION (MAIN POINTS)

- Discussion on using the parent ß-lactam and BLI for QCing the agent. Testing the strain without the BLI is an integrity check of the QC strain. There are other parent compounds that can be used for this purpose.
- In the M23 QC study, a combination of cefiderocol (non-reference method as CA-MHB was used), xeruborbactam and meropenem-xeruborbactam QC in both iron depleted CAMHB and CAMHB. They had multiple QC organisms for consideration.
- The correlation was best with iron depleted CAMHB for cefiderocol and with animal model data. Have the same studies been performed for the combination? The sponsor has yet to perform these studies.
- Discussion ensued on whether the lack of iron depleted CAMHB will reduce the time above the MIC in PK/PD models.

- Xeruborbactam does have some intrinsic activity so there is the potential to change the free drug target. There may be some model fitting issues as there are 2 populations being evaluated; those that may require iron depleted CAMHB for entry versus others that rely on passive transport of cefiderocol as xeruborbactam is an effective BLI.
- The sponsor is taking a conservative approach by using CAMHB and not requiring iron depleted media in this case. This would be the same thing in the PK/PD models to challenge it with the most conservative approach. They also want this to be more available to laboratories with the standard media.
- The discussion was that this is a reproducible method and our reference method. However, it is not understood if this will be the best method to correlate with the PK/PD models. This is a risk the sponsor needs to take.
- The sponsor will need to still pursue the studies to set the breakpoints.
- Does the iron concentration in standard CAMHB media impact the MICs? Should iron be quantified in CAMHB broth? There was a signal in the reproducibility study that iron concentration (Oxoid generally has more iron) may impact reproducibility.
- The sponsor is working on understanding the impact of iron and other variables in the CAMHB media.
- Biologically it makes sense to revert back to the CAMHB.
- EUCAST has yet to discuss this approach.

A motion to move forward with the CAMHB method for cefiderocol-xeruborbactam at 100% inhibition. Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

Abstaining Vote Reasoning:
- Conflict of interest.

CEFIDEROCOL-XERUBORBACTAM QC (PRESENTED BY C. PILLAR)
- Background

Drug: Cefiderocol-xeruborbactam (fixed 4 µg/mL)	**Abbreviation (Glossary II & III):** Pending	**Previous ID:** NA
Solvent (Table 6A): Cefiderocol = saline Xeruborbactam = sterile distilled water	**Diluent (Table 6A):** Cefiderocol = saline Xeruborbactam = sterile distilled water	**Preparation (Table 6C combination agents):** Same as aztreonam-avibactam and cefepime-taniborbactam
Route of administration (Glossary II): IV	**Class (Glossary I & II):** β-lactam combination agents	**Subclass (Glossary I & II):** NA
Study Report by: Element (JMI Labs) and Shionogi/Qpex	**Pharma Co:** Shionogi/Qpex	**Control Drugs:** Cefiderocol, meropenem, meropenem-xeruborbactam, xeruborbactam
Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, etc): The *in vitro* effect studies are planned but have not yet been performed by the sponsor. • **ISO/TS 16782 assessment of Tier 2 study materials**: Confirmed	
Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC)**: ??	
Discussion	Novel agent for serious infections caused by Acinetobacter spp, Enterobacterales, and Pseudomonas aeruginosa, including carbapenem-resistant strains. Active against isolates producing all classes of β-lactamases. Media manufacturers: Difco, BBL, Oxoid	

QC Strain	Average Inocula CFU/mL
A. baumannii #1134488 (AB1665; ADC-33, OXA-23, OXA-422)	3.8×10^5
K. pneumoniae ATCC BAA-2814	2.8×10^5
P. aeruginosa ATCC 27853	3.8×10^5

QCWG 2026 Jan 10

- Proposed MIC QC Ranges

Drug Name:	Cefiderocol-xeruborbactam (fixed 4 µg/mL)	Votes:	7/0/0/2 For/Against/Absent/Abstain

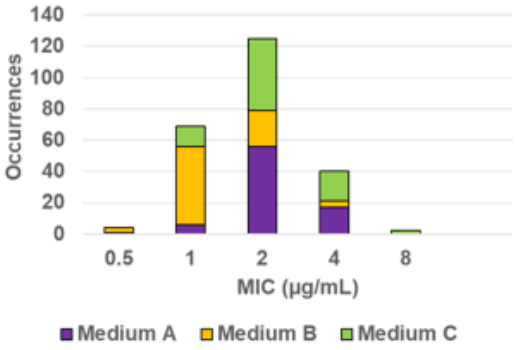
QC Strain	Range	% In	Mode	Dil	Shoulder	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
A. baumannii #1134488*	1/4 - 4/4	97.5%	2/4	3	55% @ 1/4	1/4, 2 @ 2/4	1/4, 7 @ 2/4	1/4 - 4/4, 3 dil, 97.5%	0.5/4 – 4/4, 4 dil, 99.2%	Some media variability Supplemental QC to confirm correct enantiomer of xeruborbactam is used in manufacturing.
K. pneumoniae ATCC BAA-2814	0.5/4 - 4/4	99.2%	2/4	4	70.6% @ 1/4	1/4, 2 @ 2/4	3@ 1/4, 5 @ 2/4	0.5/4- 4/4, 4 dil, 99.2%	0.5/4- 4/4, 4 dil, 99.2%	Some media and lab variability Highlight as routine QC
P. aeruginosa ATCC 27853	0.25/4 - 2/4	96.8%	1/4	4	51% @0.5/4	0.5/4, 2 @ 1/4	0.25/4, 0.5/4, 6 @ 1/4	0.5/4 - 2/4, 3 dil, 90.8% expand to 4 dil	0.25/4 - 2/4, 4 dil, 96.8%	
K. pneumoniae ATCC BAA-2814 Xeruborbactam alone	8-32 or 8-64	98.8% or 100%	16	3	50% @ 32	NA	7@16, 1@32	8-32 or 8-64		One media lot tested. Informational only.

*Pending deposit and identifier for QC strain

- Should we pursue QC range for *K. pneumoniae* ATCC BAA-2814 for Cefiderocol alone (or is it sufficient since ranges are provided for other beta lactam drugs to use as QC integrity check)?

- Inter- and intralaboratory comparisons of cefiderocol-xeruborbactam (fixed 4 µg/mL) broth microdilution MIC results for *Acinetobacter baumannii* #1134488

MIC (µg/mL)	Occurrences by media lot[a]			Laboratory code (occurrences):								Total
	A	B	C	A	B	C	D	E	F	G	H	
0.5/4	1	3		3	1							4
1/4	6	50	13	9	9	20	2	6	9	5	9	69
2/4	56	23	46	15	14	9	21	20	19	16	11	125
4/4	17	4	19	3	5	1	7	4	2	8	10	40
8/4			2		1					1		2
Total	80	80	80	30	30	30	30	30	30	30	30	240
Mean	2.3	1.4	2.5	1.8	2.2	1.4	2.4	2.1	1.8	2.6	2.4	2.1
Median	2	1	2	2	2	1	2	2	2	2	2	2
Mode	2	1	2	2	2	1	2	2	2	2	2	2
Geometric mean	2.2	1.3	2.2	1.5	1.8	1.3	2.2	1.9	1.7	2.2	2.0	1.8
Range	4	4	4	4	5	3	3	3	3	4	3	5



Method	Proposed QC range (µg/mL)	# of dilutions	% in range
RangeFinder	0.5/4 – 4/4	4	99.2% (238/240)
CLSI	**1/4 – 4/4**	**3**	**97.5% (234/240)**

Calculated QC range

CLSI

1/4 – 4/4 µg/mL

(97.5%; 234/240)

RangeFinder

0.5/4 – 4/4

(99.2%; 238/240)

QCWG 2026 Jan

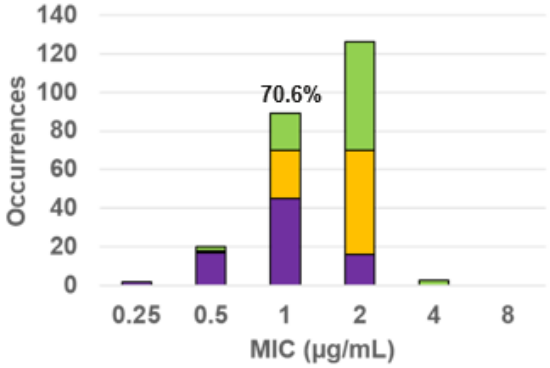
- Inter- and intralaboratory comparisons of cefiderocol-xeruborbactam (fixed 4 µg/mL) broth microdilution MIC results for *Klebsiella pneumoniae* ATCC BAA-2814

MIC (µg/mL)	Occurrences by media lot[a]			Laboratory code (occurrences):								Total
	A	B	C	A	B	C	D	E	F	G	H	
0.25/4	2			2								2
0.5/4	17	1	2	8	5				2	5		20
1/4	45	25	19	16	19	6	4	15	9	12	8	89[b]
2/4	16	54	56	4	6	24	25	15	19	13	20	126
4/4			3				1				2	3
8/4												
Total	80	80	80	30	30	30	30	30	30	30	30	240
Mean	1.1	1.7	1.8	1	1.1	1.8	1.9	1.5	1.6	1.4	1.9	1.5
Median	1	2	2	1	1	2	2	1.5	2	4	2	2
Mode	1	2	2	1	1	2	2	1	2	2	2	2
Geometric mean	1.0	1.6	1.7	0.8	1.0	1.7	1.9	1.4	1.4	1.2	1.7	1.4
Range	4	3	4	4	3	2	3	2	3	3	3	5

[a] A, Difco, Lot #275710; B, BBL (BD), Lot #221322; C, Oxoid, Lot #3566128.

[b] A 70.6% MIC shoulder at 1/4 µg/mL indicates the need for the 4th dilution.

Method	Proposed QC range (µg/mL)	# of dilutions	% in range
RangeFinder	0.5/4 – 4/4	4	99.2% (238/240)
CLSI	0.5/4 – 4/4	4	99.2% (238/240)



Calculated QC range

CLSI

0.5/4 – 4/4 µg/mL

(99.2%; 238/240)

RangeFinder

0.5/4 – 4/4

(99.2%; 238/240)

QCWG 2026 Jan

13

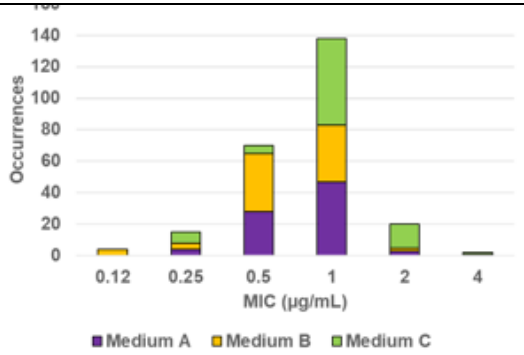
- Inter- and intralaboratory comparisons of cefiderocol-xeruborbactam (fixed 4 µg/mL) broth microdilution MIC results for *Klebsiella pneumoniae* ATCC BAA-2814

MIC (µg/mL)	Occurrences by media lot[a]			Laboratory code (occurrences):								Total
	A	B	C	A	B	C	D	E	F	G[b]	H	
0.06/4		1						1				1
0.12/4		3						1	2			3
0.25/4	4	4	7							15		15
0.5/4	28	37	5	14	16	6	4	5	10	12	3	70
1/4	47	36	55	15	16	23	24	17	22	3	18	138
2/4	3	2	15	1	1	1	2	6	2		5	18
4/4	1		1								4	4
Total	83	83	83	30	33	30	30	30	36	30	30	249
Mean	0.9	0.8	1.1	0.8	0.8	0.9	1	1.1	0.9	0.4	1.5	0.9
Median	1	0.5	1	1	1	1	1	1	1	0.4	1	1
Mode	1	0.5	1	1	0.5	1	1	1	1	0.25	1	1
Geometric mean	0.8	0.6	1	0.7	0.7	0.9	1	0.9	0.8	0.4	1.3	0.8
Range	4	6	5	3	3	3	3	6	5	3	4	7

[a] A, Difco, Lot #275710; B, BBL (BD), Lot #221322; C, Oxoid, Lot #3566128.

[b] Laboratory G is a statistical outlier for the modal cefiderocol-xeruborbactam (fixed 4 µg/mL) MIC value.

Method	Proposed QC range (µg/mL)	# of dilutions	% in range
RangeFinder	0.25/4 – 2/4	4	96.8% (241/249)
CLSI	0.5/4 – 2/4	3	90.8% (226/249)



Calculated QC range

CLSI

0.5/4 – 2/4 µg/mL

(90.8%; 226/249)

RangeFinder

0.25/4 – 2/4

(96.8%; 241/249)

QCWG 2026 Jan 14

SC DISCUSSION (MAIN POINTS)

- There was a question of whether the QC could be placed in M100 without breakpoints. The QC can be placed in M100 before the breakpoint is accepted if there is a formal drug name.

A motion to accept the cefiderocol-xeruborbactam MIC supplemental QC for *Acinetobacter baumannii* #1134488 (1/4-4/4 µg/mL). Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

Abstaining Vote Reasoning:

- Conflict of interest.

A motion to accept the cefiderocol-xeruborbactam MIC routine QC for *Klebsiella pneumoniae* ATCC BAA-2814 (0.5/4-4/4 µg/mL). Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

Abstaining Vote Reasoning:

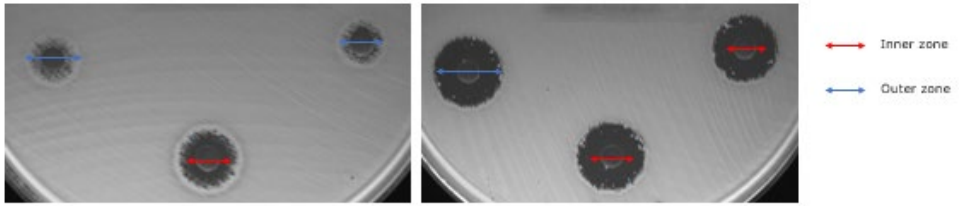
- Conflict of interest.

A motion to accept the cefiderocol-xeruborbactam MIC supplemental QC for *Pseudomonas aeruginosa* ATCC 27853 (0.25/4-2/4 µg/mL). Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

Abstaining Vote Reasoning:
- Conflict of interest.

CEFIDEROCOL AD HOC WORKING GROUP REPORT

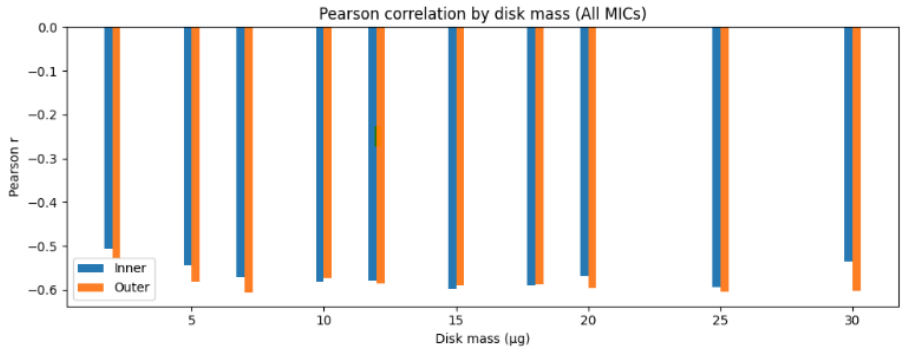
- Study Design
  - Cefiderocol disks
    - 2 µg, 5 µg, 7 µg, 10 µg, 12 µg, 15 µg, 18 µg, 20 µg, 25 µg, 30 µg
    - Inner and outer zones read in duplicate by different readers
    - One media lot
  - MIC testing
    - Cefiderocol (dilution range 0.03 to 64 µg/mL) in IDCAMHB (BD BBL media)
    - Significant reduction (SR) reading



  - Organisms

Organisms	Total isolates	Cefiderocol MIC distribution (mg/L; no. of isolates and cumulative %)												MIC_{50}	MIC_{90}
		<=0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32		
All isolates	146	21	17	25	23	18	19	10	8	3	1	0	1	0.25	2
		14.4	26.0	43.2	58.9	71.2	84.2	91.1	96.6	98.6	99.3	99.3	100.0		
A. baumannii-calcoaceticus complex	44	4	4	8	8	4	6	5	4	1				0.25	4
		9.1	18.2	36.4	54.5	63.6	77.3	88.6	97.7	100.0					
E. cloacae species complex	7			1	2	1	2	0	0	1				0.5	8
				14.3	42.9	57.1	85.7	85.7	85.7	100.0					
E. coli	28	5	6	3	2	7	2	1	1	1				0.12	2
		17.9	39.3	50.0	57.1	82.1	89.3	92.9	96.4	100.0					
K. pneumoniae	21	3	2	1	6	1	3	4	1					0.25	2
		14.3	23.8	28.6	57.1	61.9	76.2	95.2	100.0						
P. aeruginosa	24	5	1	6	5	1	3	0	2	0	1			0.12	4
		20.8	25.0	50.0	70.8	75.0	87.5	87.5	95.8	95.8	100.0				
S. maltophilia	22	4	4	6	0	4	3	0	0	0	0	0	1	0.12	1
		18.2	36.4	63.6	63.6	81.8	95.5	95.5	95.5	95.5	95.5	95.5	100.0		

- Correlation of MIC vs inner and outer zone



- Conclusions
  - If outcomes are correlated to the MIC values, the optimal disk mass (and reading method) should be the one that best correlates to the MIC
  - In many disks the outer zone readings correlate better with the MIC values when compared to the inner zone reading that is currently recommended for cefiderocol
  - All disks had elevated error rates using ≤ 15 mm as defined by M23S for wildtype isolates
  - Using a minimum of 9 mm the error rates were still elevated
  - Inner colonies and growth as ghost zones were tested and sequenced. This data is still being analyzed.
- Methods Working Group Discussion and Recommendation
  - Data shows that the disk mass is correct.
  - Reading outer zones goes against CLSI recommendation for reading guides although there are other drugs that are read differently.
  - Concerned about inner colonies could lead to resistance.
  - Inner colonies are not always reproducible.
  - Future work will focus on 30 µg cefiderocol data on inner and outer zones and correlation with clinical data. Better pictures in the reading guide.

SC DISCUSSION (MAIN POINTS)

- In general, the outer zone works better within this study.
- Discussion ensued about looking at the data using the 30 µg cefiderocol disk using the outer zone for interpretation. The proposal was to re-evaluate the data with more resistant organisms using multiple media and disk manufacturers.
- EUCAST is performing a similar study and is willing to share the data.

ALTERNATIVE MEDIA FOR MH-F

- EUCAST disk diffusion of fastidious organisms
  - MH-F: MH agar with 5% defibrinated horse blood and 20 mg/L
    - Selected to have a common medium for streptococci and *Haemophilus* (Matuschek et al, CMI 2014 Apr;20(4):O255-66)
    - Breakpoints were later developed on MH-F for several additional fastidious organisms
    - Not sufficient for anaerobes and *N. gonorrhoeae*
  - Reports on difficulties accessing the MH-F medium
- Evaluation of MH + sheep blood (MH-S) as an alternative for MH-F
  - All species with breakpoints on MH-F, except *H. influenzae*
  - 20 isolates per species/group selected to represent S and R isolates
    - Zone diameters and growth
  - QC: *S. pneumoniae* ATCC 49619 and *C. jejuni* ATCC 33560
  - Categorical agreement, correlation between zone diameters and bias using MH-F as reference
- Data Analysis
  - Visual estimation of growth between MH-S and MH-F (no difference)
  - QC strains
    - Mean values per agent and medium vs EUCAST target values
  - Clinical isolates
    - Percentage of zone diameters being within ± 1 mm
    - Bias: Difference between % zone diameters being larger (“above”) and smaller (“below”) on MH-S.
    - Categorical errors
- Correlation and bias
  - Overall trend towards larger zones on MH-S, but bias is low

Species/organism group	Zones within ± 1 mm (%)	Bias (%)
Streptococcus groups A, B, C and G	77	0.9
Streptococcus pneumoniae	78	-16
Viridans group streptococci	74	6.9
Moraxella catarrhalis	72	22
Listeria monocytogenes	81	21
Pasteurella multocida	67	16
Campylobacter jejuni and *C. coli*	84	0.8
Corynebacterium spp.[1]	79	5.6
Aerococcus sanguinicola and *A. urinae*	71	18
Kingella kingae	61	2.5

- Categorical errors
  - Categorical errors for 8 of 34 agents tested

Table 1. Categorical errors for disk diffusion on MH-S using disk diffusion on MH-F as reference.
Errors were calculated based on EUCAST Breakpoint Tables v 15.0.

Species/organism group	No of isolates tested	PCG	AMP	MOX	NOR	MIN	TET	TED	TSU
Streptococcus groups A, B, C and G	60					1	3	1	5[3]
Streptococcus pneumoniae	20					1			1
Viridans group streptococci	50	2	1						
Moraxella catarrhalis	20								
Listeria monocytogenes	20	1							
Pasteurella multocida	20								2
Campylobacter jejuni and *C. coli*	40								
Corynebacterium spp.[1]	20	1		1					
Aerococcus sanguinicola and *A. urinae*	20				2				
Kingella kingae	20	1							
Categorical errors (%)[2]		**2.4**	**0.8**	**0.6**	**2.0**	**2.0**	**1.5**	**0.9**	**5.0**
Total number of isolates tested		**210**	**130**	**170**	**100**	**100**	**200**	**110**	**160**

[1] Species other than *C. diphtheriae* and *C. ulcerans*.

[2] Based on the total number of isolates tested for each antimicrobial agent.

[3] When growth within the zone was igenored and the outer zone read.

PCG = Benzylpenicillin, AMP = Ampicillin, MOX = Moxifloxacin, NOR = Norfloxacin, MIN = Minocycline, TET = Tetracycline, TED = Tedizolid, TSU = Trimethoprim-sulfamethoxazole

- EUCAST Recommendations
  - MH-F will continue to be the EUCAST standard medium for disk diffusion of fastidious organisms.
  - EUCAST will perform a parallel, less extensive validation of MH-S for organism or antimicrobial agent developed for testing on MH-F.
  - For most species and agents, MH-S can be used instead of MH-F.
    - MH-S does not work for *H. influenzae*
  - The MH-S agar plates must contain 5% mechanically defibrinated sheep blood and have the same agar depth as MH-F (4.0 mm with a random variation of ± 0.5 mm).
  - For MH-S, specific reading instructions are needed for Streptococcus groups A, B, C and G and trimethoprim-sulfamethoxazole
    - Ignore growth within the zone and read the outer zone edge.
- Methods working Group Discussion and Recommendation
  - CLSI also looked at MH-F
  - Stated readers who read both HTM and MH-F said MH-F is easier to read.
  - Future work will focus on validation for alternative use and data will be published.

SC DISCUSSION (MAIN POINTS)

- There was discussion on whether CLSI should evaluate MH-F as an alternative media with an initial focus on ß-hemolytic streptococci.
- Liofilchem does produce MH-F media. They are based in Italy but have a Boston distributor. Thus, there is a distributor in the US that could make this available.
- Hardy Diagnostics mentioned they could also prepare the media if there is a demand.

INTRINSIC RESISTANCE AD HOC WORKING GROUP REPORT

- Reviewed decisions from June 2025 – (1) new definition for Expected Resistance, to include intrinsic resistance or expected clinical failure (2) new R* with footnote for Salmonella/Shigella with aminoglycosides, 1st and 2nd gen cephalosporins, cephamycins (same as Enterococci in Appendix B)
- Activities before June
    - Newer drugs that are not yet in the table but maybe should be
    - Organisms that are either in human M100 Appendix B or in VET01S and maybe should be in both
        - M100 has *E. hermannii* intrinsic resistance to amikacin (and tigecycline), *P. rettgeri* (numerous drugs)
        - VET01S7 has *Y. pseudotuberculosis* intrinsic resistance to colistin, and *Achromobacter* (numerous drugs) in Appendix B2
    - Discussion of some ideas for what to put in table for organisms that may be clinically resistant and are going to focus on 2 that recently had breakpoints removed, and not others yet. Will review the appropriate Breakpoints Working Group summaries for these breakpoint changes.
        - *P. aeruginosa* – gentamicin
        - *S. maltophilia* – ceftazidime
    - Liked the idea of distinguishing the R of Intrinsic Resistance with this, perhaps with R*? Or RCR?
    - Talked about a footnote for No BPs: "Therapeutic breakpoints were removed from systemic use of this antimicrobial agent/organism combination because of… leading to expected clinical resistance"

CEFTOBIOPROLE SURROGATE TESTING

- Ceftobiprole
    - Fifth-generation intravenous cephalosporin with broad-spectrum
        - Gram-positive and Gram-negative bacteria, including MRSA isolates
    - Ceftobiprole was approved by the US FDA in April 2024 for the treatment of:
        - Adults with *S. aureus* bloodstream infections (SAB), including those with right-sided infective endocarditis, caused by MSSA and MRSA isolates
        - Adults with acute bacterial skin and skin structure infections (ABSSSI) caused by susceptible isolates of certain Gram-positive and Gram-negative organisms
        - Adult and pediatric patients (aged 3 months to less than 18 years old) with community-acquired bacterial pneumonia (CABP)
    - Susceptibility testing for ceftobiprole is not currently available on commercial automated susceptibility testing systems used in many US clinical laboratories
- Study objectives: Assessed the accuracy and error rates when using to ceftaroline as a surrogate test to predict susceptibility to ceftobiprole
- Organisms
    - 45,078 clinical isolates collected during 2016 to 2020 in US hospitals:
    - *Staphylococcus aureus* (13,867)
    - MRSA (5,906)
    - Methicillin-susceptible *S. aureus* (MSSA; 7,961)
    - *Streptococcus pneumoniae* (3,245)
    - Β-hemolytic streptococci (1,768)
    - *Haemophilus influenzae* (2,248) and *Haemophilus parainfluenzae* (30) combined (2,278)
    - Enterobacterales (21,205)
    - *Escherichia coli* (9,297)

- *Klebsiella pneumoniae* (4,054)
- Coagulase-negative *Staphylococcus* (CoNS; 1,600)
- *Moraxella catarrhalis* (1,115)

- Methods
  - All isolates were tested against ceftobiprole and ceftaroline by the reference broth microdilution method according to CLSI standards (CLSI M07, 2024)
  - Frozen-form 96-well panels were produced with cation-adjusted Mueller-Hinton broth (CAMHB)
    - CAMHB supplemented with 2.5-5% lysed horse blood for streptococci
    - Haemophilus Test Medium (HTM) broth was used for *Haemophilus* spp.
  - The most recent CLSI M100 breakpoint criteria were used for ceftaroline and the US FDA breakpoint criteria were used for ceftobiprole
- Summary
  - Summary of ceftaroline test result accuracy for predicting ceftobiprole susceptibility using ceftaroline breakpoints established by CLSI

Pathogen or species group (no. tested / no. ceftaroline-susceptible)	Accuracy[a]	Minor error[b]	Very major error[c]	Categorical agreement
S. aureus (13,867 / 13,510)	99.98%	0.02%	0.00%	97.71%
MRSA (5,906 / 5,549)	99.95%	0.05%	0.00%	94.63%
MSSA (7,961 / 7,961)[d]	100.00%	0.00%	0.00%	100.00%
S. pneumoniae (3,245 / 3,245)	99.48%	0.46%	0.06%	99.48%
β-hemolytic streptococci (1,768 / 1,768)[d]	100.00%	0.00%	0.00%	100.00%
Haemophilus spp. (2,278 / 2,273)	98.86%	0.84%	0.31%	98.81%
Enterobacterales (21,205 / 15,979)	98.73%	0.87%	0.40%	89.25%
E. coli (9,297 / 7,301)	99.97%	0.03%	0.00%	94.65%
K. pneumoniae (4,054 / 3,224)	99.91%	0.06%	0.03%	96.47%

- Conclusions
  - The accuracy of the surrogate test using ceftaroline to predict susceptibility to ceftobiprole was > 99% for *S. aureus* (99.98%), including MRSA (99.95%), *S. pneumoniae* (99.48%), β-hemolytic streptococci (100.00%), *E. coli* (99.97%), and *K. pneumoniae* (99.91%)
  - Accuracy was high for *Haemophilus* spp. (98.86%) and Enterobacterales (98.73%).
  - Minor error rates (categorizing a ceftobiprole-intermediate as ceftobiprole-susceptible) were < 1%
  - Very major errors (categorizing a ceftobiprole-resistant as ceftobiprole-susceptible) were observed at very low rates (≤ 0.40%) with *S. pneumoniae* (0.06%), *Haemophilus* spp. (0.31%), Enterobacterales (0.40%), and *K. pneumoniae* (0.03%)
- Methods Working Group Discussion and Recommendation
  - Study used FDA approved breakpoints; CLSI does not have breakpoints for ceftobiprole.
  - What is the definition of surrogate and does this fit the definition? Is there a precedence for this?

- **Surrogate agent test:** test performed with an agent that replaces a test performed with the antimicrobial agent of interest and is used when the agent of interest cannot be tested due to unavailability of the agent or performance issues (eg, surrogate agent performs better than the agent of interest)

Surrogate Agent Tests

Surrogate Agent	Organisms	Test Description	Results	Table Locations
Cefazolin	• *E. coli* • *K. pneumoniae* • *P. mirabilis*	Broth microdilution or disk diffusion	When used for therapy of uncomplicated UTIs, predicts results for the following oral antimicrobial agents: cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef Cefazolin tested as a surrogate may overcall resistance to cefdinir, cefpodoxime, and cefuroxime. If cefazolin tests resistant, test these drugs individually if needed for therapy.	1A-1, 2A-1
Cefoxitin	• *S. aureus* • *Staphylococcus lugdunensis* • *Staphylococcus epidermidis* • Other *Staphylococcus* spp. (except ***Staphylococcus coagulans,*** *Staphylococcus pseudintermedius,* and *Staphylococcus schleiferi*)	• Broth microdilution: – *S. aureus* – *S. lugdunensis* • Disk diffusion: – *S. aureus* – *S. lugdunensis* – Other *Staphylococcus* spp. (except ***S. coagulans,*** *S. pseudintermedius* and *S. schleiferi*)	Predicts results for *mecA*-mediated methicillin (oxacillin) resistance.	1C, 2C, 3H
Oxacillin	*S. pneumoniae*	Disk diffusion	Predicts penicillin susceptibility if oxacillin zone is ≥ 20 mm. If oxacillin zone is ≤ 19 mm, penicillin MIC must be performed.	1G, 2G
Pefloxacin	*Salmonella* spp.	Disk diffusion	Predicts reduced susceptibility to ciprofloxacin	2A-2

Abbreviations: MIC, minimal inhibitory concentration; UTI, urinary tract infection.

- Asking for a surrogate when CLSI has not agreed the breakpoint is not appropriate.
- This is useful for laboratories but not for M100.
- Recommendation to publish this data and revisit surrogate if/when sponsor brings data for a breakpoint.

DIRECT BLOOD AST AD HOC WORKING GROUP REPORT

- *Acinetobacter* spp. tobramycin breakpoint reassessment
  - Updated direct disk data applying 36th edition standard disk breakpoints will be circulated to AHWG for approval by email
  - Present to Methods Working Group at June 2026 meeting
- Manuscript status
  - Enterobacterales manuscript draft in progress; targeting submission by summer 2026 (per ARLG request)
  - *P. aeruginosa* and *Acinetobacter* manuscript draft to be initiated (using template of Enterobacterales draft); target submission by end of year

EARLY GROWTH AST AD HOC WORKING GROUP REPORT

- Objectives
  - Demonstrate that early growth AST (6 hours) by disk diffusion and BMD compares well to standard 18-24 hour growth using current breakpoints
  - Gram positive organisms
  - Enterobacterales

- Nonfermenters
- Study Design: Phase I
  - Assess a small subset of isolates (QC and clinical) on different commercially available CLSI recommended media and broths
  - Goals:
    - Determine whether performance of all different manufacturers' media and broths are consistent
    - Select a single media and broth to use in Phase II of testing (clinical isolates)
  - 3 study sites
  - Antibiotics tested
  - BMD
    - Perform at 6 hour and 18-24 hour isolate(s) incubation
    - 18-24 hour isolate incubation will be the reference method
    - 3 media manufacturers
  - Disk Diffusion
    - Perform at 6 hour isolate(s) incubation
    - 3 media manufacturers and one disk manufacturer
  - All testing will be performed in triplicate
- Quality Control
  - Performed on each day of testing. Perform in triplicate on Day 1 of testing only.
  - BMD: Perform QC at 6 hour and 18-24 hour isolate(s) incubation
  - Disk Diffusion: Perform QC at 6 hour and 18-24 hour isolate(s) incubation
  - Colony count will be performed on a total of 3 ATCC strain (1 Gram positive, 1 Enterobacterales and 1 non-fermenter)
- Update
  - All testing is now complete.
  - Analyzed data will be presented to AHWG in February
- Methods Working Group Discussion and Recommendation
  - Can one of the QC isolates be an ESBL? No, but there is likely an ESBL in the clinical isolates.
  - Can ceftazidime-avibactam be included? No, not for Phase 1 as panels have already been made. Could consider for Phase 2.
  - Colony counts for Enterococcus
  - Recommend doing 18-24 hour disk diffusion QC

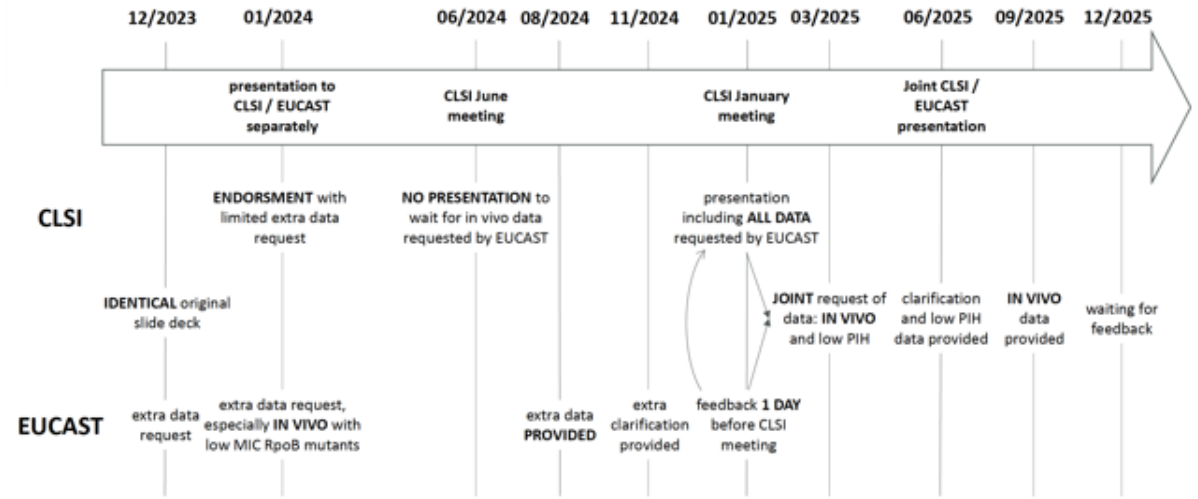
SC DISCUSSION (MAIN POINTS)

- Would this be a replacement to the standard method or an alternative method? It would be proposed as an alternative method that laboratories can consider and not a modification to the standard method.
- There was concern about a single disk being used for the Phase I study.

RIFABUTIN SUSCEPTIBILITY TESTING METHOD UPDATE

- Goal: To receive approval from CLSI and EUCAST to use a modified susceptibility testing method using agar dilution in Mueller Hinton medium supplemented with the iron chelator pyridoxal isonicotinoyl hydrazone (PIH) as the reference susceptibility testing method for rifabutin against *A. baumannii*.

- Summary of Discussions with CLSI/EUCAST



- January 2024 Decision
  - Discussion by AST Subcommittee:
    - Is determination of the level of iron important after chelation?
    - Would like to see data on the agar dilution method with different media manufacturers and different PIH manufacturers
    - EUCAST discourages modifications to the reference method and had not, at the time, reviewed this method yet
    - Modifications to the reference method need to be taken seriously
    - CLSI was cautiously optimistic pending further data
    - CLSI needs to provide clear recommendations to the sponsor
- Methods Working Group Discussion (January 2025)
  - Resistance to rifabutin in *Acinetobacter baumannii* is primarily due to *fhuE* mutation. Can the method detect other resistance mechanisms?
  - RNA polymerase (*rpoB*) mutations have also been observed and MIC varies by type of mutation (0.016-4 ug/mL in data shown). All *FhuE* active/rpoB mutant strains tested in the *in vivo* model (all MIC=0.016 ug/mL) showed rifabutin efficacy.
  - Could an *fhuE* mutation detection test be sufficient to test efficacy of rifabutin vs a phenotypic test? No, as there are rpoB mutants that likely confer resistance and rare ADP ribosyltransferase mutants.
  - EUCAST
    - Concerned about rpoB mutants and whether enough data has been presented for these. (rpoB mutants represent less than 10% of *A. baumannii* strains)
    - Could modifying the PIH concentration better separate *rpoB* mutants from WT population?
  - Do we feel confident that this method separates out the WT from the NWT?
    - Group would like to see any data that the sponsor can provide at different PIH concentrations.

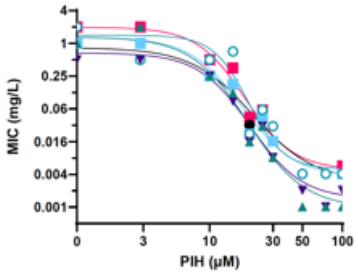
- Group would like to see EUCAST data requests and responses.
- The group would like to standardize method with EUCAST if possible.

- Sponsor's ask: What additional data does CLSI want to see and what is the goal of producing that data? Ideally, the sponsor would like to move forward with a single method for their development work.

- February – October 2025
  - Worked together with EUCAST on joint responses to BioVersys
  - Primary additional data requests:
    - *In vivo* data on rpoB mutants that test at high MICs
      - Justification required for 3mg/kg optimal dose in animal model and how does it relate to human dosing?
      - rpoB mutant information
      - Information on frequency of ADP-ribosyltransferase (arr) mutants and levels of expression of Arr.
    - Test isolates with high and low MICs at 50uM, 75uM and 100uM PIH across a variety of media manufacturers, in addition to an iron titration experiment
    - Request for more disk diffusion data
      - Testing of more than 16 strains using disks with and without PIH
      - Data on impacts of PIH on other agents on the disk agar plate.
- PIH Concentration

→Can PIH be used at lower concentrations?

→ 6 WT strains, PIH titration:



→ 36 strains (18 WT, 10 FhuE, 5 RpoB, 2 Arr and 1 combi), one media manufacturer (Difco):

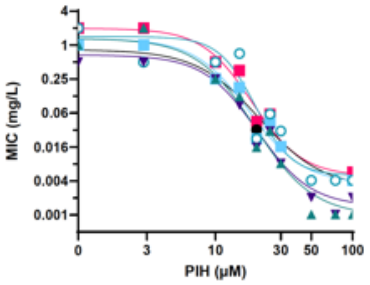
Essential agreement (%) to 0.1 mM PIH			
100 µM PIH	75 µM PIH	50 µM PIH	30 µM PIH
baseline	100%	88.9	33.6

Agar dilution MIC in Mueller-Hinton agar supplemented with the indicated PIH concentration

PIH concentrations above 30 µM are required to accurately measure rifabutin activity

→Can PIH be used at lower concentrations?

→ 6 WT strains, PIH titration:



→ 36 strains (18 WT, 10 FhuE, 5 RpoB, 2 Arr and 1 combi), one media manufacturer (Difco) with iron supplementation:

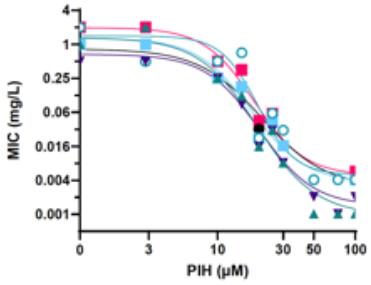
Fe supplementation (mg/L)	Essential agreement (%) to 0 mg/L Fe	
	100 µM PIH	30 µM PIH
0	baseline	baseline
0.05	100	88.9
0.14	97.2	61.1
0.46	38.9	33.3
1.4	16.7	25
4.6	16.7	25

Agar dilution MIC in Mueller-Hinton agar supplemented with the indicated PIH concentration

0.1 mM PIH provides the best MIC reproducibility

→Can PIH be used at lower concentrations?

→ 6 WT strains, PIH titration:



→ 34 strains (16 WT, 10 FhuE, 5 RpoB, 2 Arr and 1 combi), 3 media lots and 4 manufacturers:

Media	Essential agreement (%) to Difco Lot. 1		
	100 µM PIH	75 µM PIH	50 µM PIH
Difco Lot. 1	baseline	baseline	baseline
Difco Lot. 2	100	100	100
Difco Lot. 3	97.1	70.6	88.2
BBL	100	97.1	79.4
Condalab	100	97.1	88.2
Oxoid	82.3	58.8	38.2

Agar dilution MIC in Mueller-Hinton agar supplemented with the indicated PIH concentration

0.1 mM PIH provides the best MIC reproducibility

- Arr Mutants

e) How frequent are the arr mutants in Acinetobacter baumannii clinical strains?
f) Could you provide more detail on the arr mutant strains: Are these clinical isolates or lab-derived isolates? Are the levels of expression of the arr mutants known?

The rate of Arr positive Acinetobacter clinical strain is low:
- **3 strains out of 293 global CRAB clinical isolates (2017-2019)**
- **3 strains out of 100 Chinese CRAB clinical isolates (2021)**

Arr is not intrinsically present in Acinetobacter but can be acquired through class 1 integron HGT (1).
Our Arr strains are clinical isolates harboring a class 1 integron containing the *arr* gene.
The expression of *arr* is driven by the common promoter (Pc) of the class 1 integron (1).
Arr containing strains have elevated RBT MIC, correlating with *arr* expression levels.

Strain	*arr* variant (class 1 integron type)	RBT MIC (mg/L)	Relative expression (qRT-PCR)
BV200	*arr-2* (1)	0.06 - 0.125	1
BV840	*arr-2* (2)	0.25 - 0.5	3
BV1617	*arr-3* (3)	0.25 - 2	15

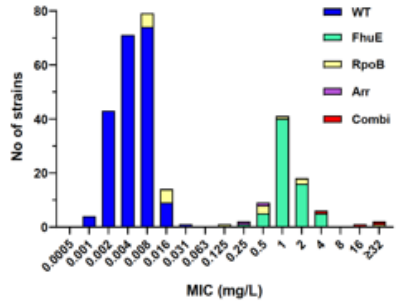
RBT (rifabutin) MIC determined in MHA + 0.1 mM PIH

(1) Coyne et al. 2009; doi:10.1128/AAC.01037-09

***Acinetobacter baumannii* strains harboring *arr* are rare**

- *In vitro* Activity/Genotype

→Is rifabutin activity in iron-limited conditions predictive of *in vivo* efficacy, including against RpoB mutants?



Strain population	Strain ID	FhuE status	RpoB status	Rifabutin MIC (mg/L) no PIH	Rifabutin MIC (mg/L) 0.1 mM PIH
WT	BV378	active	WT	0.06	0.004
	BV557	active	WT	0.125	0.008
	BV562	active	WT	0.5	0.002
	BV558	active	WT	1	0.001
RpoB / low MIC	BV710	active	S583L	> 32	0.016
	BV845	active	N527D	8	0.016
	BV683	active	I581M	16	0.016
RpoB / elevated MIC	BV1011	active	H535C	> 32	0.5
	BV1068	active	H535L	> 32	1
FhuE inactive	BV556	inactive	WT	8	2
	BV565	inactive	WT	2	1
	BV559	inactive	WT	4	1
	BV566	inactive	WT	8	2

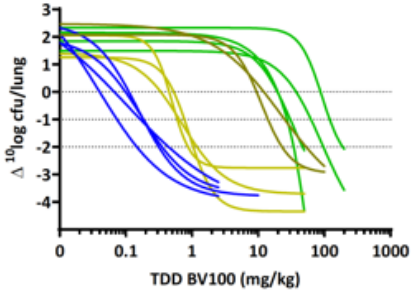
293 carbapenem resistant A. baumannii clinical isolates
Agar dilution MIC in Mueller-Hinton supplemented with 0.1 mM PIH

Rifabutin MIC in the presence of 0.1 mM PIH separates WT from non-WT isolates

- *In vitro* Activity/ *in vivo* Efficacy

→Is rifabutin activity in iron-limited conditions predictive of *in vivo* efficacy, including against RpoB mutants?

→ Neutropenic 24 hours lung infection model:



BV100: rifabutin infusion
TDD: total daily dose

Strain population	Strain ID	FhuE status	RpoB status	Rifabutin MIC (mg/L) no PIH	Rifabutin MIC (mg/L) 0.1 mM PIH
WT	BV378	active	WT	0.06	0.004
	BV557	active	WT	0.125	0.008
	BV562	active	WT	0.5	0.002
	BV558	active	WT	1	0.001
RpoB / low MIC	BV710	active	S583L	> 32	0.016
	BV845	active	N527D	8	0.016
	BV683	active	I581M	16	0.016
RpoB / elevated MIC	BV1011	active	H535C	> 32	0.5
	BV1068	active	H535L	> 32	1
FhuE inactive	BV556	inactive	WT	8	2
	BV565	inactive	WT	2	1
	BV559	inactive	WT	4	1
	BV566	inactive	WT	8	2

MIC in the presence of 0.1 mM PIH accurately measures rifabutin susceptibility, including against RpoB mutant isolates

- Progress
  - Based on this data, CLSI provided feedback to EUCAST in order to draft a joint response back to EUCAST at end of October 2025
  - Awaiting EUCAST feedback as of January 6, 2026 virtual meeting
- Newly Proposed Combination of Rifabutin and Polymyxin B for *Acinetobacter*

Rifabutin/polymyxin B MIC method

BIOVERSYS

Proposal

In concertation with the regulatory agencies, we have decided to develop BV100 (rifabutin for infusion) not as a standalone drug but as a fixed combination drug with polymyxin B for the treatment of *A. baumannii* infections. A fixed dose combination of BV100 and polymyxin B will be tested in a global clinical phase III trial. Consequently, the final reference testing method should ideally test the activity of rifabutin in combination with polymyxin B.

Do CLSI and EUCAST agree with the following approach to develop the testing method for the combination?

- All previous findings demonstrating that MIC in the presence of 0.1 mM accurately measure rifabutin susceptibility remain valid, indicating that the combination should be tested in the presence of 0.1 mM PIH
- The combination should be tested using a fixed concentration ratio rather than using a fixed concentration of polymyxin B since both agents have antibacterial activity
- The optimal testing concentration ratio should lead to MICs that best reflect the mode of action of the combination:
  - synergy on FhuE inactive strains due to polymyxin B-mediated increase uptake of rifabutin
  - no interaction on WT strains due to FhuE-mediated active uptake of rifabutin

Do CLSI and EUCAST have any other data requirement to determine the susceptibility testing method for a combination of two active agents?

- Methods Working Group Discussion and Recommendation (January 2026)

- Any idea on when further feedback from EUCAST would be available? No.
- Clarification on rpoB mutants. rpoB mutants are found in the WT and NWT MIC distributions and respond appropriately in the murine model.
- Concerns from WG about use of agar dilution for polymyxin B and rifabutin given what is known about polymyxin susceptibility testing and *Acinetobacter baumannii*.
  - Sponsor indicates that this is a problem for disk diffusion, but not agar dilution.
  - Sponsor has reproducible agar dilution data so far: rifabutin is the main driver of activity and polymyxin B provides synergy on *fhuE* inactive strains
- BMD is the only CLSI-approved method for polymyxin B and *A. baumannii*. Colistin agar test is not approved for *A. baumannii*.
- Currently no resistant isolates to test, but they plan to generate these *in vitro*.
- Really important to have good QC given how sticky polymyxin is. Loss of drug is especially prominent in the 0.5-2 µg/mL range.
- No point in developing disk diffusion for this method given the issues seen with polymyxin B disk diffusion.
- How will labs test this?
  - May be better to develop a critical endpoint assay to distinguish S from R.
- Sponsor asked for advice on method development. Should this be fixed concentration ratio since both agents have activity and what should the ratio be?
  - CLSI does not have specific guidance in this scenario.
  - Need to determine what is scientifically defensible for the drug.
  - Use M23 as much as possible in development of the method.
- Motion to approve the modified method for rifabutin and *Acinetobacter baumannii* complex using CA-MHA supplemented with 100 µM PIH. WG Vote: 8-0-1-3. Passed.

SC DISCUSSION (MAIN POINTS)

- EUCAST is still waiting for feedback from BioVersys. They asked if 3 mg/kg is the same exposure in humans. If it is then, it might not be needed to add PIH. There is Phase 2 clinical data so they should have some exposure data to guide EUCAST response.

A motion to approve the modified method for rifabutin and *Acinetobacter baumannii* complex using CA-MHA supplement with 100 µM PIH in Appendix H. Vote: 13 for, 1 against, 0 abstain, 0 absent (Pass)

Against Vote Reasoning:

- Wanted agreement with EUCAST.

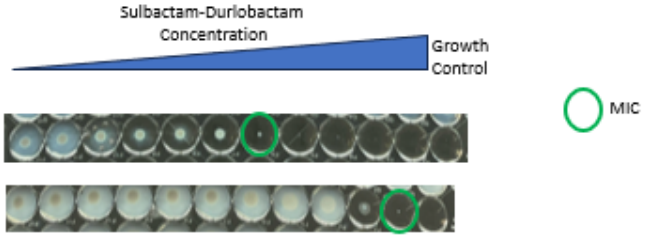
NOTE: Following the January 2026 meeting, it was agreed that no additions would be made to M100. Both CLSI and BioVersys are aligned with this decision.

SULBACTAM-DURLOBACTAM MIC READING

- Sulbactam-durlobactam (SUL-DUR) background
  - Sulbactam-durlobactam (XACDURO®) was approved by the FDA in May 2023

- Indications – 18+ years of age for hospital-acquired and ventilator-associated pneumonia (HABP/VABP) caused by *Acinetobacter baumannii-calcoaceticus* complex
- Broth MICs - doubling dilutions of sulbactam with fixed 4 µg/mL of durlobactam
- Broth QC ranges approved by CLSI for (M23 Tier 2 study)
  - *Acinetobacter baumannii* NCTC 13304 – 0.5/4 - 2/4
- Interpretive criteria approved by CLSI in June 2023 (aligns with FDA STIC)
  - S ≤ 4/4 µg/mL / I = 8/4 µg/mL / R ≥ 16/4

- Broth MIC testing of SUL-DUR (IHMA Experience)
  - Surveillance with SUL-DUR vs *A. baumannii-calcoaceticus* complex global clinical isolates from 2016 through 2024 - 6,000+ isolates
  - Central lab in Phase 3 pivotal trial (ATTACK), testing all isolates from the trial
  - Data used in setting breakpoints for broth MICs and disk diffusion
  - IHMA has not experienced regular trailing with SUL-DUR, therefore reading guide was not initially proposed
  - For testing, IHMA applies this standard; however, used rarely:
    - MIC endpoint corresponds to lowest concentration that shows growth inhibition to a button of < 1mm (otherwise clear) or when there is a light haze. Button of ≥ 1 mm or turbidity is considered as growth
- SUL-DUR *A. baumannii* challenge set (CDC AR Bank Experience)
  - AR Bank has been creating and validating a challenge set of *A. baumannii* for SUL-DUR MIC testing
    - IHMA sent isolates from SUL-DUR surveillance that had higher SUL-DUR MICs
    - JMI shared SUL-DUR MIC testing results of CDC AR Bank *A. baumannii* panel
  - CDC saw essential agreement with results shared by IHMA and JMI
    - caveat: IHMA and JMI results are single MIC values
  - CDC has encountered some trailing, particularly with SUL-DUR non-susceptible isolates, therefore has suggested a reading guide would be helpful
- Recommendation for next steps
  - Review and revise wording based on IHMA reading criteria
  - Obtain better pictures to include with reading guide
  - Present draft wording and images at June 2026 CLSI meeting
- Images for Reading Guide Samples (from AR Bank pictures)

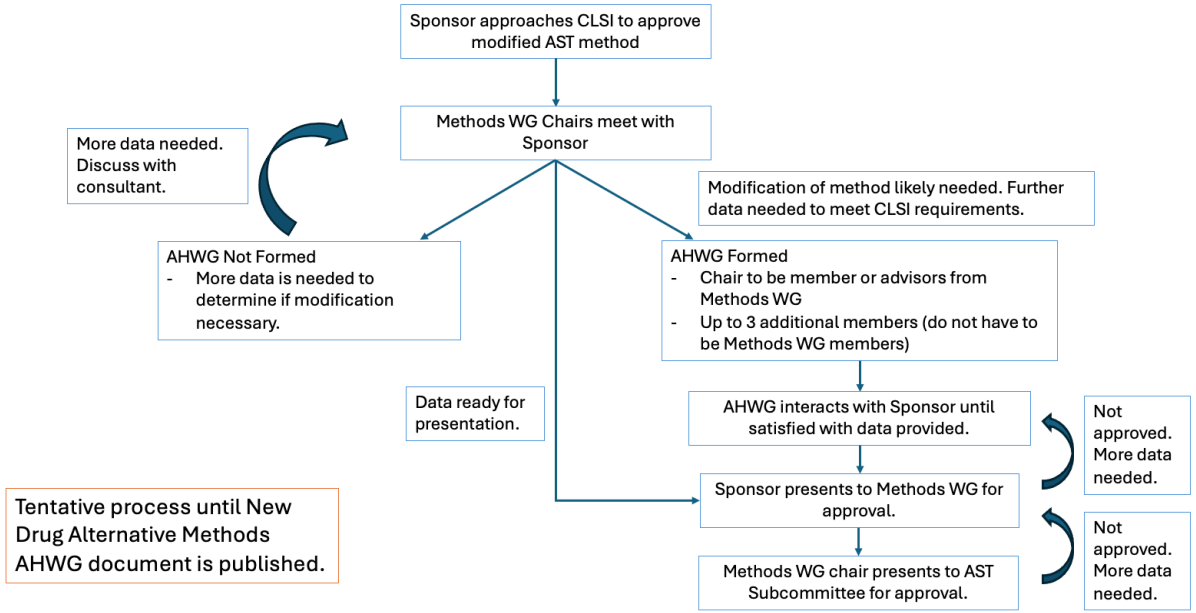


- Methods Working Group Discussion and Recommendation
  - Trailing does not change interpretations.
  - Would be helpful to understand how big an issue this is.

        - Was seen very rarely in surveillance studies at IHMA.
    - Reading guide needed as different reference labs are reading the trailing differently.
    - JMI and CDC can provide some pictures for the reading guide.
    - Use guidance from the cefiderocol reading guide and try to align reading of the buttons as much as possible.
    - Does this overlap with the reading guide in M07? If it differs from information provided in M07, then a reading guide would be helpful.
    - Could also put this information in the troubleshooting section of the M100.

WORKING GROUP PROCESS FOR METHOD DEVELOPMENT OF NEW AGENTS

- A flowchart was presented on how to manage the requests for alternative reference methods prior to the availability of the M23 supplement.



3.	**OUTREACH WORKING GROUP (J. HINDLER)**
	
	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
	• Meeting Education Workshops
	• News updates
	• Webinars
	  ○ Annual M100 Update
	  ○ 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, ADLM, 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
	
	CONTENT PRIORITIES 2025-2026
	• Newer Quality Control recommendations
	  ○ News Update article November 2025
	  ○ Pending
	  ▪ Update ASM-CAP-CLSI MIC IQCP (based on revisions to CAP checklist MIC.21910)
	  ▪ Update disk diffusion IQCP
	  ▪ Add more anecdotal examples
	• *Burkholderia cepacia* complex
	  ○ News Update article November 2025
	  ○ JCM Commentary – in progress
	  ○ Publication targeted for clinicians
	• Aztreonam-avibactam
	• Revisit updating breakpoint needs

AST SUBCOMMITTEE MEETING EDUCATION WORKSHOPS

- CLSI January 2026 Education Workshop
  - ECVs in Focus: Bridging Epidemiology, Research, and Clinical Practice
  - January 24, 2026
  - Speakers: Alexandra Bryson, Philippe Dufresne, and Amanda Kreuder
  - Moderator: Stella Antonara
- Maintain January workshop
- Discontinue June workshop
  - AFST and VAST Subcommittees do not meet in June
  - Fewer attendees
  - June has other meetings limiting speaker availability (eg, ASM)

NEWS UPDATE

- Publication goals: March, September
- AST News Updates are located under “Resources” tab of CLSI homepage
- Spring 2026
  - Feature: Patient perspective on fungal infection and value of AFST guidance
  - Case: Aztreonam-avibactam
  - Practical tips: Aztreonam-avibactam methods and validation of updated disk diffusion breakpoints
  - Hot topic: Increase in NDM in US
  - Recent developments: ß Streptococci and trimethoprim-sulfamethoxazole breakpoints
  - More News!: What’s in CLSI AST’s pipeline?

ATTENDEE ORIENTATION

- Updated June 2024. Will be updated again for June 2026
- On demand via YouTube as CLSI New Member Orientation
- 342 views on YouTube

WEBINARS/PRESENTATIONS

- CLSI-CAP Annual Webinar
  - Getting in the Zone: Maximizing AST Proficiency Testing
  - October 9, 2025
  - Speakers: Laurel Glaser and Samia Naccache
  - Moderator: Audrey Schuetz
  - Stats:
    - Registration: 321
    - Live Attendance: 173
    - On-Demand Views (not unique): 107

- Antibiotic Awareness Week Webinar
  - Advancing Standards to Fight Antimicrobial Resistance
  - November 19, 2025
  - Speakers: Dubraska Diaz-Campos, Philippe Dufresne, and Amy Mathers
  - Moderator: Romney Humphries
  - Stats:
    - Registration: 212
    - Live Attendance: 118
    - On-Demand Views (not unique): 32
- CLSI Annual Update (24th)
  - The Latest in Antimicrobial Susceptibility Testing (What's new in 2026 M100 36th edition)
  - February 25, 2026
  - Speakers: April Bobenchik and Virginia Pierce
  - Moderator: Janet Hindler
- CLSI/SIDP/ACCP Annual Webinar: Planning for Spring
- CLSI/CAP Annual Webinar: Planning for Fall
- Updated Antifungal Documents Webinar: Planning for Late Summer

NON-CLSI CONFERENCES AND EVENTS

- ASM Microbe 2026
  - Ur-ine or Ur-out: Body Site Considerations When Reporting and Interpreting Antimicrobial Susceptibility Test Results
  - Micro Session Tentative June 6, 2026
  - Speaker: James Lewis
- ADLM 2026
  - Confronting Antimicrobial Susceptibility Testing Challenges: Tools from CLSI
    - Speakers: Alexandra Bryson, Elizabeth Garrett, and Elizabeth Palavecino
    - Moderator: Alexandra Bryson
  - Fungal Taxonomy Updates, Candida auris, and Antifungal Resistance Testing in Clinical Laboratories
    - Speaker: Amir Seyedmousavi
- ID Week 2026
  - Taxonomical Instabilities and Managing Recent Changes in Microbial Nomenclature - Including Fungi, Bacteria, and Mycobacteria
  - Speakers: Amir Seyedmousavi, Romney Humphries, and TBD
  - Moderators: Amir Seyedmousavi and Juan Gea-Banacloche

CLSI M100 EDUCATIONAL PROGRAM

- Update anticipated Spring 2026
- No fee
- Enhance user ease of access
- Great for laboratory directors, training technologists and other trainees in laboratory

- Current version released March 2025
- Stats (January 1, 2025 – January 1, 2026)
  - Total Registrations: 3,975
  - 1,660 registrations (883 prior year)
  - 1,423 learners accessed the course (475 prior year)
  - 493 users completed the course (209 prior year)

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.
- CLSI M100 36th edition
- CLSI vs EUCAST Methods for Antifungal Susceptibility Testing
- *Burkholderia cepacia* (for clinicians)

BREAKPOINT IMPLEMENTATION TOOLKIT (BIT)

- Launched June 2023
- Updated in September 2025
- Align with upcoming CLSI M68 (Validation of AST System Breakpoints) and CLSI M52 (Verification of AST Systems) document updates. Need to address disk diffusion.
- Stats (January 1, 2025 – January 1, 2026):
  - 40,263 website page views
  - 14,310 visitors
  - 8,631 downloads
- Stats (past year):
  - 5,982 website page views
  - 1,441 downloads

CLSI M02 /M07 EDUCATIONAL PROGRAM

- In progress
- Based on CDC Antimicrobial Susceptibility Testing Training “Master” CD ROM from 2002
  - Subsequently on CDC’s website
  - Removed 2019 due to lack of resources to maintain the program
- Interactive overview of M02/M07
- Reflect “how to” for bench techs

PODCAST/SOCIAL MEDIA OPPORTUNITIES/OTHER

- Social media – where can AST fit?

- Trivia Tuesdays
- Laboratory jokes
- Facebook

- CLSI introducing podcasts
- CLSI making website landing page more user-friendly
- Let's Talk Micro with Luis Plaza
  - 180: CLSI M100-Ed35 Updates and More
  - Guest: April Bobenchik

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
  - Suggest topics
- Webinars / Workshops / Lectures
  - Suggest content
  - Speakers
- Other Projects

4.	**JOINT WORKING GROUP (E. MATUSCHEK)**
	
	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.
	• Goal #3: Respond to differences between CLSI and EUCAST AST methods to determine if harmonization can be achieved.
	
	WORKING GROUP DOCUMENTS
	• M23S (November 2024 2nd ed): Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria
	• M23S2 (November 2024 2nd ed): Process to Submit Disk Content (Potency) Data for Joint CLSI-EUCAST Working Group Review and Approval
	• M23S3 (June 2023 1st ed): Procedure for Confirming the Acceptability of Mueller-Hinton Agar Sources for Subsequent Use in CLSI and/or EUCAST Studies to Establish Disk Diffusion Quality Control Ranges (pending addition of paragraph re: pre-QC of CAMHB)
	
	DISK CONTENT SELECTION AND PRE-QC STUDIES
	• Disk content selection (M23S and M23S2)
	  ○ Phase 1: 10 disks with a wide variety in disk potency
	  ○ Phase 2: 2-4 selected disks
	  ○ A sub-group with CLSI and EUCAST members analyze the data together with the pharma company
	• Pre-QC studies (M23S3)
	  ○ Evaluation of disks from 2-3 manufacturers on media from 4 manufacturers
	  ○ Designed to disclose differences between media manufacturers
	
	M23S DISK SOURCES FOR PHASE 1 AND PHASE 2 ADDITION
	2.3
	Phase 1 (bullet 3)
	• Testing can be performed using one disk lot per content (potency) on Mueller-Hinton media from one manufacturer. These disks can be commercially produced or obtained from small-scale production by the pharmaceutical company or a contract laboratory. It may be preferred to use manually prepared disks rather than commercially produced disks in Phase 1 since the potency, disk QC range, and stability of the compound in the disk may not yet be known at this stage. A procedure for manual preparation of disks is provided in Appendix B.

2.4.1
Phase 2 (bullet 1)

- Testing should be performed using commercially produced disks ~~(one disk lot per disk content [potency])~~ or disks from small-scale production by the pharmaceutical company or a contract laboratory (two disk lots per disk content [potency]). For both commercially produced and manually prepared disks, it is recommended that at least 20 replicate tests are performed for each provisional QC strain to generate reference data that commercial disk manufacturers can use as a benchmark. Reproducibility between each lot of disk could be evaluated by comparing Phase 2 to Phase 1 results when the same isolates and disk potencies are used in each study. ~~It is preferred that all testing is performed from a single batch of both disk lots, but if isolate numbers are high it is acceptable to use more than one batch of disks.~~ A procedure for manual preparation of disks is provided in Appendix B.

A motion to add the proposed revisions to CLSI M23S disk sources for Phase 1 and Phase 2. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

DISK CONTENT SELECTION STUDIES

WG Assigned Study #	Agent	Sponsor	Status
JWG-2023-1	BWC0977	Bugworks (JMI)	Phase 2 on hold
JWG-2023-2	Piperacillin-tazobactam (reassessment)	JMI, EDL	Updated data presentation 1/12/26
JWG-2024-1	GDC0829	Genentech (IHMA)	Phase 2 on hold
JWG-2024-4	FG-960	Blacksmith Medicines (Forge Therapeutics) (JMI)	Phase 2 in progress with 30, 50, 75 µg disks
JWG-2025-2	Cefiderocol (reassessment)	JMI, EDL	Phase 1 data presented to Cefiderocol Ad Hoc WG. To be discussed at Methods WG.
JWG-2025-4	Cefiderocol-xeruborbactam	Qpex Biopharma (JMI)	Will share Phase 1 results with sub-WG soon

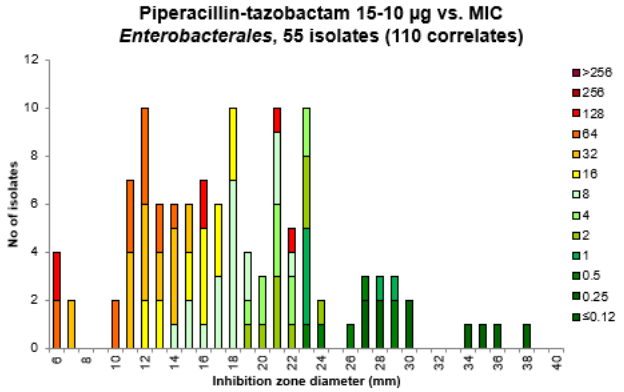
PIPERACILLIN-TAZOBACTAM PHASE 1 DISK POTENCY STUDY

- Data generated by JMI/Element

- Phase 1 (JMI/Element)
- 55 Enterobacterales
- 13 *P. aeruginosa*
- Piperacillin-tazobactam disks
  - 5-0.5 µg, 5-1 µg
  - 7-0.5 µg, 7-1 µg, 7-2 µg
  - 10-1 µg, 10-2 µg
  - 15-10 µg
  - 20-5 µg, 20-10 µg
  - 30-6 µg (EUCAST disk)
- Selected for phase 2:
  - 15-5 µg (not included in phase 1), 15-10 µg
  - 20-5 µg, 20-10 µg

Enterobacterales

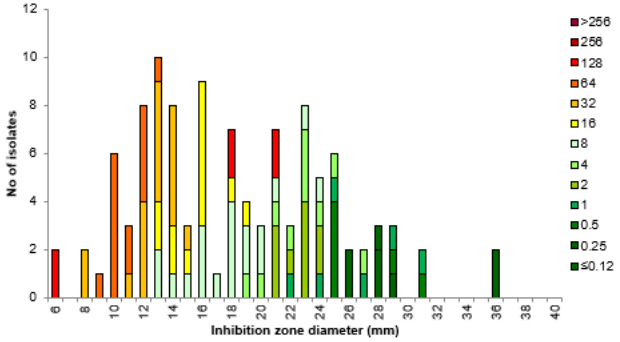
EUCAST S≤8, R>8 mg/L
CLSI S≤8, I=16, R≥32 mg/L



Enterobacterales

EUCAST S≤8, R>8 mg/L
CLSI S≤8, I=16, R≥32 mg/L

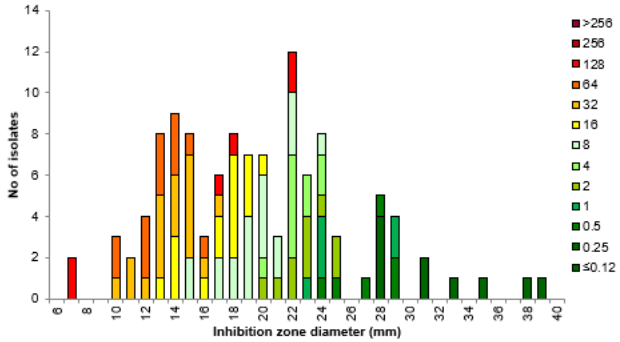
Piperacillin-tazobactam 20-5 µg vs. MIC
***Enterobacterales*, 55 isolates (110 correlates)**



Enterobacterales

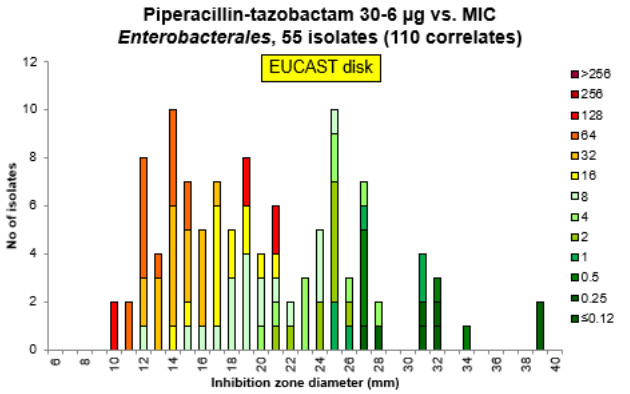
EUCAST S≤8, R>8 mg/L
CLSI S≤8, I=16, R≥32 mg/L

Piperacillin-tazobactam 20-10 µg vs. MIC
***Enterobacterales*, 55 isolates (110 correlates)**



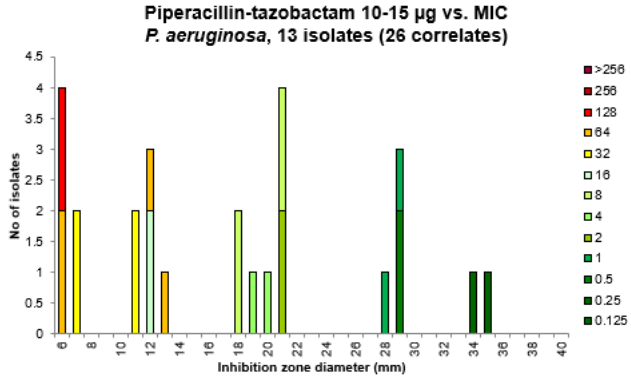
Enterobacterales

EUCAST S≤8, R>8 mg/L
CLSI S≤8, I=16, R≥32 mg/L



P. aeruginosa

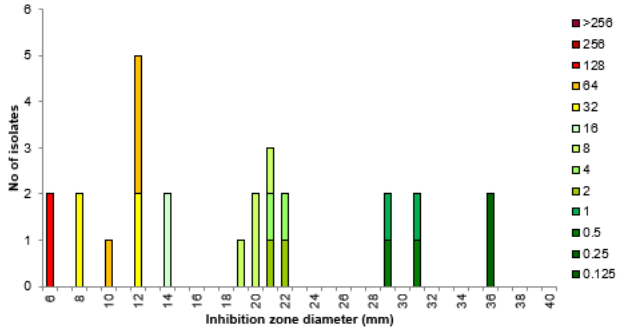
EUCAST S≤0.001, R>16 mg/L
CLSI S≤16, I=32, R≥64 mg/L



P. aeruginosa

EUCAST S≤0.001, R>16 mg/L
CLSI S≤16, I=32, R≥64 mg/L

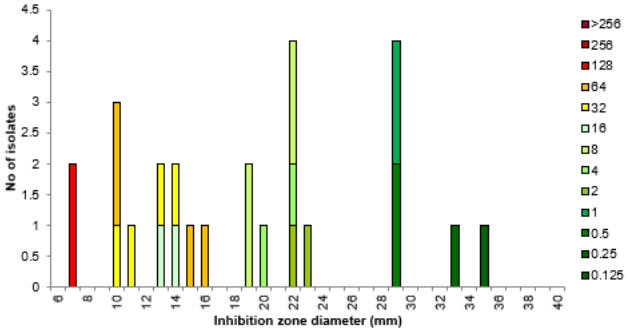
Piperacillin-tazobactam 20-5 µg vs. MIC
***P. aeruginosa*, 13 isolates (26 correlates)**

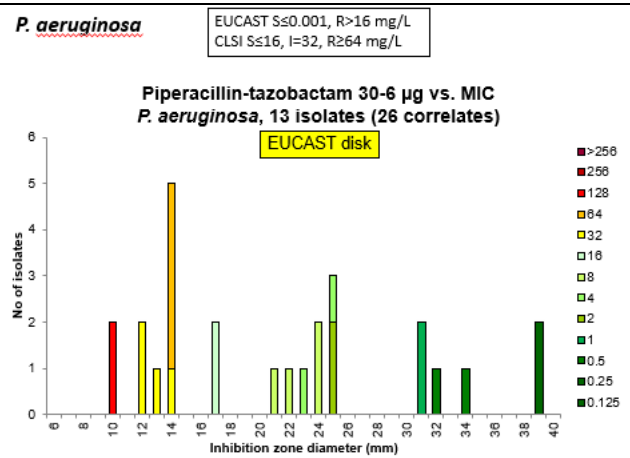


P. aeruginosa

EUCAST S≤0.001, R>16 mg/L
CLSI S≤16, I=32, R≥64 mg/L

Piperacillin-tazobactam 20-10 µg vs. MIC
***P. aeruginosa*, 13 isolates (26 correlates)**





MHA AGAR EVALUATIONS (M23S)

- New Action item: Expand M23S3 to look at differences in disk performance when there are zone diameter differences of > 1 mm between disks from two or more manufacturers.

WG Assigned Study #	Agent	Sponsor	Status
JWG-2025-3	Zoliflodacin	GARDP (IHMA)	Evaluating various media more closely
JWG-2026-1	Meropenem-ANT3310	Antabio (IHMA)	Evaluating disk performance differences

FINALIZED ACTION ITEMS

Action	Status
Further address source of disks for Phase 1 and Phase 2 disk content selection studies	Finalized 1/12/26 (in preparation for presentation to AST SC)
Provide more Tier 2 QC disk diffusion data to John to validate the use of Range Finder to find **statistical** lab/manufacturer outliers	John Turnidge's' statistical tools were unable to identify disk manufacturers' outliers among Tier 2 QC data

PENDING ACTION ITEMS

Action	Status
Explore the need for further describing performance of **colony counts** on AST inocula for reference-type AST procedures (e.g., QC studies)	In discussion at QC WG
QC ranges for beta-lactam agents vs **beta-lactam/beta-lactamase inhibitor combinations** for susceptible QC strains	In discussion at QC WG
CLSI potential addition of **QC targets**	In discussion at QC WG

NEW DRUG ALTERNATIVE METHODS AD HOC WORKING GROUP REPORT (PRESENTED BY I. MORRISSEY AND J. PATEL)

- AHWG Charge
  - Develop a standard for determining when the reference method for antimicrobial susceptibility testing, 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
- Subchapter 4.1 Lack of reproducibility of the AST method
  - Trailing endpoints

- If trailing endpoints occur for the antimicrobial agent in development, it should be investigated if alternative reading instructions, and primarily those already recommended by CLSI and EUCAST, produce more reproducible MICs rather than modifying the test medium.

- Skipped wells
  - It is not unusual that a 'skipped well', a well of no bacterial growth followed by one or several wells with growth, may occur when testing a dilution series of any antimicrobial agent (old or new). There are several possible explanations including incorrect inoculation, contamination, heterogenous resistance, plate preparation errors etc. Examples from the EUCAST reading guide are shown in Figure 2. However, if skipped wells repeatedly occur, resulting in many MIC test results being invalid when testing a new antimicrobial agent, there may be a need to modify the testing method to overcome this problem.
- Medium interference
  - Some antimicrobial agents are more affected by the composition of the test medium than others. BMD shall be performed using CAMHB and when needed, modified as described in Table 1 to achieve sufficient growth for some species or to accurately measure antimicrobial activity. The CAMHB used should meet the specifications in international standards. If irreproducible MICs are observed when performing BMD with CAMHB from one manufacturer, CAMHB from additional manufacturers should be tested to investigate if this is related to the CAMHB brand used. If preliminary data suggests that a modification of the reference method is needed, then modifications already approved by CLSI and EUCAST should be evaluated first (see Table 1).
- Instability of the antimicrobial agent in the test medium
  - Instability of the antimicrobial agent in the test medium is another possible reason for irreproducible MICs. Tests to investigate this should include testing different solvents, diluents, or different salts of the active antimicrobial agent. Investigations into possible supplementation or modification of CAMHB should be secondary to these tests.
- Inoculum effect
  - The target final inoculum in BMD is $5x10^5$ CFU/mL with an acceptable range of $2x10^5$ - $8x10^5$ CFU/mL. Modifying the inoculum is not suggested as a modification of the AST method but could be an important part of investigating irregular MICs. When assessing the inoculum effect, it is advisable to start by determining if small differences within the specified inoculum range ($2x10^5$ - $8x10^5$ CFU/mL) influence the MICs.

- Subchapter 4.2 Unexpected results from the AST method
  - Regards large panel of organisms incl. QC and antimicrobial agent test range. Use reference method and proposed method.
- Subchapter 4.3 *In vivo* pharmacodynamic studies
  - Perform PK/PD studies ASAP.
  - If no correlation between MIC and efficacy relative to *in vivo* exposure
    - Consider modified MIC method
    - Consider alternative PK/PD indices
- Reasons that Do Not Justify a Change
  - Modifying the AST method without sound physiological justification.
    - The physiological justification is a rationale based upon the antimicrobial agent's mechanism of action and data, described above, indicating that modification of the AST method is necessary for accurate MIC determination.
  - Lowering MIC values to demonstrate greater activity than competitor antimicrobial agents.
    - There may be multiple drivers or misunderstandings that can result in this activity. The following are two clarifications that can help to address concerns.

- Irrespective of the method used, the MIC is a relative value, and the activity of an antimicrobial agent cannot be determined solely by MIC values. Instead, activity is determined by applying data derived from relevant *in vitro* and *in vivo* infection models to MIC data.
- Some antimicrobial agents, like peptides, have a higher molecular weight than traditional small molecule antimicrobial agents. This results in a higher MIC per molecule when the MIC is expressed as µg/mL or mg/L, both traditional expressions of MIC values. For educational purposes, this problem can be addressed by expressing the MIC in both weight per volume units and molar units per volume when questions regarding MIC values occur.

- Modification of the AST method to support growth of both fastidious and non-fastidious bacteria.
  - It is preferred to use BMD with CAMHB for non-fastidious aerobic bacteria and an accepted reference method for fastidious and anaerobic bacteria. AST methods for fastidious and anaerobic bacteria can be found in CLSI M100, CLSI M45, and EUCAST Clinical Breakpoint Tables.

- Decision Flowchart

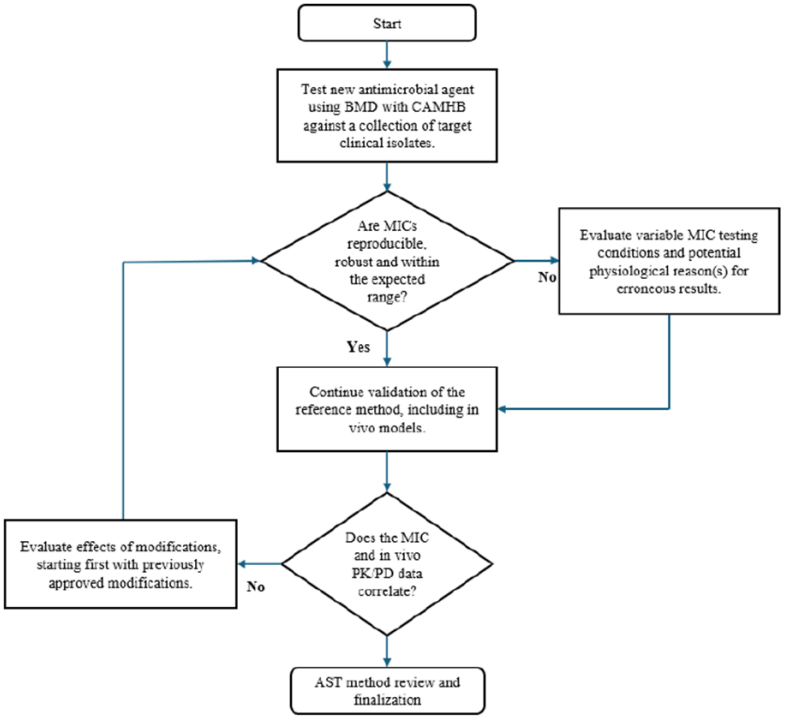


Figure 7. Workflow and decision process for evaluating AST methods.

	SC DISCUSSION (MAIN POINTS) • The draft will be placed into the supplement template in Word and added to Edaptive. Then, to be voted on by the AST Subcommittee. • The Methods Working Group would oversee the process with sponsors coming forward and requesting alterations to the reference method. • EUCAST would like to propose a joint CLSI/EUCAST working group similar to the disk content evaluation. • The document needs to be considered for review by CLSI and EUCAST. The open consultation through EUCAST received feedback with the disk mass document. • An education session on methods development was recommended.
5.	**ADJOURNMENT** Dr. Mathers provided an update on: • Working groups will be creating mission statements to define the scope of the groups. The mission statements will be presented in June 2026. • There will be an update on the subcommittee and working group term limits at the June meeting. Dr. Mathers thanked the participants for their attention. The meeting was adjourned at 11:17 AM Mountain Standard (US) time.

PLENARY ATTENDEES

Plenary 1	Plenary 2	Plenary 3
Abdul Azim Ahmed	Abdul Azim Ahmed	Abdul Azim Ahmed
Adams Jennifer K.	Adams Jennifer K.	Adams Jennifer K.
Alby Kevin	Alby Kevin	Alby Kevin
Andermann Tessa	Andermann Tessa	Andermann Tessa
Asempa Tomefa	Arone Lisa	Asempa Tomefa
Austerman Ashley	Asempa Tomefa	Austerman Ashley
Bala Shukal	Austerman Ashley	Bala Shukal
Balbuena Rocio	Bala Shukal	Balbuena Rocio
Banerjee Bose Dithi	Balbuena Rocio	Banerjee Bose Dithi
Berkow Elizabeth	Banerjee Bose Dithi	Berkow Elizabeth
Bhatti Micah M.	Berkow Elizabeth	Bhatti Micah M.
Bobenchik April M.	Bhatti Micah M.	Bobenchik April M.
Boswell Malcolm	Bobenchik April M.	Bovill James
Bovill James	Boswell Malcolm	Bowden Robert
Bowden Robert	Bowden Robert	Boyle Bridget
Boyle Bridget	Boyle Bridget	Brandt Maryann
Brandt Maryann	Brandt Maryann	Brent Lonnie
Brent Lonnie	Brent Lonnie	Brocco Fabio
Brocco Fabio	Brocco Fabio	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.	Burgess David S
Burgess David S	Burgess David S	Burnham Carey-Ann
Burnham Carey-Ann	Burnham Carey-Ann	Campbell Davina
Bursens Jeroen	Bursens Jeroen	Campeau Shelley
Campbell Davina	Campbell Davina	Capraro Gerald A.
Campeau Shelley	Campeau Shelley	Carpenter Darcie E.
Capraro Gerald A.	Capraro Gerald A.	Carvalhaes M.D., Ph.D., D ABMM Cecilia
Carpenter Darcie E.	Carpenter Darcie E.	Castanheira Mariana
Carvalhaes M.D., Ph.D., D ABMM Cecilia	Carvalhaes M.D., Ph.D., D ABMM Cecilia	Chandrasekaran Sukantha
Castanheira Mariana	Castanheira Mariana	CHEN YAMIN
Chandrasekaran Sukantha	Chandrasekaran Sukantha	Cintron Cotto Melvili
CHEN YAMIN	CHEN YAMIN	Clayton Nicola
Cintron Cotto Melvili	Cintron Cotto Melvili	Copsey-Mawer Sarah
Clayton Nicola	Clayton Nicola	Cullen Sharon K.
Copsey-Mawer Sarah	Copsey-Mawer Sarah	DeDonder Keith
Cullen Sharon K.	Cullen Sharon K.	DeJonge Boudewijn
DeDonder Keith	DeDonder Keith	Dial Courtney
DeJonge Boudewijn	DeJonge Boudewijn	Dingle Tanis

Dial Courtney
Diaz-Campos Dubraska V.
Dickinson Drew
Dien Bard Jennifer
Dingle Tanis
Donohue Lindsay
Dressel Dana C.
Dulfer Kaitlyn
Dumm Rebekah
Duncan Elaine
Esparza German
Estrada Sandy
Fedorenko Marianna
Ferrell Andrea L.
Fisher Mark A.
Frascarelli Maria
Fratoni Andrew
Gancarz Barb
Garrett Elizabeth
Gatermann Sören
Gefroh Sarah
Gill Christian
Giske Christian G.
Glasgow Heather
Gomez Emily J.
Gray Alice
Greninger Alex
Griffin Natasha
Haddock Christopher
Hamilton Lauren
Hara Takafumi
Hastey Christine
Hawser Stephen
Hendrix Megan
Hernandez Esther
Hill Brandon
Hindler Janet A.
Hirsch Elizabeth
Hoffard Rita
Holliday Nicole
Hope Katie
Howell Nicholas
Hsiung Andre
Dial Courtney
Diaz-Campos Dubraska V.
Dien Bard Jennifer
Dingle Tanis
Donohue Lindsay
Dressel Dana C.
Dulfer Kaitlyn
Dumm Rebekah
Duncan Elaine
Esparza German
Estrada Sandy
Fedorenko Marianna
Ferrell Andrea L.
Fisher Mark A.
Frascarelli Maria
Fratoni Andrew
Gancarz Barb
Garrett Elizabeth
Gatermann Sören
Gefroh Sarah
Gill Christian
Giske Christian G.
Glasgow Heather
Gomez Emily J.
Gray Alice
Greninger Alex
Griffin Natasha
Haddock Christopher
Hamilton Lauren
Hara Takafumi
Hastey Christine
Hawser Stephen
Hendrix Megan
Hernandez Esther
Hill Brandon
Hindler Janet A.
Hirsch Elizabeth
Hoffard Rita
Holliday Nicole
Hope Katie
Howell Nicholas
Hsiung Andre
Huband Michael D.
Donohue Lindsay
Dressel Dana C.
Dulfer Kaitlyn
Dumm Rebekah
Duncan Elaine
Esparza German
Estrada Sandy
Fedorenko Marianna
Ferrell Andrea L.
Fisher Mark A.
Frascarelli Maria
Fratoni Andrew
Gancarz Barb
Garrett Elizabeth
Gatermann Sören
Gefroh Sarah
Gill Christian
Giske Christian G.
Glasgow Heather
Gomez Emily J.
Greninger Alex
Griffin Natasha
Haddock Christopher
Hamilton Lauren
Hara Takafumi
Hastey Christine
Hawser Stephen
Hendrix Megan
Hernandez Esther
Hindler Janet A.
Hirsch Elizabeth
Hoffard Rita
Holliday Nicole
Howell Nicholas
Hsiung Andre
Huband Michael D.
Humphries Romney M
Ilges Dan
Jimenez Pearson Antonieta
Johnson Kristie
Jones Barb
KANWAR NEENA
Karlsson Asa

Huband Michael D.
Humphries Romney M
Jimenez Pearson Antonieta
Johnson Kristie
Jones Barb
KANWAR NEENA
Karlsson Asa
Kavanaugh Logan
Khan Ayesha
Killian Scott B.
Kirn Thomas
Klavins Anna
Koeth Laura M.
Kumaraswamy Monika
La Forge Michael H
Lam Christine M.
LaVoie Stephen
Law Ashley
Leung Beth
Lewis James S.
Li Xian-Zhi
Liesman Rachael
Lutgring Joseph
Maddock Kelli
Malmberg Christer
Malysa Michelle
Marion Solenne
Martin Isabella
Mason Russell
Mathers Amy
Matuschek Erika
McCurdy Sandra
McDaneld Patrick
McLeod Sarah
Miller Jennifer
Miller Linda A.
Miller William
Mitchell Stephanie L.
Moeck Greg
Monogue Maggie
Moore Nicholas M.
Morales Yesenia
Morrissey Ian
Humphries Romney M
Jimenez Pearson Antonieta
Johnson Kristie
Jones Barb
KANWAR NEENA
Karlsson Asa
Kavanaugh Logan
Khan Ayesha
Killian Scott B.
Kirn Thomas
Klavins Anna
Koeth Laura M.
Kumaraswamy Monika
La Forge Michael H
Lam Christine M.
LaVoie Stephen
Leung Beth
Lewis James S.
Li Xian-Zhi
Liesman Rachael
Lutgring Joseph
Maddock Kelli
Malmberg Christer
Malysa Michelle
Marion Solenne
Martin Isabella
Mason Russell
Mathers Amy
Matuschek Erika
McCurdy Sandra
McDaneld Patrick
McLeod Sarah
Miller Jennifer
Miller Linda A.
Miller William
Mitchell Stephanie L.
Moeck Greg
Monogue Maggie
Moore Nicholas M.
Morales Yesenia
Morrissey Ian
Moussa Samir
Naccache Samia N.
Kavanaugh Logan
Khan Ayesha
Killian Scott B.
Kirn Thomas
Klavins Anna
Koeth Laura M.
Kumaraswamy Monika
La Forge Michael H
Lam Christine M.
LaVoie Stephen
Law Ashley
Leung Beth
Lewis James S.
Li Xian-Zhi
Liesman Rachael
Lutgring Joseph
Maddock Kelli
Malmberg Christer
Malysa Michelle
Marion Solenne
Martin Isabella
Mathers Amy
McDaneld Patrick
McLeod Sarah
Miller Jennifer
Miller Linda A.
Miller William
Mitchell Stephanie L.
Mitteer Hayden
Moeck Greg
Morales Yesenia
Morrissey Ian
Moussa Samir
Naccache Samia N.
Narayanan Navaneeth
Nicolau David P.
Nielsen Lindsey
Ohkusu Kiyofumi
OKADE HAYATO
Onishi Motoyasu
Otto Caitlin
Oyarzun Sebastian Cifuentes
Palavecino Elizabeth

Moussa Samir
Naccache Samia N.
Narayanan Navaneeth
Nicolau David P.
Nielsen Lindsey
Ohkusu Kiyofumi
OKADE HAYATO
Onishi Motoyasu
Otto Caitlin
Oyarzun Sebastian Cifuentes
Palavecino Elizabeth
Patel Jean B.
Perez Katherine
Pierce Virginia M.
Pillar Chris
Pischel Kelsey
Pomponio Stefano
Puumala Emily
Ramos Karl Anthony
Rice Felicia
Rossi Flavia
Sanchez Belkys
Satlin Michael
Scangarella-Oman Nicole
Scheetz Marc H.
Schmerer Matthew
Schneider Cynthia
Shaeer Kristy
Shannon Samantha
Shurland Simone M
Simner Patricia J.
Simon Sam
Slaughter Jennifer
Snippes Vagnone Paula M.
Staats Dylan
Steenbergen Judith
Stevenson John Scott
Stone Gregory G.
SUZUKI EISUKE
Takemura Miki
Tamma Pranita D.
Tekle Tsigereda
Tenllado Jolyn
Narayanan Navaneeth
Nicolau David P.
Nielsen Lindsey
Ohkusu Kiyofumi
OKADE HAYATO
Onishi Motoyasu
Otto Caitlin
Oyarzun Sebastian Cifuentes
Palavecino Elizabeth
Patel Jean B.
Perez Katherine
Pierce Virginia M.
Pillar Chris
Pischel Kelsey
Pomponio Stefano
Puumala Emily
Ramos Karl Anthony
Rice Felicia
Rossi Flavia
Sanchez Belkys
Satlin Michael
Scangarella-Oman Nicole
Scheetz Marc H.
Schmerer Matthew
Schneider Cynthia
Shaeer Kristy
Shannon Samantha
Shurland Simone M
Simner Patricia J.
Simon Sam
Slaughter Jennifer
Snippes Vagnone Paula M.
Staats Dylan
Steenbergen Judith
Stevenson John Scott
Stone Gregory G.
SUZUKI EISUKE
Takemura Miki
Tamma Pranita D.
Tekle Tsigereda
Tenllado Jolyn
Thomson Susan
Thrupp Lauri D.
Patel Jean B.
Perez Katherine
Pierce Virginia M.
Pillar Chris
Pischel Kelsey
Pomponio Stefano
Puumala Emily
Ramos Karl Anthony
Rice Felicia
Sanchez Belkys
Satlin Michael
Scangarella-Oman Nicole
Schmerer Matthew
Schneider Cynthia
Shaeer Kristy
Shannon Samantha
Shurland Simone M
Simner Patricia J.
Simon Sam
Slaughter Jennifer
Snippes Vagnone Paula M.
Staats Dylan
Steenbergen Judith
Stevenson John Scott
Stone Gregory G.
SUZUKI EISUKE
Takemura Miki
Tamma Pranita D.
Tekle Tsigereda
Tenllado Jolyn
Thomson Susan
Thrupp Lauri D.
Truong Thao
Turng Ben
Usongo Valentine
Van Tam T.
Weinstein Melvin P.
Wikler Matthew A.
Winkler Marisa
Yamano Yoshinori
Yanagihara Katsunori
Young Katherine
Zappas Kristie

Thrupp Lauri D.
Truong Thao
Turng Ben
Uprety Priyanka
Usongo Valentine
Van Tam T.
Weinstein Melvin P.
Wikler Matthew A.
Winkler Marisa
Yamano Yoshinori
Yanagihara Katsunori
Young Katherine
Zappas Kristie
Zhuo Ran
Zimmer Barbara L.

Truong Thao
Turng Ben
Uprety Priyanka
Usongo Valentine
Van Tam T.
Weinstein Melvin P.
Wikler Matthew A.
Winkler Marisa
Yamano Yoshinori
Yanagihara Katsunori
Young Katherine
Zappas Kristie
Zhuo Ran
Zimmer Barbara L.

Zhuo Ran
Zimmer Barbara L.