Meeting Title:	**Subcommittee on Antimicrobial Susceptibility Testing (AST)**	**Contact:**	egomez@clsi.org
Meeting Location:	Chicago, Illinois, USA		
Meeting Dates and Times: All times are Central Time (US).	**Plenary 1:** Monday, 1 June 2026, 7:30 AM – 12:00 PM **Plenary 2:** Monday, 1 June 2026, 1:00 – 5:30 PM **Plenary 3:** Tuesday, 2 June 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**
Alexandra L. Bryson, PhD, D(ABMM) AST Subcommittee Secretary	**Virginia Commonwealth University Health**
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 R. 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:	
Advisors Present:	
Mariana Castanheira, PhD	Element/JMI Laboratories
Sharon K. Cullen, BS, RAC	Beckman Coulter, Inc. Microbiology Business
Lindsay Donohue, PharmD, BCIDP	University of Virginia Medical Center
Rebekah Dumm, PhD	Washington University School of Medicine
Andrea L. Ferrell, MLS(ASCP)	BD
Andrew Fratoni, PharmD	Hartford Hospital
Marcelo Galas, BSc	Pan American Health Organization
Elizabeth Garrett, PhD, D(ABMM)	Penn State Health, Milton S. Hershey Medical Center
Sören Gatermann	EUCAST
Elizabeth Hirsch, PharmD	University of Minnesota
Andre Hsiung, MBA, MS	Hardy Diagnostics
Dmitri Iarikov, MD, PhD	FDA Center for Drug Evaluation and Research
Antonieta Jimenez Pearson, MQC, PhD	INCIENSA

Thomas J. Kirn, MD, PhD	Rutgers Robert Wood Johnson Medical School
Samia Naccache, PhD, M(ASCP)CM, D(ABMM)	LabCorp
Mike Satlin, MD	Weill Cornell Medicine
Ribhi Shawar, PhD, D(ABMM), F(AAM)	FDA Center for Devices and Radiological Health
Patricia Simner, PhD, D(ABMM)	Mayo Clinic
Jolyn Tenllado	bioMerieux
Melvin P. Weinstein, MD	Robert Wood Johnson University Hospital
Katsunori Yanagihara	Japanese Society for Clinical Microbiology
Barbara L. Zimmer, PhD	Beckman Coulter
Advisors Absent:	
Amelia S. Bhatnagar, MPH	Centers for Disease Control and Prevention
Romney M. Humphries, PhD, D(ABMM), FIDSA, FAAM	Vanderbilt University Medical Center
Kristie Johnson, PhD, D(ABMM)	University of Maryland
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
Michael La Forge, MHA, MLS	CLSI
Christine Lam, MT(ASCP)	CLSI
Lori Selden, MS, MT(ASCP)	CLSI

Plenary Agendas

PLENARY AGENDA: SESSION 1 – IN PERSON Monday, 1 June 2026 7:30 AM – 12:00 PM Central Time (US)			
Time	**Item**	**Presenter**	**Page**
7:30 AM – 7:35 AM (5 min)	Opening Remarks	A. Mathers	8
7:35 AM – 7:40 AM (5 min)	Approval of Meeting Agenda	A. Mathers	8
7:40 AM – 7:45 AM (5 min)	CLSI Welcome and Update	J. Adams	8
7:45 AM – 7:50 AM (5 min)	AST Subcommittee Update	E. Gomez	9
7:50 AM – 8:00 AM (10 min)	Subcommittee on Veterinary Susceptibility Testing (VAST) Update	R. Bowden	12
8:00 AM – 8:10 AM (10 min)	Expert Panel on Microbiology Update	A. Schuetz	18
8:10 AM – 8:20 AM (10 min)	STMA Introduction and Update	C. Brown	21
8:20 AM – 8:40 AM (20 min)	Text and Tables WG	A. Bobenchik S. Campeau	23
8:40 AM – 9:25 AM (45 min)	Investigational Breakpoints AHWG	A. Khan M. Wikler	31
9:25 AM – 9:45 AM (20 min)	Break		
9:45 AM – 11:15 AM (1 hr 30 min)	Quality Control WG	S. Cullen E. Garrett C. Pillar	45
11:15 AM – 11:45 AM (30 min)	Anaerobes WG	D. Carpenter S. Copsey-Mawer	73
11:45 AM – 12:00 PM (15 min)	*Staphylococcus aureus* Tetracycline Breakpoints	J. Lutgring	75

PLENARY AGENDA: SESSION 2 - IN PERSON Monday, 1 June 2026 1:00 PM - 5:30 PM Central Time (US)			
Time	**Item**	**Presenter**	**Page**
1:00 PM – 3:00 PM (2 hr)	Breakpoints WG: Part 1	N. Narayanan M. Satlin	81
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	81
PLENARY AGENDA: SESSION 3 - IN PERSON Tuesday, 2 June 2026 7:30 AM - 12:00 PM Central Time (US)			
Time	**Item**	**Presenter**	**Page**
7:30 AM – 9:30 AM (2 hr)	Methods WG: Part 1	T. Dingle A. Ferrell	170
9:30 AM – 9:50 AM (20 min)	Break		
9:50 AM – 10:50 AM (1 hr)	Methods WG: Part 2	T. Dingle A. Ferrell	170
10:50 AM – 11:20 AM (30 min)	Joint CLSI-EUCAST WG	J. Hindler	223
11:20 AM – 11:50 AM (30 min)	Outreach WG	J. Hindler A. Schuetz	229
11:50 AM - 12:00 PM (10 min)	Closing Remarks	A. Mathers	234

Summary of Voting Decisions and Action Items

Summary of Passing Votes			
#	**Motion Made and Seconded**	**Results[a]**	**Page[b]**
1.	To approve the June 2026 meeting agenda.	15-0-0-1	8
2.	To remove the cefazolin comment 17 from Table 2A-1.	14-2-0-0	23
3.	To remove the media prep general comments from Table 2E and 2F.	16-0-0-0	24
4.	To remove the cefepime comments 12 and 13 from Table 2G.	16-0-0-0	25
5.	To add the proposed NOTE 2, "This test has only been evaluated for assessing the *in vitro* activity of the combination of aztreonam plus ceftazidime-avibactam; CLSI has not yet evaluated data for correlation to the *in vitro* activity of aztreonam-avibactam." to Table 3D.	16-0-0-0	26
6.	To add the proposed cefiderocol-xeruborbactam footnote, "QC ranges reflect MICs obtained using CAHMB (ie, not iron-depleted CAMHB)." to Table 5A-2.	16-0-0-0	26
7.	To remove the ceftibuten MIC and disk diffusion breakpoints for Enterobacterales and *Haemophilus*.	16-0-0-0	35
8.	To remove the cefetamet MIC and disk diffusion breakpoints for Enterobacterales and *Haemophilus*.	16-0-0-0	36
9.	To keep the pefloxacin disk diffusion breakpoints for *Salmonella* and remove the Inv designation.	16-0-0-0	37
10.	To remove the fleroxacin MIC and disk diffusion breakpoints from Enterobacterales, *Haemophilus*, and *Staphylococcus*.	16-0-0-0	39
11.	To remove the teicoplanin MIC and disk diffusion breakpoints for *Staphylococcus* and *Enterococcus*, add a comment that states "temporarily removed pending further review", remove the Inv designation, and form an ad hoc working group to review.	16-0-0-0	43
12.	To approve the proposed process for investigational breakpoints.	13-3-0-0	44
13.	To accept the zoliflodacin disk diffusion (0.5 µg) QC range for *Neisseria gonorrhoeae* ATCC 49226 (18-26 mm).	16-0-0-0	49
14.	To accept the clindamycin disk diffusion (2 µg) QC range for *Bacteroides fragilis* ATCC 25285 (22-28 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update.	16-0-0-0	65
15.	To accept the clindamycin disk diffusion (2 µg) QC range for *Clostridium perfringens* ATCC 13124 (20-26 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update.	15-0-0-1	67
16.	To accept the meropenem disk diffusion (10 µg) QC range for *Bacteroides fragilis* ATCC 25285 (32-39 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update.	15-0-0-1	68
17.	To accept the meropenem disk diffusion (10 µg) QC range for *Clostridium perfringens* ATCC 13124 (34-40 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update.	16-0-0-0	69

Summary of Passing Votes			
18.	To accept the metronidazole disk diffusion (5 µg) QC range for *Bacteroides fragilis* ATCC 25285 (29-36 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update.	16-0-0-0	71
19.	To accept the metronidazole disk diffusion (5 µg) QC range for *Clostridium perfringens* ATCC 13124 (20-26 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update.	16-0-0-0	72
20.	To form an ad hoc working group to reassess the tetracycline *S. aureus* breakpoints.	16-0-0-0	78
21.	To form an ad hoc working group to review cefiderocol breakpoints for *Burkholderia cepacia* complex.	16-0-0-0	81
22.	To accept the aztreonam-avibactam MIC breakpoints (S ≤ 8/4, I 16/4, R ≥ 32/4 µg/mL) for *Stenotrophomonas maltophilia* with a comment stating, "Breakpoints are based on stasis PK/PD target, MIC distributions, and limited clinical data.", a comment stating to not use as monotherapy, and the sponsor dosage (2 g (1.5 g aztreonam + 0.5 g avibactam) IV q6h over 3h).	14-1-1-0	93
23.	To accept the aztreonam-avibactam disk diffusion breakpoints (S ≥ 22, I 17-21, R ≤ 16 mm) for *Stenotrophomonas maltophilia*.	16-0-0-0	98
24.	To accept the gepotidacin MIC breakpoints (S ≤ 16, I 32, R ≥ 64 µg/mL) for Enterobacterales with a U designation to report only on organisms isolated from the urinary tract and the FDA approved dose (1,500 mg PO q12h).	15-1-0-0	133
25.	To accept the gepotidacin disk diffusion breakpoints (S ≥ 12, I 8-11, R ≤ 7 mm) for Enterobacterales.	16-0-0-0	135
26.	To accept the gepotidacin MIC breakpoints (S ≤ 0.25 µg/mL) for *Staphylococcus saprophyticus* with a U designation to report only on organisms isolated from the urinary tract.	16-0-0-0	137
27.	To accept the gepotidacin disk diffusion breakpoints (S ≥ 23 mm) for *Staphylococcus saprophyticus*.	16-0-0-0	138
28.	To place gepotidacin in Table 1 Tier 3 for Enterobacterales.	16-0-0-0	142
29.	To place gepotidacin in Table 1 Tier 4 for *Staphylococcus*.	16-0-0-0	142
30.	To accept the gepotidacin MIC breakpoints (S ≤ 1, I 2, R ≥ 4 µg/mL) for *Neisseria gonorrhoeae* for urogenital isolates only.	16-0-0-0	149
31.	To accept the gepotidacin disk diffusion breakpoints (S ≥ 28, I 23-27, R ≤ 22 mm) for *Neisseria gonorrhoeae*.	16-0-0-0	149
32.	To place gepotidacin in Table 1 Tier 1 for *Neisseria gonorrhoeae*.	16-0-0-0	150
33.	To remove the ceftazidime disk diffusion breakpoints for *Acinetobacter* spp. and add a comment stating, "The disk diffusion breakpoints have been temporarily removed pending additional review."	16-0-0-0	154
34.	To keep the current cefepime MIC breakpoints (S ≤ 8, I 16, R ≥ 32 µg/mL) for *Acinetobacter* spp. and add dosing comment for a dose of 2 g IV q 8 h over 3 hours.	16-0-0-0	159
35.	To remove the piperacillin-tazobactam MIC and disk diffusion breakpoints for *Acinetobacter* spp. with a comment in the Overview of Changes in M100 stating, "temporarily removed pending additional review".	13-3-0-0	166
36.	To remove the piperacillin breakpoints MIC and disk diffusion breakpoints for *Acinetobacter* spp. with a comment in the Overview of Changes in M100 stating, "temporarily removed pending additional review" and remove comment 4 in Table 2B-2.	15-0-0-1	168
37.	To approve the alternative broth microdilution method using CAA medium for testing of the GangaGen Klebicin cocktail against *Klebsiella* spp.	16-0-0-0	175

Summary of Passing Votes			
38.	To remove quinupristin-dalfopristin throughout M100.	15-0-0-1	188
39.	To approve the revised Table 6A pending CLSI style formatting, change to “gentle heat” in new footnote, and remove surotomycin.	16-0-0-0	189
40.	To accept the direct from blood culture tobramycin disk diffusion breakpoints (S ≥ 17, I 13-16, R ≤ 12) for *Acinetobacter* spp. at 16-18 hours and 8-10 hours.	16-0-0-0	191
41.	To remove ceftriaxone direct blood culture disk diffusion breakpoints with no comment and to remove ceftazidime and piperacillin-tazobactam direct blood culture disk diffusion breakpoints for *Acinetobacter* spp. with a comment in the Overview of Changes in M100 stating, “temporarily removed pending additional review.”.	16-0-0-0	192
42.	To approve the trimethoprim-sulfamethoxazole disk diffusion study for beta-hemolytic streptococci as outlined.	16-0-0-0	198
43.	To approve option 1 text for Table 2B-1 regarding *Pseudomonas aeruginosa* isolates that should be considered for carbapenemase testing with the meropenem + ceftolozane-tazobactam-NS testing as the preferred testing and either imipenem + ceftolozane-tazobactam-NS or meropenem + ceftazidime-NS as the less preferred testing to accommodate laboratories that do not test ceftolozane-tazobactam pending wordsmithing.	15-0-1-0	212
44.	To approve the expected resistance definition and introduction text with the addition of “systemic administration” instead of “systemic infection”, consideration to move “(ie, this does not apply for topical agents or inhaled antimicrobial agents)” to the definition, add a sentence regarding ECF and institution antibiograms with the M39 statement, and to change to “this may not apply for topical agents or inhaled antimicrobial agents.”	16-0-0-0	215
45.	To add intrinsic resistance to *Yersinia pseudotuberculosis* for colistin in Appendix B.	16-0-0-0	221
46.	To change *Citrobacter freundii* to *Citrobacter freundii* complex in Appendix B with the footnote stating, “*C. freundii* complex includes *C. freundii, C. braakii, C. gillenii, C. murliniae, C. sedlakii, C. youngae, C. werkmanii, C. cronae,* and *C. portucalensis*”.	16-0-0-0	222

[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 JUNE AST MEETING SUMMARY MINUTES PLENARY 1: Monday, 1 June 2026 7:30 AM – 12:00 PM Central Time (US)	
#	**Description**
1.	**OPENING REMARKS (A. MATHERS)** Dr. Mathers opened the meeting at 7:30 AM Central Time (US) by welcoming the participants to the CLSI meeting in Chicago, Illinois.
2.	**APPROVAL OF MEETING AGENDA**
	A motion to approve the June 2026 meeting agenda was made and seconded. Vote: 15 for, 0 against, 0 abstain, 1 absent (Pass)
3.	**CLSI WELCOME AND UPDATE (J. ADAMS)** Ms. Adams provided an update on CLSI activities.

4.	**AST SUBCOMMITTEE UPDATE (E. GOMEZ)**
	Ms. Gomez provided an update on the AST Subcommittee. The main points included:
	• AST Subcommittee Chairholders and Secretary
	  ○ Chairholder: Amy J. Mathers, MD, D(ABMM)
	  ○ Vice-Chairholder: James S. Lewis II, PharmD, FIDSA
	  ○ Secretary: Alexandra L. Bryson, PhD, D(ABMM)
	• AST Subcommittee Members
	  ○ Kevin Alby
	  ○ April Bobenchik
	  ○ Shelley Campeau
	  ○ Tanis Dingle
	  ○ German Esparza
	  ○ Mark Fisher
	  ○ Joseph Lutgring
	  ○ William Miller
	  ○ Stephanie Mitchell
	  ○ Navaneeth Narayanan
	  ○ Elizabeth Palavecino
	  ○ Virginia Pierce
	  ○ Paula Snippes Vagnone
	  ○ Pranita Tamma
	• Disbanded Ad Hoc Working Groups
	  ○ Breakpoints Working Group
	  ▪ AST ECVs AHWG
	  ▪ Mero-vab OXA 48 AHWG
	  ▪ *Stenotrophomonas* AHWG
	• New Ad Hoc Working Groups
	  ○ Breakpoints Working Group
	  ▪ Gepotidacin AHWG
	  ▪ Nacubactam AHWG
	  ○ Methods Working Group
	  ▪ Klebicins Alternative Method AHWG
	  ▪ *P. aeruginosa* Carbapenemase Testing AHWG
	• AST Subcommittee Working Group Application
	  ○ Apply to participate on an AST Subcommittee working group
	  ○ Applications will be used for consideration of ad hoc working group members
	  ○ Antimicrobial Susceptibility Testing Subcommittees and Working Groups Application
	• Subcommittee Voting Rules
	  ○ Subcommittee chairholder and vice-chairholder vote

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

Subcommittee on Antimicrobial Susceptibility Testing
Chairholder's Rules on Voting

June 2026 AST Subcommittee Roster
16 voting members (including Chairholder and Vice-chairholder)

Committee Status	"Pass" Vote
All members present and voting	16-0; 15-1; 14-2; 13-3; 12-4; 11-5
One member not present or abstaining	15-0; 14-1; 13-2; 12-3; 11-4
Two members not present or abstaining	14-0; 13-1; 12-2; 11-3
Three members not present or abstaining	13-0; 12-1; 11-2
Four members not present or abstaining	12-0; 11-1
If a two-thirds majority are not present	Chairholder's discretion to table until sufficient members are present or an electronic vote is taken.

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

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

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

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

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

- M100 37th Edition is publishing in January 2027.
- M100 Subcommittee Reminders

- January and June 2026 meeting decisions are incorporated into M100 37th edition
- No additional decisions after the June 2026 meeting
- Any pending M100 comments or revisions need to be provided to the Text and Table Working Group Chairholders (April Bobenchik and Shelley Campeau) by end of day on Tuesday
- Meet set review deadlines

- Important Upcoming Dates
  - Second Text and Tables Working Group Review: 23 June to 6 July
  - AST Subcommittee Supplement Review: 13 August to 27 August
- Supplement Review
  - Vote and Comment: AST Subcommittee Chairholders and Members
    - Need 2/3 approval votes
  - Comment Only: AST Subcommittee Secretary, Advisors, and Reviewers
    - Working group members and advisors are AST Subcommittee Reviewers
  - Completed using the Edaptive Platform
  - Focus on tracked changes (new revisions to 37th edition)
  - Comments regarding content outside the tracked changes, email Text and Tables Working Group Chairholders or the applicable working group chairholders
- 2027 Meeting Dates
  - January 2027
    - 24 - 26 January 2027 in St. Petersburg, Florida
    - Virtual Only Working Group Meetings in weeks of 4 January and 11 January 2027
    - Meeting materials due 4 December 2026
- A celebration of dedication was held for James S. Lewis, II, PharmD, FIDSA.
- Dr. Mathers announced Romney M Humphries, PhD, D(ABMM), FIDSA as the 2027 AST Subcommittee Vice-Chairholder.

5. **SUBCOMMITTEE ON VETERINARY SUSCEPTIBILITY TESTING UPDATE (R. BOWDEN)**

Mr. Bowden provided an update on the activities of the Subcommittee of Veterinary Susceptibility Testing (VAST SC). The main points included:

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

Subgroup 1: Breakpoint Comment Review and Harmonization
- Standardize verbiage of comments throughout document – *many updates approved in Jan. 2025*

Subgroup 2: Human Breakpoint Addition and Removal (inclusion/exclusion)
- Develop criteria for evaluating if an M100 breakpoint should or should not be included in VET01S
  tentative criteria presented in Jan. 2025 -> implementation and vote at Jan. 2026 meeting

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

Subgroup 4: *Staphylococcus* subgroup
- Oxacillin BP work continues for *S. pseudintermedius*-group, *S. coagulans*, and *S. schleiferi*
  new oxacillin and beta-lactam data presented at Jan. 2026 meeting

AHWG on Table 1 Reorganization
- *work will begin in ~~late fall 2025~~* ***summer/fall 2026***

- Subgroup 2: Human Breakpoint Addition and Removal (Inclusion/Exclusion)
  - An example of VET01S Tables 2
  - Originally only human breakpoints
  - Now >200 vet-specific breakpoints
  - Vet-specific breakpoints = white boxes
  - Human breakpoints = grey boxes
  - Longterm goal has been to "make the grey go away!"

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

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

working criteria, not a set of new categories for publication

Category 1
Human BPs included for use to detect important mechanisms of resistance that impact clinical efficacy
(ex. PEN for beta-lactamase and OX for methicillin-resistance detection among *Staphylococcus* spp.)

Category 2
Human BPs included because there are no or few vet-specific BPs *and* there is some evidence that the BPs have applicability in predicting clinical outcomes in veterinary species
- (ex. trimethoprim-sulfamethoxazole)

Category 3
Human BP entries included without BPs
- (ex. chloramphenicol BPs, which canine and equine PK data suggests are inapplicable to dogs and horses)

Category 4
Human BP that should be fully removed from VET01S, either because there are a sufficient number of vet-specific BPs, or because there is no clinical applicability to veterinary medicine
- (ex. Enterobacterales BPs for amikacin and streptomycin)

- Human breakpoints to be retained in VET01S-Ed8

Organism Group	
Enterobacterales	Ceftazidime, Imipenem, Levofloxacin, Meropenem, Nitrofurantoin, Trimethoprim-sulfamethoxazole
P. aeruginosa	Ceftazidime, Imipenem, Levofloxacin, Meropenem, Piperacillin-tazobactam
Staphylococcus spp.	Azithromycin, Clarithromycin, Erythromycin, Linezolid, Nitrofurantoin, Rifampin, Trimethoprim-sulfamethoxazole, Vancomycin
Enterococcus spp.	Linezolid, Minocycline, Nitrofurantoin, Vancomycin
Streptococcus spp.	Azithromycin, Clarithromycin, Erythromycin, Trimethoprim-sulfamethoxazole, Vancomycin

- Human breakpoints to be removed from VET01S-Ed8

Organism Group	PK data suggests inapplicable to veterinary species	sufficient number of vet-specific BPs	no clinical applicability to veterinary medicine
Enterobacterales	Amoxicillin-clavulanate Ampicillin Chloramphenicol Doxycycline Minocycline Tetracycline	Cefazolin Amikacin Gentamicin	Kanamycin Streptomycin
Staphylococcus spp.	Chloramphenicol Tetracycline		
Enterococcus spp.	Ampicillin Penicillin Chloramphenicol Doxycycline Tetracycline		Erythromycin Rifampin
Streptococcus spp.	Chloramphenicol Doxycycline Tetracycline	Ampicillin Clindamycin Penicillin	

- Mockup of new VET01S Appendix. Criteria for how to make inclusion and exclusion decisions will be placed in VET02.

Appendix H-2. CLSI Human Breakpoints Included in or Removed from CLSI VET01 Supplements[a,b] Since 2024

Antimicrobial Agent	Table	BP Types	Organisms	Reason for Inclusion of or Removal from VET01S	Supplement Edition (Date of Inclusion or Removal)[a]
Amikacin	2A	ZD, MIC	Enterobacterales	Removed because there are sufficient veterinary species-specific breakpoints.	CLSI VET01S-Ed8 (20XX)
Amikacin	2A	ZD, MIC	Enterobacterales	Revised breakpoints per M100[1]	
Chloramphenicol	2A	ZD, MIC	Enterobacterales	Agent included without breakpoints, noting they should not be used.	CLSI VET01S-Ed8 (20XX)
Gentamicin	2B	ZD, MIC	*Pseudomonas aeruginosa*	Removed because human gentamicin BPs against P. aeruginosa were removed from M100[1]	CLSI VET01S-Ed7 (2024)
Oxacillin	2C-1, 2L	ZD, MIC	*Staphylococcus* spp.	Included for detection of resistance.	CLSI VET01S-Ed8 (20XX)
Penicillin	2C-1, 2L	ZD, MIC	Staphylococcus spp.	Included for detection of resistance.	CLSI VET01S-Ed8 (20XX)

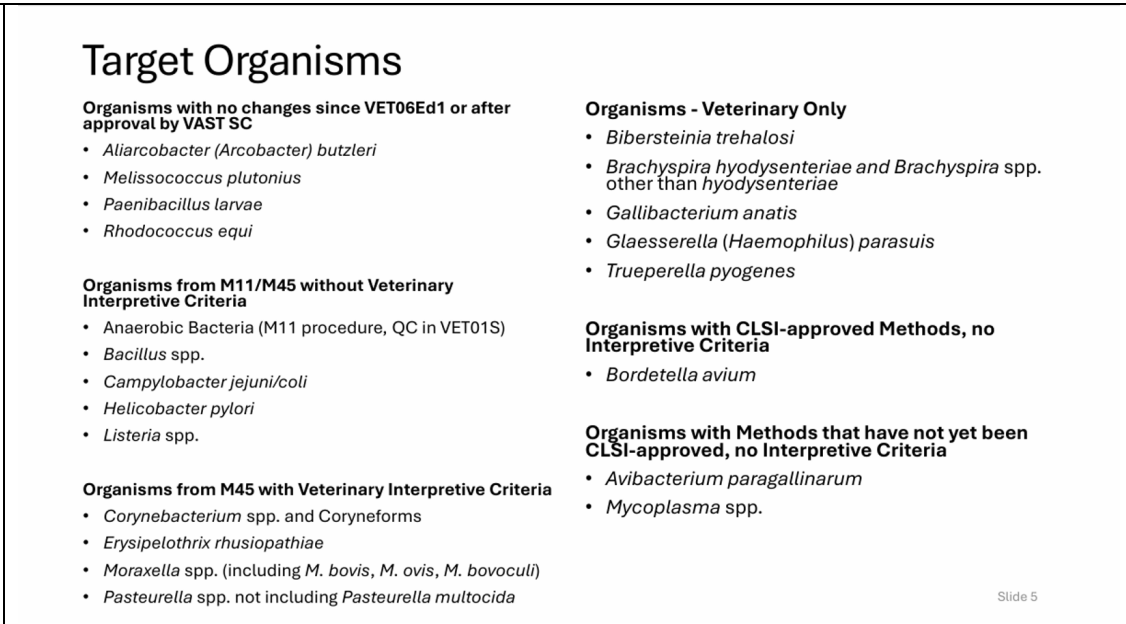
- Subgroup 4: *Staphylococcus* subgroup
  - VET01S currently includes M100 "human" breakpoints for PEN, OX, FOX
  - Also veterinary-specific breakpoints for individual ß-lactam agents
  - This creates confusion: use surrogate agents or test each individual agent?
  - Removed vet-specific ß-lactam MICs, zone diameters, and disk content from Table 2C-1
  - Row entries will remain, with a comment referring users to PEN, OX, and FOX breakpoints

- PEN, OX, and FOX breakpoints will change from grey-shaded ("Human") to a white background with a heading "All Veterinary Species"
- OX BPs: more news coming January 2027

- Working Group on Breakpoint-Setting Requirements (VET02)
  - Most recently published in 2021
  - Project proposal for revision
  - VAST SC voted to form AHWG to investigate and address the following issues before moving forward:
    - Setting breakpoints in the wildtype
    - Setting breakpoints in the absence of adequate QC
  - Voted to remove existing Streptococcus spp. veterinary fluoroquinolone breakpoints for enrofloxacin, marbofloxacin, orbifloxacin, and pradofloxacin due to lack of established QC range for *S. pneumoniae* ATCC 49619
  - Voted to accept proposed oxytetracycline QC ranges
- Working Group on Aquatic Animals (AWG)
  - Next editions of VET03 and VET03S (formerly VET04S) anticipated for late 2026
  - *Flavobacterium columnare* ECVs previously set based on 229 isolates. Now split into 4 species.
  - 120 isolates available for re-identification = no significant differences in MICs -> Table 2C will be renamed to clarify that ECVs apply to *F. columnare*, *F. covae*, and *F. davisii*.
  - *F. oreochromis* excluded due to lack of isolates and overall rarity.
  - Disk diffusion ECVs established:

Organism	Disk Diffusion ECVs
V. parahaemolyticus	Ceftazidime (35°C) Enrofloxacin (35°C) Florfenicol (28°C and 35°C) Gentamicin (28°C and 35°C) Meropenem (35°C) Oxolinic acid (28°C) Oxytetracycline (28°C)

  - Working on methods and QC for streptococci that require temp <35°C, aquaculture *Francisella* spp.
- Working Group on Methods for AST of Infrequently Isolated or Fastidious Bacteria Isolated from Animals (VET06)



Target Organisms

Organisms with no changes since VET06Ed1 or after approval by VAST SC

- *Aliarcobacter (Arcobacter) butzleri*
- *Melissococcus plutonius*
- *Paenibacillus larvae*
- *Rhodococcus equi*

Organisms from M11/M45 without Veterinary Interpretive Criteria

- Anaerobic Bacteria (M11 procedure, QC in VET01S)
- *Bacillus* spp.
- *Campylobacter jejuni/coli*
- *Helicobacter pylori*
- *Listeria* spp.

Organisms from M45 with Veterinary Interpretive Criteria

- *Corynebacterium* spp. and Coryneforms
- *Erysipelothrix rhusiopathiae*
- *Moraxella* spp. (including *M. bovis*, *M. ovis*, *M. bovoculi*)
- *Pasteurella* spp. not including *Pasteurella multocida*

Organisms - Veterinary Only

- *Bibersteinia trehalosi*
- *Brachyspira hyodysenteriae and Brachyspira* spp. other than *hyodysenteriae*
- *Gallibacterium anatis*
- *Glaesserella* (*Haemophilus*) *parasuis*
- *Trueperella pyogenes*

Organisms with CLSI-approved Methods, no Interpretive Criteria

- *Bordetella avium*

Organisms with Methods that have not yet been CLSI-approved, no Interpretive Criteria

- *Avibacterium paragallinarum*
- *Mycoplasma* spp.

Slide 5

- Working Group on Education
  - Working on methods and QC for: streptococci that require temp <35°C, aquaculture *Francisella* spp.
  - Webinars to be revived, coinciding with each new document publication (next up: VET03/VET03S, VET06)

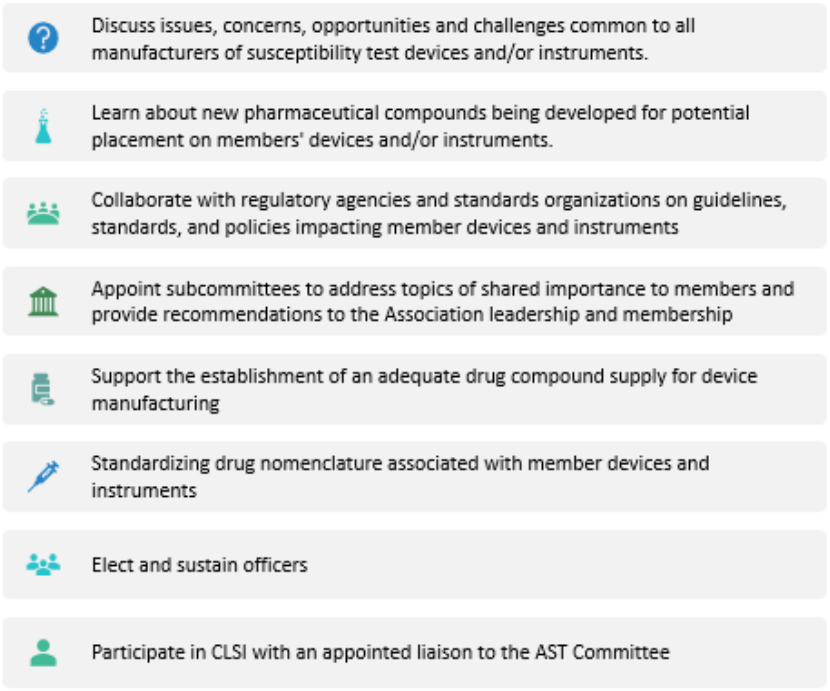
6.	**EXPERT PANEL ON MICROBIOLOGY UPDATE (A. SCHUETZ)**
	Dr. Schuetz provided an update on the activities of Expert Panel on Microbiology. The main points included:
	• 2026 Expert Panel Members
	  ○ Chairholder: Audrey Schuetz
	  ○ Vice-Chairholder: Susie Sharp
	  ○ Kevin Alby
	  ○ Robyn Atkinson Dunn
	  ○ Sara Blosser
	  ○ Tanis Dingle
	  ○ Romney Humphries
	  ○ Antonieta Jimenez Pearson
	  ○ Cristian Lozano
	  ○ Stephanie Mitchell
	  ○ Elizabeth Palavecino
	  ○ Glenn Patriquin
	  ○ Virginia Pierce
	  ○ Amity Roberts
	  ○ Dylan Staats
	  ○ Michael Sweeney
	• 2026 Expert Panel Advisors
	  ○ Sophie Arbefeville
	  ○ April Bobenchik
	  ○ Victoria Campodonico
	  ○ Brandon Hill
	  ○ Kayla Kirby
	  ○ Paula Snippes Vagnone
	  ○ Priyanka Uprety
	  ○ Nicole Zitterkopf
	• CLSI Governance Structure
	  ○ Expert panels are constituted for various technical subject areas. Currently, there are 10—one for each of CLSI's technical areas. Each panel is made up of subject-matter experts who provide advice to document development committees and the consensus council, as needed.
	  ○ The expert panels report to the Consensus Council and may take directives from CLSI's Board of Directors.
	  ○ Document development committees and subcommittees report to the Expert Panels and Consensus Council.
	• Expert Panel Responsibilities
	  ○ Identifying consensus document and product development projects
	  ○ Creating or soliciting the creation of a completed Project Proposal Form for consolidating or dividing a consensus document
	  ○ Reviewing proposals from other sources
	  ○ Endorsing project proposals and presenting them to CC for authorization as applicable
	  ○ Monitoring progress of documents in its technical area

- Reviewing and commenting on consensus documents and products within their area of expertise during the Proposed Draft vote
- Reviewing documents within their area of expertise to recommend reaffirmation, revision, consolidation or division, or withdrawal
- Serving as advisors and subject matter experts for document development groups within its technical area

- Active Microbiology Documents
  - M29: Protection of Laboratory Workers From Occupationally Acquired Infections (Revision)
  - M35: Abbreviated Identification of Bacteria and Yeasts (Revision)
  - M40: Quality Control of Microbiological Transport Systems (Revision)
  - M48: Laboratory Detection and Identification of Mycobacteria (Revision)
  - M52: Verification of Commercial Microbial Identification and AST Systems (Revision)
  - M56: Principles and Procedures for Detection of Anaerobes in Clinical Specimens (Revision)
  - M58: Methods of Identification Using MALDI-TOF MS (Limited Revision)
  - M63: Principles and Procedures for Gram Stains (New)
  - M66: Methods for Active Surveillance of MDROs (New)
  - M68: Validation of Commercial AST Breakpoints (New)
  - M69: Principles of Serologic Testing for Infectious Diseases (New)
  - M70: Laboratory Testing for Lyme Borreliosis (New)
- Additional Microbiology Documents
  - M15: Laboratory Diagnosis of Blood-borne Parasitic Diseases
  - M22: Quality Control for Commercially Prepared Microbiological Culture Media
  - M28: Procedures for the Recovery and Identification of Parasites From the Intestinal Tract
  - M34: Western Blot Assay for Antibodies to *Borrelia burgdorferi*
  - M36: Clinical Use and Interpretation of Serologic Tests for *Toxoplasma gondii*
  - M41: Viral Culture
  - M47: Principles and Procedures for Blood Cultures
  - M50: Quality Control for Commercial Microbial Identification Systems
  - M53: Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection
  - M54: Principles and Procedures for Detection and Culture of Fungi in Clinical Specimens
  - M64: Implementation of Taxonomy Changes
  - M67: Verification of Laboratory Automation in Microbiology
- Recent Publications
  - M64-ED1 Implementation of Taxonomy Nomenclature Changes
    - Published December 2024
  - M67-ED1: Verification of Laboratory Automation of Microbiology
    - Published October 2025
- Upcoming Publications
  - M52-ED2: Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems
  - M66-ED1: Methods for Active Surveillance of Multidrug-Resistant Organisms
  - M68-ED1: Validation of Commercial Antimicrobial Susceptibility Testing Breakpoints
  - GP50-ED1: Clinical Testing for Early Detection and Management of Sepsis

	▪ Joint document with chemistry, hematology, and microbiology • How can you help? ○ Reach out to Microbiology Expert Panel members or advisors with ideas for documents ○ Respond to requests for volunteers for document development committees (DDCs)

7.	**STMA INTRODUCTION AND UPDATE (C. BROWN)**
	Dr. Brown provided an update on the activities of STMA. The main points included:
	• Who is STMA: The **STMA** is an association who represents susceptibility testing manufacturers who have products commercially available under appropriate regulatory clearance (IVDR, CE marking, FDA cleared, etc.).
	• Current Members
	  ○ Affinity Biosensors
	  ○ Astrego Diagnostics AB
	  ○ Beckman Coulter, Inc.
	  ○ bioMérieux, Inc.
	  ○ Bio-Rad Laboratories
	  ○ Hardy Diagnostics
	  ○ Liofilchem, Inc.
	  ○ Mast Group Ltd.
	  ○ Thermo Fisher Scientific
	  ○ Waters (formerly BD Diagnostic Systems)
	• Executive Committee
	  ○ Darcie Carpenter (IHMA)-Executive Director
	  ○ Andrea Ferrell (Waters)-CLSI-STMA Liaison
	  ○ Carrine Brown (Thermo Fisher)-President
	  ○ Anna Klavins (Hardy)-Vice President
	  ○ Karl Ramos (Waters)-Secretary
	• Membership Criteria: General membership in the Association is open to all employees or representatives of manufacturers/distributors of susceptibility test devices and/or instruments that provide an MIC or SIR result. Employees or representatives of pharmaceutical companies, clinical and microbiological research organization, academic institutions, hospital and community microbiology laboratories and other organizations with a globally marketed (FDA cleared, CE Marked, etc.) device related to antimicrobial susceptibility or resistance shall be admitted as members by a vote of current members on a case-by-case basis.
	• STMA Objectives



- STMA Engagement with FDA
  - 2017–2023: Initial discussions began in 2017 through engagement with the FDA and AdvaMed Dx. Meaningful progress was made prior to the pandemic; however, momentum slowed in subsequent years, with the exception of a 2021 FDA/STMA teleconference focused on breakpoint protocols.
  - September 2023: The FDA published updated guidance for AST system devices related to breakpoint and device labeling updates. The guidance formally allows pre-determined change control plans (PCCPs) previously approved in 510(k)s to be applied to legacy devices, a positive step forward.
  - 2024–Present: STMA sought to re-engage the FDA on broader industry-wide topics; however, the Agency indicated that the FDA is unable to provide formal feedback outside of device-specific submissions, placing the responsibility on individual manufacturers.

8. **TEXT AND TABLES WORKING GROUP (A. BOBENCHIK AND S. CAMPEAU)**

TABLE 2A-1 CEFAZOLIN COMMENT

- Do systemic breakpoints apply specifically for these 3 organisms or do they apply to rest of the Enterobacterales?
- Manufacturers may interpret differently and apply breakpoints in different ways
- Comment 17 states "Breakpoints when cefazolin is used for therapy of infections other than uncomplicated UTIs due to *E. coli*, *K. pneumoniae*, and *P. mirabilis*."

Cefazolin	30 µg	≥ 23	–	20-22	≤ 19	≤ 2	–	4	≥ 8	**(17)** Breakpoints when cefazolin is used for therapy of infections other than uncomplicated UTIs due to *E. coli, K. pneumoniae,* and *P. mirabilis.* See comment (15).
Cefazolin (U)[a]	30 µg	≥ 15	–	–	≤ 14	≤ 16	–	–	≥ 32	**(18)** Breakpoints when cefazolin is used for therapy of uncomplicated UTIs due to *E. coli, K. pneumoniae,* and *P. mirabilis.* See additional information in CEPHEMS (ORAL).

- Text and Tables Working Group Discussion and Recommendation
  - The systemic breakpoints do not need to be restricted to only those 3 species, as some have interpreted
    - Cefazolin can be used for other Enterobacterales (that are not intrinsically resistant)
  - The comment got adopted when the urine breakpoint for cefazolin introduced
  - The Oral Cephalosporins AHWG may have decisions that could impact comments of parenteral breakpoints
    - However, the AHWG's work will not be completed/resolved for 37th edition
    - Implications for manufacturers to have to wait another year
  - Options:
    - Remove comment (17) completely, but would users know to look at the U breakpoint for those uUTI use?
    - Amend comment (17) to refer to comment (18) for use in uUTI but not mention the organisms.
    - Amend comment (17) to clarify that it can apply to Enterobacterales (other than just these 3) – Recommended by the Working Group
      - Proposed clarification: (17) Breakpoints when cefazolin is used for therapy of infections due to Enterobacterales, other than uncomplicated UTIs due to *E. coli, K. pneumoniae*, and *P. mirabilis*

SC DISCUSSION (MAIN POINTS)

- Consider removing comment 17 completely.
- The comment was added in 2016, and it was not in prior editions.
- CAP received several questions about this topic.
- The comment does not say anything relevant and should be removed.
- Need to differentiate complicated vs uncomplicated.

A motion to remove the cefazolin comment 17 from Table 2A-1 was made and seconded. Vote: 14 for, 2 against, 0 abstain, 0 absent (Pass)

Against Vote Reasoning:

- Value in keeping the comment. Laboratories will be confused about this change.

MEDIA PREP INSTRUCTIONS IN M100 AND CLSI M07

- We do not provide these details for any other media in M100
- These details/steps for preparation are provided in CLSI M07
- Text and Tables Working Group Recommendation: To remove these general comments

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

(7) To make HTM: Prepare a fresh hematin stock solution by dissolving 50 mg of hematin powder in 100 mL of 0.01 mol/L NaOH with heat and stirring until the powder is thoroughly dissolved. Add 30 mL of the hematin stock solution and 5 g of yeast extract to 1 L of MHA, and autoclave. After autoclaving and cooling, add 3 mL of an NAD stock solution (50 mg NAD dissolved in 10 mL distilled water, filter sterilized) aseptically.

Table 2F. *Neisseria gonorrhoeae* (Continued)

(5) The recommended medium for testing *N. gonorrhoeae* consists of GC agar to which a 1% defined growth supplement (1.1 g L-cystine, 0.03 g guanine HCl, 0.003 g thiamine HCl, 0.013 g para-aminobenzoic acid, 0.01 g vitamin B12, 0.1 g cocarboxylase, 0.25 g NAD, 1 g adenine, 10 g L-glutamine, 100 g glucose, 0.02 g ferric nitrate, 25.9 g L-cysteine HCl [in 1 L water]) is added after autoclaving.

A motion to remove the media prep general comments in Table 2E and Table 2F was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

COMMENT REMOVALS

- Comment 12 states "In the United States for CSF isolates, report only nonmeningitis interpretations. There is not an FDA-approved indication for the use of cefepime for meningitis in the United States."
- Comment 13 states: "In the United States, report only interpretations for nonmeningitis and include the nonmeningitis notation on the report."
- FDA STIC now recognizes M100 for these breakpoints but meningitis is not listed in the FDA drug label for cefepime and it was suggested these comments should be removed
- Discussed whether used clinically in the US (or ex-US) to treat CNS infections and if the meningitis breakpoints could be used
    - It would not be first line drug; in Latin America, cefepime would not be used
    - Cefepime (meningitis) has * asterisk, which is "Other" and not included in Tables 1 (but is that clear enough)
- In general, do not include other indications in Tables 2
- Because these are US-centric comments, may be more appropriate for Table 1, if want to retain

Table 2G. *Streptococcus pneumoniae* (Continued)

Cefepime (meningitis)*	–	–	–	–	≤ 0.5	1	≥ 2	**(12)** In the United States, for CSF isolates, report only nonmeningitis interpretations. There is not an FDA-approved indication for the use of cefepime for meningitis in the United States.
Cefepime (nonmeningitis)	–	–	–	–	≤ 1	2	≥ 4	**(13)** In the United States, report only interpretations for nonmeningitis and include the nonmeningitis notation on the report.

- Text and Tables Working Group Discussion and Recommendation
  - Recommendation: Remove from Tables 2 and add footnote to Table 1G.
  - Proposed footnote in Table 1 G: e. Report only nonmeningitis interpretations for isolates from the CSF and include nonmeningitis notation on the report. Cefepime is not indicated for meningitis in the United States.

SC DISCUSSION (MAIN POINTS)

- Cefepime does get used clinically for meningitis and the comment should remain as is in Table 2G.
- There is PK/PD data to support the meningitis breakpoints.
- CLSI does not have comments in other places that comment on lack of FDA approval.
- Ceftriaxone is used for community acquired meningitis but cefepime is used for neurosurgical cases.
- It is an unusual circumstance that someone would need the meningitis breakpoint plus the tier 4 category indicates to laboratories to not routinely report.
- Cefepime is on panels, so laboratories need guidance.

A motion to remove the cefepime comments 12 and 13 from Table 2G was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

NOTE: After the vote and during review of the table, comments consistent with other drugs in Table 2G that have meningitis/nonmeningitis breakpoints were also added for cefepime (as discussed by the Subcommittee Chairholder and Text and Tables Working Group Chairholders).

AZTREONAM PLUS CEFTAZIDIME-AVIBACTAM BROTH DISK ELUTION METHOD

- With FDA-approval and wider availability of FDA-cleared aztreonam-avibactam devices for this combination, should it be made clear that this method was evaluated for aztreonam and ceftazidime-avibactam susceptibility, but does not necessarily translate for aztreonam-avibactam

Table 3D. Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution Method[1]

Due to limited therapeutic options, there may be a clinical need to assess the *in vitro* activity of the combination of aztreonam and ceftazidime-avibactam t guide therapeutic management of multidrug-resistant gram-negative bacterial infections, especially those caused by MBL producers.

The aztreonam plus ceftazidime-avibactam broth disk elution method was established with limited disk and/or media manufacturers and is considered provisional until additional data are evaluated by CLSI and shown to meet CLSI M23[2] guidance.

NOTE 1: Manufacturer-related issues were observed with different combinations of antimicrobial disks and CAMHB when the aztreonam plus ceftazidime-avibactam broth disk elution method was performed. QC of the method must be performed with every new lot or shipment of reagents to ensure the accuracy of results.

- Text and Tables Working Group Discussion and Recommendation

- Some laboratories (though rare?) may only be able to test using the broth disk elution method, so it would be restrictive if stated that it should not or cannot be used for aztreonam-avibactam susceptibility
- Better to just state what this method was evaluated for and stop there
- This method is still termed as 'provisional'
- If data exist correlating the broth disk elution method with aztreonam-avibactam to know one way or the other, CLSI could review it to provide additional language to this table for inferring (or advising against inferring) susceptibility to aztreonam-avibactam
- In some cases, institutions are using aztreonam plus ceftazidime-avibactam for treatment (eg, not bringing aztreonam-avibactam onto formulary) and wonder if aztreonam-avibactam tests can be used to infer susceptibility
- Recommendation to add the proposed language to clarify: "NOTE 2: This test has only been evaluated for assessing the *in vitro* activity of the combination of aztreonam plus ceftazidime-avibactam; CLSI has not yet evaluated data for correlation to the *in vitro* activity of aztreonam-avibactam."

SC DISCUSSION (MAIN POINTS)

- CLSI needs to be clear about what was and was not looked at, so CLSI should state that this triple drug method should be used for the triple drug therapy, not the double drug therapy.
- There are data to look at the three drugs method vs the two drugs method. CLSI can look at that data in the future.

A motion to add the proposed NOTE 2, "This test has only been evaluated for assessing the *in vitro* activity of the combination of aztreonam plus ceftazidime-avibactam; CLSI has not yet evaluated data for correlation to the *in vitro* activity of aztreonam-avibactam." to Table 3D was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

CEFIDEROCOL-XERUBORBACTAM FOOTNOTE

- Does Cefiderocol-Xeruborbactam Need a Footnote to State Use of CAMHB, not ID-CAMHB?

Cefiderocol[g,h]	0.06-0.5	0.06-0.5	-	-	-	-	-	-	-	-
Cefiderocol-xeruborbactam	-	0.25/4-2/4	-	-	-	-	-	-	0.5/4-4/4	-

g. QC ranges reflect MICs obtained when CAMHB is iron depleted. Chelation is used for iron depletion, which also removes other cations (ie, calcium, magnesium, and zinc). Following this process, cations are added back to concentrations of calcium 20-25 mg/L, magnesium 10-12.5 mg/L, and zinc 0.5-1.0 mg/L.

- Text and Tables Working Group Discussion and Recommendation
  - Cefiderocol (alone) has a footnote regarding iron-depleted CAMHB
  - There are no footnotes for cefiderocol-xeruborbactam, because it uses "normal" CAHMB
  - Working group with reference laboratory and manufacturer representatives said yes that it would be helpful to include a note about this to be clear, especially given there was discussion/confusion when this method was originally presented in January
  - Proposed new footnote for cefiderocol-xeruborbactam: "i. QC ranges reflect MICs obtained using CAHMB (ie, not iron-depleted CAMHB)."

A motion to add the proposed cefiderocol-xeruborbactam footnote, "QC ranges reflect MICs obtained using CAHMB (ie, not iron-depleted CAMHB)." to Table 5A-2 was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

NEW LANGUAGE FOR VALIDATING NEW SYSTEMS

- Instructions for Use discusses Quality Control and Verification
- New CLSI M68 document publishing in Fall 2026 is guidance for validations (reminder CLSI M52 is guidance for verifications)
- Text and Tables Working Group Recommendation: Proposed revisions to incorporate 'validation' and reference to the new CLSI M68

IX. Quality Control, ~~and~~ Verification, and Validation

Recommendations for QC are included in various tables and appendixes. Acceptable ranges for QC strains are provided in Tables 4A-1 through 4B for disk diffusion and Tables 5A-1 through 5E for MIC testing. Guidance for QC frequency, selection of QC strains, and modifications of AST systems is found in Table 4C for disk diffusion and Table 5F for MIC testing. Guidance for troubleshooting out-of-range results is included in Table 4D for disk diffusion and Table 5G for MIC testing. Additional information is available in Appendix C (eg, QC organism characteristics, QC testing recommendations).

The Centers for Medicare & Medicaid Services (CMS) requires laboratories in the United States to perform appropriate QC testing for AST with each lot/batch or shipment of media and antimicrobial agent(s) before, or concurrent with initial use.[7] Thereafter, QC must be performed with each day of testing (subsequently referred to as "daily" QC testing). The specific QC strains required for daily QC testing are not specified by CMS. Other regulatory agencies may have alternative QC requirements. A laboratory in the United States must develop an individualized quality control plan (IQCP) if it wishes to deviate from CMS's daily AST QC requirement. If an IQCP is acceptable to the laboratory's director and accreditation requirements, an IQCP can be designed to reduce AST QC frequency and to determine which QC strains to test. Refer to Appendix I for additional guidance on selection of QC strains and QC testing frequency.

Implementing any new diagnostic test requires verification or establishment of performance specifications (ie, validation).[8] Each laboratory that introduces a new AST system or adds a new antimicrobial agent to an existing AST system must verify or establish that, before reporting patient test results, the system meets performance specifications for that system established by either the manufacturer (ie, verification of an unmodified, FDA-cleared test) or established by the laboratory (ie, validation of a modified, FDA-cleared test or a laboratory-developed test). ~~Verification generally involves testing patient isolates with the new AST system and comparing results to those obtained with an established reference method, a system that has been previously verified, or in some cases the standard disk diffusion method. Testing patient isolates may be done concurrently with the two systems. Alternatively, organisms with known MICs or zone sizes may be used for the verification.~~ Guidance on verification and validation studies are not included in CLSI M100. Other publications describe AST system verification (eg, CLSI M52[9] and Patel et al.[10]) and validation (eg, CLSI M68[11]).

SC DISCUSSION (MAIN POINTS)

- Are there unintended consequences of removing validation? The goal was to remove all this a few years ago so FDA would accept CLSI breakpoints.
- Majority wanted to keep the document as is and not add the proposed text.
- Decision made not to include the new text.

CSF VS CNS INFECTIONS

- Initial question as to whether the "Warning" box pertains to isolates from brain tissue or not?
  - Some laboratory directors interpreting this to mean anything related to CNS
  - Issue of disrupted BBB and impact on CSF concentration is likely beyond CLSI purview
- Brought up question of whether "CSF infections" or "CNS infections" is more accurate?
  - CLSI M27M44S uses both CNS (brain tissue/abscess material) and CSF
- Text and Tables Working Group Recommendation: Keeping as is.

"Warning": Do not report the following antimicrobial agents for bacteria isolated from CSF. These are not the drugs of choice and may not be effective for treating CSF infections caused by the bacteria included in Tables 2A through 2J:

- Agents administered by oral route only
- First- and second-generation cephalosporins and cephamycins
- Doripenem, ertapenem, and imipenem
- Clindamycin
- Lefamulin
- Macrolides
- Tetracyclines

Refer to Glossary I for individual agents within the drug classes listed above.

SC DISCUSSION (MAIN POINTS)

- The intent of the additional language during the interim is understandable, but will likely be more confusing to users and is probably unnecessary.
- Agreement to leave the text as written.

UPDATES RELATED TO OTHER NON-ENTEROBACTERALES

- Updates to footnotes in Table 1B-5 and 2B-5 will be needed once CLSI M45 is published
- Issue is the timing of publications – it is anticipated that CLSI M45 will publish after M100 37th edition – what to do given this interim gap?

Table 2B-5 general comment (2)

Current	Other non-Enterobacterales include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli but exclude *Pseudomonas aeruginosa, Acinetobacter* spp., *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia*. Recommendations for testing and reporting *Aeromonas* spp. (including members of *A. caviae* complex, *A. hydrophila* complex, and *A. veronii* complex), *Burkholderia mallei, Burkholderia pseudomallei,* and *Vibrio* spp. (including *V. cholerae*) are found in CLSI M45.[1]
Proposed	Other non-Enterobacterales include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli but exclude *Pseudomonas aeruginosa, Acinetobacter* spp., *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia* (refer to Tables 2B-2, 2B-3, and 2B-4, respectively), as well as organism groups currently included in CLSI M45[1] (*Aeromonas* spp., *Burkholderia mallei, Burkholderia pseudomallei,* and *Vibrio* spp.) or those under evaluation for inclusion in an upcoming revision to CLSI M45 (*Achromobacter* spp. and *Pseudomonas* spp. Other than *P.* aeruginosa).

Table 1B-5 footnote a (align with edits to Table 2 comment)

Current	Other non-Enterobacterales include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli but exclude than *Pseudomonas aeruginosa, Acinetobacter* spp., *Burkholderia cepacia* complex, and *Stenotrophomonas*. Refer to each respective Table 1 for suggested antimicrobial agents to test and report.
Proposed	Other non-Enterobacterales include ~~*Pseudomonas* spp. and other~~ nonfastidious, glucose-nonfermenting, gram-negative bacilli but exclude *Pseudomonas aeruginosa, Acinetobacter* spp., *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia* (refer to Tables 1B-2, 1B-3, and 1B-4, respectively), as well as organism groups currently included in CLSI M45[1] (*Aeromonas* spp., *Burkholderia mallei, Burkholderia pseudomallei,* and *Vibrio* spp.) or those under evaluation for inclusion in an upcoming revision to CLSI M45 (*Achromobacter* spp. and *Pseudomonas* spp. Other than *P.* aeruginosa). ~~Refer to each respective Table 1 for suggested antimicrobial agents to test and report.~~

SC DISCUSSION (MAIN POINTS)

- Agreement to leave the text as written and revisit when CLSI M45 is published.

NOMENCLATURE UPDATE – *K. PNEUMONIAE* ATCC 700603

- The species has been officially updated by ATCC
- CLSI M64 provides recommendations for implementing nomenclature changes in organisms identified from clinical specimens, not necessarily QC strain names
- *K. pneumoniae* ATCC® 700603 occurs 46 times in M100
- Text and Tables Working Group Discussion and Recommendation
    - Update *K. pneumoniae* ATCC 700603 to *K. quasipneumoniae* ATCC 700603 throughout with note in the Major Additions and/or Revisions section stating that *K. pneumoniae* ATCC 700603 was reclassified by ATCC to *K. quasipneumoniae* ATCC 700603 and M100 reflects this new name
    - Additional edits in Tables 4A-2 and 5A-2 QC Tables to include a footnote for added clarity where users will mostly see this name change

Table 5A-2. MIC QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents[a]

Antimicrobial Agent	QC Organisms and Characteristics									
	QC Strains Not Recommended for Routine QC of β-Lactam Combination Agents				QC Strains Recommended for Routine QC of β-Lactam Combination Agents					
	Escherichia coli ATCC® 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[c,f]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,f]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,f]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,f]
	β-Lactamase Negative	Inducible AmpC	Weak β-Lactamase, mecA Negative		TEM-1	OXA-2 Mutations in OmpK35 and OmpK37	CTX-M-15 OXA-1	KPC-2 TEM SHV	KPC-3 SHV-11 TEM-1	OXA-27
	MIC QC Ranges, µg/mL									

Klebsiella quasipneumoniae ATCC® 700603[c,d,e,f]

f. Formerly *Klebsiella pneumoniae* ATCC® 700603.

SC DISCUSSION (MAIN POINTS)

- Agreement to update *K. pneumoniae* ATCC 700603 to *K. quasipneumoniae* ATCC 700603.

QC INTEGRITY CHECK RANGE FOR CEFIDEROCOL

- Should *K. pneumoniae* BAA-2814 have a range for cefiderocol that is shaded orange? Most (all?) other parent drugs do.
- Send to Quality Control Working Group for consideration.

Table 5A-2. (Continued)

Antimicrobial	QC Organisms and Characteristics									
	QC Strains Not Recommended for Routine QC of β-Lactam Combination Agents				QC Strains Recommended for Routine QC of β-Lactam Combination Agents					
	Escherichia coli ATCC® 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
	β-Lactamase Negative	Inducible AmpC	Weak β-Lactamase, *mecA* Negative		TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37	CTX-M-15 OXA-1	KPC-2 TEM SHV	KPC-3 SHV-11 TEM-1	OXA-27
Cefiderocol[g,h]	0.06-0.5	0.06-0.5	-	-	-	-	-	-	-	-
Cefiderocol-xeruborbactam	-	0.25/4-2/4	-	-	-	-	-		0.5/4-4/4	-

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

INVESTIGATIONAL AGENTS

- New CLSI definition of investigational breakpoints established in June 2025
- Agents currently listed as investigational in M100 do not meet the new definition
- Breakpoints originally set when these agents were new and may not have undergone an extensive CLSI M23-level review process

CEFTIBUTEN

- Background
  - 3rd generation oral cephalosporin (suspension) developed in the 1980s
    - Approved initially in the United States and Europe at 400 mg once-daily
    - Indications: mild-to-moderate infections like acute exacerbation of chronic bronchitis, otitis media, pharyngitis, tonsillitis
    - Oral bioavailability: 75-90%
  - Limited active global usage (based on available literature)
- Discontinuation
  - Discontinued in 2016 in the United States
  - FDA review in 2017 determined that discontinuation was NOT due to issues with safety or efficacy
- CLSI ceftibuten breakpoints set in 1995, not updated since
  - Data used to set original breakpoints unavailable.
  - *Haemophilus* not labeled as Inv.

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm				Interpretive Categories and MIC Breakpoints, µg/mL			
		S	SDD	I	R	S	SDD	I	R
CEPHEMS (ORAL)									
Ceftibuten (U, Inv.)[a]	30 µg	≥ 21	–	18-20^	≤ 17	≤ 8	–	16^	≥ 32

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

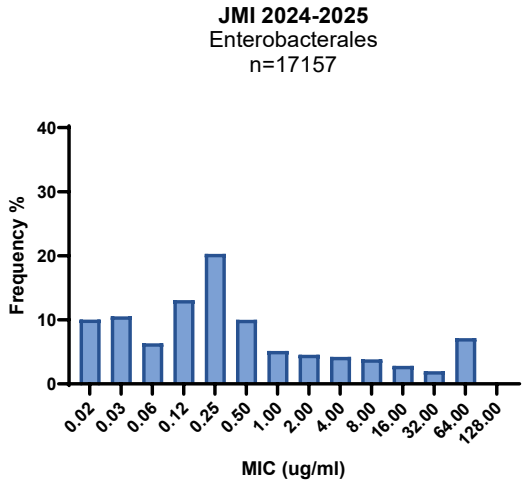
Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm			Interpretive Categories and MIC Breakpoints, µg/mL		
		S	I	R	S	I	R
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)							
Ceftibuten*	30 µg	≥ 28	–	–	≤ 2	–	–

- CLSI and EUCAST ceftibuten breakpoints are different
  - Limited studies show that discordance between in CLSI and EUCAST Enterobacterales breakpoints contributes to high variability in % S/R rates

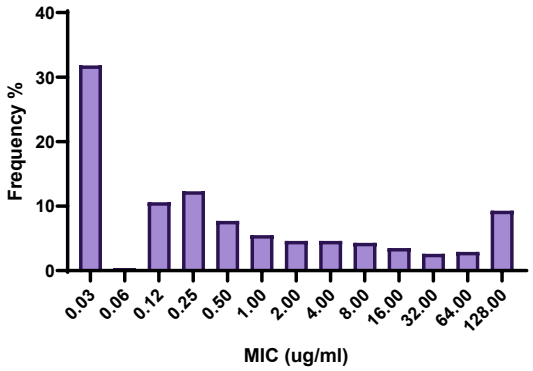
- CLSI: *H. influenzae* and *H. parainfluenzae*; EUCAST: *H. influenzae* only

Organisms		CLSI			EUCAST	
		S	I	R	S	R
Enterobacterales (Urine only)	MIC	≤ 8	16^	≥ 32	≤ 1	> 1
	Disk	≥ 21	18-20^	≤ 17	≥ 23	≤ 23
*Haemophilus**	MIC	≤ 2	-	-	≤ 1	> 1
	Disk	≥ 28	-	-	≥ 25	≤ 25

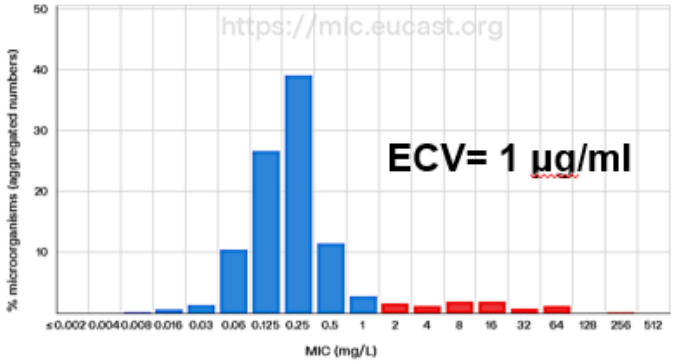
- Ceftibuten and Enterobacterales
  - Drug label states that ceftibuten is inactive against some Enterobacterales (AmpC producers?)
  - Ceftibuten contemporary MIC data



IHMA 2018-2025
Enterobacterales
n=77378



EUCAST
E. coli
n= 5112

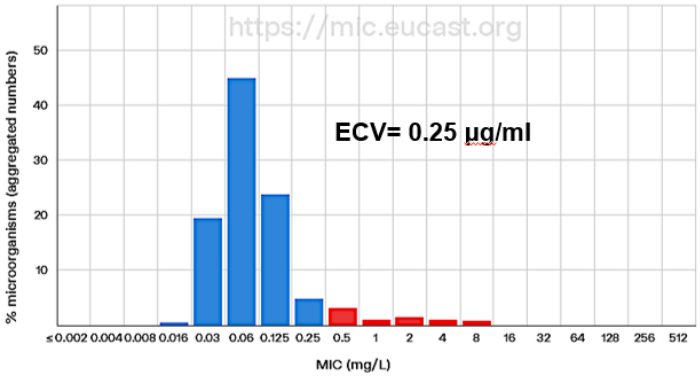


- AmpC producers are less susceptible to ceftibuten

Agent	Species	N	MIC50	MIC90
JMI data	All Enterobacterales	17157	0.25	16
	E. Coli	9582	0.25	8
	K. Pneumoniae	4471	0.06	32
	P. Mirabilis	1288	≤0.015	0.06
	E. Cloacae complex	1432	2	32
	C. Freundii	384	1	>32

- EUCAST ceftibuten data for *Haemophilus influenzae*

n= 444



- Limited contemporary literature, mainly in context of ceftibuten-based novel oral BL/BLI combinations
  - Ceftibuten-avibactam
    - In early development for oral treatment of cUTIs
    - *In vitro* activity against ESBLs, AmpC, KPC, OXA-48
  - Ceftibuten-ledaborbactam
    - For oral treatment of cUTIs, phase 1 complete
    - *In vitro* activity against ESBLs, AmpC, KPC, OXA-48
  - Ceftibuten-xeruborbactam
    - For oral treatment of cUTIs and pyelonephritis, phase 1
    - *In vitro* activity against serine and metallo-ß-lactamases
- Investigational Breakpoints AHWG Discussion and Recommendation
  - Breakpoints set in 1995, not reviewed since and data used to set breakpoints missing
  - Discontinued in U.S. in 2016 and limited global use as a standalone agent
  - Current focus is on ceftibuten-based BL/BLI combinations

- Enterobacterales CLSI breakpoints are higher than EUCAST
- Current CLSI breakpoints (especially for Enterobacterales) may be unreliable
- Motion to remove ceftibuten breakpoints from M100. AHWG Vote: 4-0-0-0.

SC DISCUSSION (MAIN POINTS)

- Do QC ranges need to be removed? No, want to keep the QC ranges because this is used for new combination drugs.

A motion to remove the ceftibuten MIC and disk diffusion breakpoints for Enterobacterales and *Haemophilus* was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

CEFETAMET

- Background
  - 3rd generation oral cephalosporin developed in the 1980s (as oral prodrug cefetamet pivoxil)
    - Approved initially in Japan and parts of Europe
    - Indication: respiratory and urinary tract infections
  - Never FDA or EMA approved
    - Gradually withdrawn from most markets in 90s/ 2000s
    - No evidence of widespread active usage, potential niche use in China/ South Korea
    - No EUCAST breakpoints
- CLSI cefetamet breakpoints set in 1991, not updated since
  - Note: ceftibuten Enterobacterales breakpoints are Urine only but NOT cefetamet

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm				Interpretive Categories and MIC Breakpoints, µg/mL			
		S	SDD	I	R	S	SDD	I	R
CEPHEMS (ORAL)									
Cefetamet (Inv.)	10 µg	≥ 18	–	15-17^	≤ 14	≤ 4	–	8^	≥ 16

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (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
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)							
Cefetamet (Inv.)	10 µg	≥ 18	15-17	≤ 14	≤ 4	8	≥ 16

- Evidence used to set CLSI cefetamet breakpoints
  - Based on relatively solid PK and disk-to-MIC data in 1991 (better than many agents reviewed at the time)
  - Breakpoints initially set for *Neisseria gonorrhoeae* also (same breakpoints as *Haemophilus*) but removed in 2018 along with other cephalosporins

A motion to delete the 6 cephalosporins (Cefoxitin, Cefuroxime, Cefmetazole, Cefotetan, Ceftazidime, Cefetamet) from Table 2F and move them to the archive table on the website was made and seconded. Vote: 12 for - 0 against; 1 absent (Pass).
– Cefepime is being retained due to its better activity. – Cefepime, Ceftizoxime, and Cefpodoxime would be retained as they are used frequently in other countries and their usage needs to be researched more extensively. – The ß-lactamase comments will be reviewed for consideration for revision.

- Limited contemporary literature
  - Prospective, multicenter, randomized, double-blind, phase 4 trial in South Korea 2017-2019
    - Cefetamet 500mg 2x/day vs cefdinir 100mg 3x/day for 2 weeks, n=249 patients
    - Cefetamet 82.4% cure vs 84.7% cefdinir (noninferiority)
  - Follow up clinical trial terminated in 2020
- Investigational Breakpoints AHWG Discussion and Recommendation
  - Initial breakpoints set in 1991 based on review of microbiology, PK, limited clinical data available at the time
  - Have not been reviewed since 1991, limited global use signal
  - No contemporary literature except 2021 phase 4 trial results from South Korea, follow up trial was terminated
  - Motion to remove cefetamet breakpoints from M100. AHWG Vote: 4-0-0-0.

SC DISCUSSION (MAIN POINTS)

- Since there is 2021 publication, does there need to be caution with removing if being used in South Korea? The AHWG consulted with representatives in China and South Korea who said cefetamet is not in use for routine clinical practice.
- CLSI keeps an archived table available on the website.

A motion to remove the cefetamet MIC and disk diffusion breakpoints for Enterobacterales and *Haemophilus* was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

PEFLOXACIN

- Background
  - Developed in 1979, approved in France but not U.S.
    - Most commonly used in veterinary settings
  - Discontinued in France from reimbursement in 2015
    - Enoxacin, lomefloxacin, norfloxacin were also similarly discontinued by 2019
    - Major reduction in usage after this
- 2015: CLSI established pefloxacin as a surrogate agent for *Salmonella* spp. to predict susceptibility to ciprofloxacin

Surrogate Agent Tests

Surrogate Agent	Organisms	Test Description	Results	Table Locations
Pefloxacin	*Salmonella* spp.	Disk diffusion	Predicts reduced susceptibility to ciprofloxacin	2A-2

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

- CLSI: pefloxacin is a back-up option if direct MIC or disk testing is unavailable
  - Note: Pefloxacin disks not available in the U.S.

FLUOROQUINOLONES for *Salmonella* spp.

(13) For testing and reporting of *Salmonella* spp. (including *S. enterica* ser. Typhi and *S. enterica* ser. Paratyphi A-C). Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources.

(14) The preferred test for assessing fluoroquinolone susceptibility or resistance in *Salmonella* spp. is a ciprofloxacin MIC test. A levofloxacin or ofloxacin MIC test can be performed if either agent, respectively, is the fluoroquinolone of choice in a specific facility. If a ciprofloxacin, levofloxacin, or ofloxacin MIC or ciprofloxacin disk diffusion test cannot be done, pefloxacin disk diffusion may be used as a surrogate test to predict ciprofloxacin susceptibility.

(15) No single test detects resistance resulting from all possible fluoroquinolone resistance mechanisms that have been identified in *Salmonella* spp.

Pefloxacin (Inv.) (surrogate test for ciprofloxacin)	5 µg	≥ 24	–	–	≤ 23	–	–	–	–	**(17)** Report results as ciprofloxacin susceptible or resistant based on the pefloxacin result. Pefloxacin will not detect resistance in *Salmonella* spp. due to *aac(6')-Ib-cr*. Pefloxacin disks are not available in the United States. See comment (15).

- EUCAST: pefloxacin can be used to infer fluoroquinolone susceptibility in *Salmonella, Shigella,* Enterobacterales

Fluoroquinolones	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)		
	S ≤	R >	ATU		S ≥	R <	ATU
Pefloxacin (screen only)	NA	NA		5	24[A,B,C]	24[A,B,C]	

2/B. In meningitis, where all fluoroquinolone resistance mechanisms must be excluded, either perform an MIC test, or infer susceptibility from the pefloxacin 5 µg screening test.
3. Fluoroquinolone breakpoints are available for other agents.

A. Tests with a ciprofloxacin 5 µg disk will not reliably exclude all fluoroquinolone resistance mechanisms in *Salmonella* spp. Perform an MIC test, or infer susceptibility from the pefloxacin 5 µg screening test.
C. The pefloxacin screening test can also be used to detect fluoroquinolone resistance mechanisms in other *Enterobacterales* such as *E. coli, K. pneumoniae* and *Shigella* spp.

- Investigational Breakpoints AHWG Discussion and Recommendation
  - CLSI disk breakpoints established in 2015 for surrogate testing only for *Salmonella* species
  - Studies as of 2020 indicate CLSI pefloxacin breakpoints are reliable
  - CLSI disk breakpoints same as EUCAST but EUCAST breakpoints are applicable to all Enterobacterales
  - Motion to keep in M100 and remove from Table 2 and maintain current breakpoints in surrogate agent table only. AHWG Vote: 4-0-0-0.

SC DISCUSSION (MAIN POINTS)

- There are 2020 studies that indicate pefloxacin breakpoints are reliable.
- There are not any breakpoints in the surrogate table, so is there a need to keep in Table 2.

A motion to keep the pefloxacin disk diffusion breakpoints for *Salmonella* spp. and remove the Inv. designation was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

FLEROXACIN

- Background
  - Approved in Japan in 1980s, not in U.S.
    - Also approved in some Western European countries
  - Approval withdrawn in Japan in 1990 due to phototoxicity and other adverse effects
  - Limited market presence, limited global use since except some niche use in China
  - No EUCAST breakpoints
- CLSI fleroxacin breakpoints set in 1993
  - Based on relatively significant microbiology, PK/PD, clinical data

Fleroxacin (Inv.)	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm			Interpretive Categories and MIC Breakpoints, µg/mL		
		S	I	R	S	I	R
Enterobacterales	5 µg	≥ 19	16-18^	≤ 15	≤ 2	4^	≥ 8
Haemophilus		≥ 19	–	–	≤ 2	–	–
Staphylococcus		≥ 19	16-18	≤ 15	≤ 2	4	≥ 8

- CLSI considered removing fleroxacin breakpoints in 2014 due to limited use
  - January 2014

2. Consideration of Fluoroquinolone Listings in Tables 2F and 2G

Text and Tables WG requested that the FQ WG review Tables 2F (*Neisseria gonorrhoeae*) and 2G (*Streptococcus pneumoniae*) FQ antimicrobial agents listed to see if the tables can be cleaned up and unused agents removed.

- Table 2F -- *Neisseria gonorrhoeae*

Agent	FDA Status (Drugs@FDA)
Ciprofloxacin	Active
Enoxacin	Discontinued
Gatifloxacin	Ophthalmic use only
Grepafloxacin	Discontinued
Lomefloxacin	Discontinued
Ofloxacin	Ophthalmic use only
Trovafloxacin	Discontinued
Fleroxacin	Not Available

- FQ WG Vote to retain only ciprofloxacin: 13 Yes, 0 No;
- **Subcommittee Vote: Approved 11-0; 1 Absent**

  - CLSI ultimately retained fleroxacin breakpoints in 2014 since use in China was flagged

f. Previously it was approved to remove from Tables 2F and 2G the following fluoroquinolones: Enoxacin, lomeflox, sparflox fleroxacin, and ofloxacin. It was brought to the subcommittees attention by Dr. Hui Wang that these drugs are still used in China so the subcommittee agreed to not remove these at this time (no vote taken).

  - AHWG asked Dr. Hui Wang and another expert Dr. Hu Fupin who are clinical microbiologists based in China – both confirmed no current active wide usage in China

- Investigational Breakpoints AHWG Discussion and Recommendation
  - Breakpoints set in 1993 based on relatively significant data
  - CLSI discussed removal in 2014, retained due to concern for active use in China
  - AHWG reached out to experts based in China and two confirmed lack of current wide usage in China
  - Motion to remove fleroxacin breakpoints. AHWG Vote: 4-0-0-0.

A motion remove the fleroxacin MIC and disk breakpoints from Enterobacterales, *Haemophilus*, and *Staphylococcus* was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

TEICOPLANIN

- Background
  - Glycopeptide (IV, IM) approved for use in Europe, Asia, Latin America but not U.S.
    - Inhibits peptidoglycan synthesis by binding to PG precursor terminal D-ala-D-ala
    - Indication: serious gram-positive infections
    - cSSTIs/ pneumonia/ cUTIs, bone and joint infections/ endocarditis (12 mg/kg/day)
  - Advantages compared to vancomycin:
    - Better tissue penetration (> 90% bioavailability)
    - Longer half life (> 50 hours) and once daily dosing
    - Lower risk of nephrotoxicity/ ototoxicity vancomycin flushing syndrome
    - Routine serum monitoring not required
- Teicoplanin is active against *vanB* mediated vancomycin resistance

	vanA	*vanB*
Modified target	D-ala-D-Lac	D-ala-D-Ser
Vancomycin MIC	64 to >512	4 to >512
Teicoplanin MIC	16 to 512	0.5-1

- CLSI teicoplanin breakpoints set in 1991 (January 1991 agenda book and data used for this review unavailable)

	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm			Interpretive Categories and MIC Breakpoints, µg/mL		
		S	I	R	S	I	R
Staphylococci	-	-	-	-	≤ 8	16	≥ 32
Enterococci	30 µg	≥ 14	11-13	≤ 10	≤ 8	16	≥ 32

- CLSI versus EUCAST teicoplanin breakpoints for staphylococci

CLSI

Organism	Disk	Disk Breakpoints (mm)				MIC Breakpoints (µg/mL)			
		S	SDD	I	R	S	SDD	I	R
Staphylococci	–	–	–	–	–	≤ 8	–	16	≥ 32

EUCAST

Organism	Disk	Disk Breakpoints (mm)		MIC Breakpoints (µg/mL)	
		S	R	S	R
S. aureus	–	–	–	≤ 2	> 2
CoNS	–	–	–	≤ 4	> 4

CLSI (not updated since 1991)	EUCAST (revised in 2010)
• All staphylococci • No disk breakpoints	• Specific breakpoints for *S. aureus* & CoNS

- CLSI versus EUCAST teicoplanin breakpoints for enterococci

CLSI

Organism	Disk	Disk Breakpoints (mm)				MIC Breakpoints (µg/mL)			
		S	SDD	I	R	S	SDD	I	R
Enterococci	30 µg	≥ 14	–	11-13	≤ 10	≤ 8	–	16	≥ 32

EUCAST

Organism	Disk	Disk Breakpoints (mm)		MIC Breakpoints (µg/mL)	
		S	R	S	R
Enterococci	30 µg	≥ 16	< 16	≤ 2	> 2

CLSI (not updated since 1991)	EUCAST (revised in 2010)
• Enterococci MIC breakpoints are same as staphylococci (higher than EUCAST)	• Enterococci MIC BPs are lower than CLSI

- CLSI does not have teicoplanin breakpoints for streptococci but EUCAST does

CLSI									
Organism	Disk	Disk Breakpoints (mm)				MIC Breakpoints (µg/mL)			
		S	SDD	I	R	S	SDD	I	R
Staphylococci	–	–	–	–	–	≤ 8	–	16	≥ 32
Enterococci	30 µg	≥ 14	–	11-13	≤ 10	≤ 8	–	16	≥ 32

EUCAST					
Organism	Disk	Disk Breakpoints (mm)		MIC Breakpoints (µg/mL)	
		S	R	S	R
S. aureus	–	–	–	≤ 2	> 2
CoNS	–	–	–	≤ 4	> 4
Enterococci	30 µg	≥ 16	< 16	≤ 2	> 2
Strep grp A, B, C, G	30 µg	≥ 15	< 15	≤ 2	> 2
S. pneumoniae	30 µg	≥ 17	< 17	≤ 2	> 2
VGS	30 µg	≥ 16	< 16	≤ 2	> 2

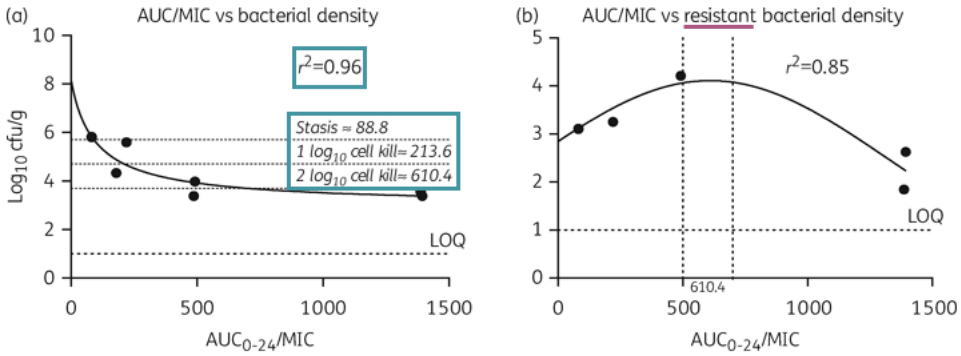
- Microbiology Data Summary

	MIC50	MIC90	ECV	CLSI S/I/R	EUCAST S/R
Staphylococcus aureus	0.5	0.5	2	≤8 / 16 / ≥32	≤2 / >2
CoNS	2	4	16-32		≤4 / >4
Enterococcus faecium	1	>16	2		≤2 / >2
Enterococcus faecalis	0.5	0.5	2		
Streptococcus pneumoniae	0.06*	0.25*	0.25	-	
Streptococcus agalactiae	0.125	0.5	0.5	-	
Streptococcus pyogenes	0.125	0.5	0.25	-	

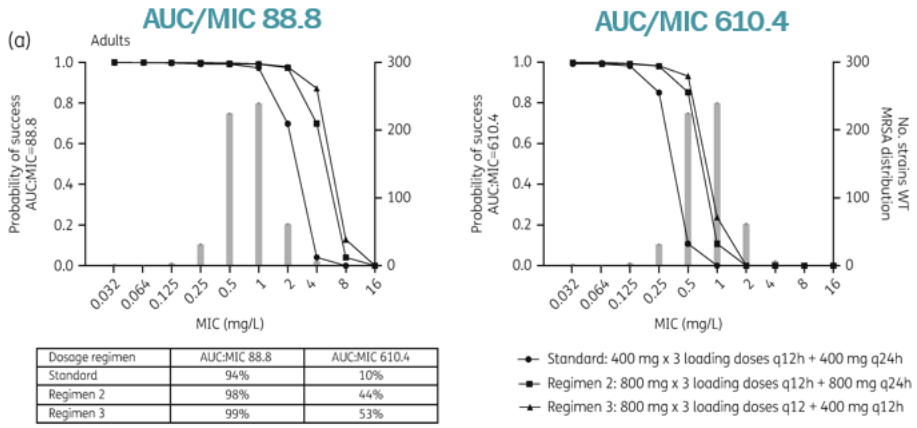
* Calculated from EUCAST data only

- PK/PD
  - J Antimicrob Chemother, Volume 72, Issue 12, December 2017, Pages 3382–3389

Mouse thigh infection model



- Monte Carlo simulations and PTA analysis



Dosage regimen	AUC:MIC 88.8	AUC:MIC 610.4
Standard	94%	10%
Regimen 2	98%	44%
Regimen 3	99%	53%

At *S. aureus* MIC 2, only 60-70% achieve a stasis target at standard dose

- Clinical Data
  - Lower RRs (in favor of teicoplanin) for clinical failure were observed with a lower risk of bias and when treatment was initiated for infections caused by gram-positive organisms
  - Total adverse events (RR, 0.61; 95% CI, 0.50 to 0.74), nephrotoxicity (RR, 0.44; 95% CI, 0.32 to 0.61), and red man syndrome were significantly less frequent with teicoplanin.
- Investigational Breakpoints AHWG Discussion and Recommendation
  - Teicoplanin is still a widely used agent with favorable safety profile compared to vancomycin
  - CLSI teicoplanin breakpoints are higher than EUCAST and may be unreliable

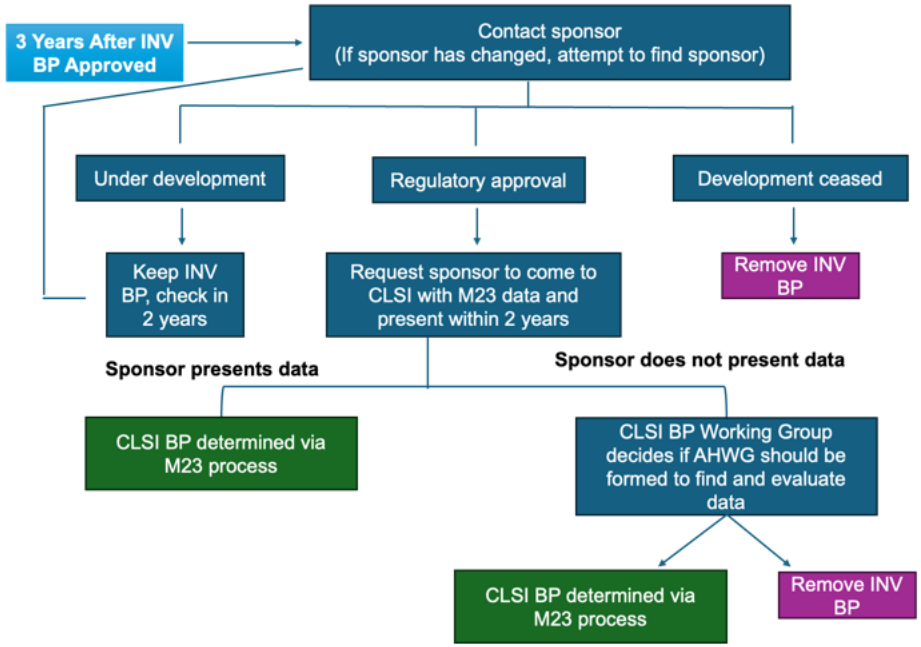
- CLSI breakpoints do not differentiate between *S. aureus* and CoNS
- No CLSI streptococci breakpoints
- Teicoplanin has good activity against streptococci, lowest ECVs
- Motion to create ad hoc working group (under Breakpoints Working Group) to review teicoplanin breakpoints and remove from M100 in the meantime. AHWG Vote: 4-0-0-0.
- Second alternative scenario: AHWG was also okay with staphylococci breakpoints being removed only, leaving enterococci in until AHWG reviews breakpoints

SC DISCUSSION (MAIN POINTS)

- There was a concern not to leave current breakpoints for the CoNS because they are not accurate.
- It is important to differentiate *vanA* and *vanB*.
- Text and Tables Working Group does not remember why it got the "investigational" designation. It may have been intended to remove the "investigational" previously and was never done.
- CLSI needs to be consistent, there are other breakpoints under review that have not removed.
- The task was to clean up the investigational breakpoints.
- The process moving forward is not to put the investigational drugs in the M100.
- Consider a comment that says breakpoint is "pending review"

A motion to remove the teicoplanin MIC and disk diffusion breakpoints for *Staphylococcus* and *Enterococcus*, add a comment that states "temporarily removed pending further review", remove the Inv. designation, and form an ad hoc working group to review was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

PROPOSED CLSI PROCESS FOR HANDLING INVESTIGATIONAL BREAKPOINTS



SC DISCUSSION (MAIN POINTS)

- Sponsors should be responsible for coming back to CLSI.
- Example for concern: cefepime/zidebactam received a different FDA approved breakpoint compared to what CLSI has for the Inv breakpoint.
- Should the term Inv be removed from M100 since all the breakpoints were removed? The definition will remain in M100 for now. Text and Tables Working Group will review.
- The process will be added to the next edition of CLSI M23.

A motion to approve the proposed process for investigational breakpoints was made and seconded. Vote: 13 for, 3 against, 0 abstain, 0 absent (Pass)

Against Vote Reasoning:

- Concerns with some of the details of the process.
- Leaves opportunity for sponsors to not return and leave work for CLSI.

10. **QUALITY CONTROL WORKING GROUP (S. CULLEN, E. GARRETT, AND C. PILLAR)**

TIER 2 QC

ZOLIFLODACIN

- Background

Drug: Zoliflodacin 0.5 µg	**Abbreviation** (Glossary II & III): ZFD	**Previous ID:** AZD0914; ETX0914 CAS # : 1620458-09-4
Solvent (Table 6A): DMSO	**Diluent (Table 6A):** Water	**Preparation (Table 6C combination agents):** NA
Route of administration (Glossary II): PO	**Class (Glossary I & II):** Spiropyrimidinetrione	**Subclass (Glossary I & II):** NA
Study Report by: IHMA	**Pharma Co:** Innoviva Specialty Therapeutics and GARDP	**Control Drugs:** Azithromycin 15 µg, Media evaluation with ciprofloxacin 5 µg disk
Additional Information/ M23 requirements	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, etc): NA for disk diffusion • **ISO/TS 16782 assessment of Tier 2 study materials**: Yes, confirmed	
Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):**	
Discussion	• Growth issues were observed with media 2. • Disk mfg: Liofilchem and MAST. Media manufacturers: Remel, Criterion, and BD BBL. • Colony counts for *N. gonorrhoeae* ATCC 49226 range 3.7 x 10^7 to 2.0 x 10^8 , average was 1.1 x 10^8 • Laboratories 7 and 9 were identified as outliers for mode only by Rangefinder so data is included in the analysis. • Despite a favourable media evaluation, some media variability was noted with medium lot 1. Lot 1 consistently produced zone diameters with mean, median and mode values 2 mm larger than the in-house prepared media lots. Laboratory variability was also observed: mode 20 mm (3 labs), mode 21 mm (4 labs), mode 23 mm (2 labs). This may be attributed to difficulty discerning zone margins. • Frequent out-of-range (OOR) QC results for AZM on media lot 1 and Laboratories 5 and 8 prompted further analysis of the ZFD disk data. Despite these QC discrepancies, the recommended Rangefinder and Gavan statistic ranges remained stable across all subsets, including those where failed replicates or specific laboratories were removed.	

Medium Lot, Disk Lot, and Interlaboratory Comparisons of Zoliflodacin Disk Diffusion Testing Results for *N. gonorrhoeae* ATCC 49226, all data

16															
17			1	1							1				1
18			2	2						1	1				2
19	1	16	17	12	22	2		1	8	7	15			1	34
20	9	57	53	45	74	24	11	19	11	22	21		10	1	119
21	21	49	42	59	53	26	20	11	13	8	9		19	6	112
22	29	30	22	37	44	7	7	10	9	9	9	7	11	12	81
23	48	21	28	52	45	1	11	11	11	7	4	24	6	22	97
24	42	6	10	36	22		6	7	4	4		17	9	11	58
25	22	1	4	19	8		2	1	3	2		10	3	6	27
26	7			5	2		3					2	2		7
27	1		1	2					1					1	2
28															
TOTAL	180	180	180	270	270	60	60	60	60	60	60	60	60	60	540
Mean	23	21	21	22	21	21	22	22	22	21	20	24	22	23	22
Median	23	21	21	22	21	21	21	21	21	21	20	23	22	23	22
Mode	23	20	20	21	20	21	21	20	21	20	20	23	21	23	20
Range	9	7	11	11	8	5	7	7	9	8	7	5	7	9	11

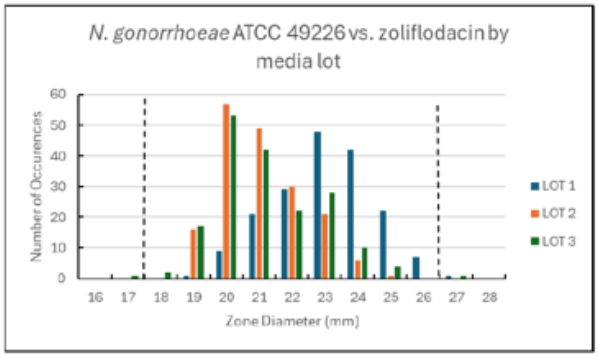
a-Lab included in analysis; identified as outlier for mode only by Rangefinder

Medium lot 1, Remel; medium lot 2, Criterion; medium lot 3, BD/BBL medium lot 3, BD/BBL

All lab median	22
Median of ranges {MR}	7
1/2/MR rounded up {R}	4
All lab median +/- R	18-26

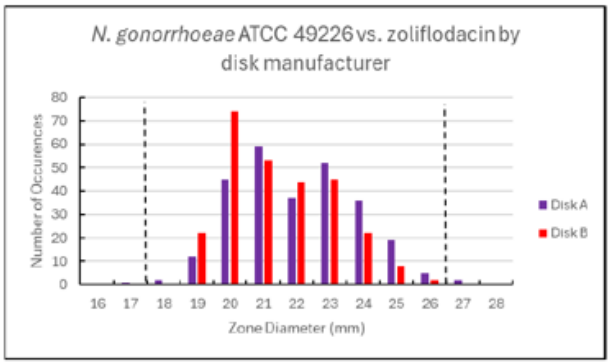
Gavan Statistic	18-26	9	99.4% (537/540)
Rangefinder	18-26	9	99.4% (537/540)
	19-26	8	99.1% (535/540)
	19-25	7	97.8% (528/540)

Figure 1. Distribution of Zoliflodacin Disk Diffusion Zones (mm) for *N. gonorrhoeae* ATCC 49226 by Medium Lot, all data



Black dash line represents calculated range from Rangefinder and Gavan Statistic.
Medium lot 1, Remel; medium lot 2, Criterion; medium lot 3, BD/BBL

Figure 2. Distribution of Zoliflodacin Disk Diffusion Zones (mm) for *N gonorrhoeae* ATCC 49226 by Disk Lot, all data



Black dash line represents calculated range from Rangefinder and Gavan Statistic.

Medium Lot, Disk Lot, and Interlaboratory Comparisons of Zoliflodacin Disk Diffusion Testing Results for *N. gonorrhoeae* ATCC 49226, Lab 5 and Lab 8 excluded

16													
17			1	1						1			1
18			1	1						1			1
19	1	15	11	7	20	2		1	8	15		1	27
20	9	41	37	36	51	24	11	19	11	21		1	87
21	19	36	30	48	37	26	20	11	13	9		6	85
22	19	23	19	25	36	7	7	10	9	9	7	12	61
23	39	19	26	43	41	1	11	11	11	4	24	22	84
24	30	5	10	29	16		6	7	4		17	11	45
25	17	1	4	15	7		2	1	3		10	6	22
26	5			3	2		3				2		5
27	1		1	2					1			1	2
28													
TOTAL	140	140	140	210	210	60	60	60	60	60	60	60	420
Mean	23	21	21	22	22	21	22	22	22	20	24	23	22
Median	23	21	21	22	21	21	21	21	21	20	23	23	22
Mode	23	20	20	21	20	21	21	20	21	20	23	23	20
Range	9	7	11	11	8	5	7	7	9	7	5	9	11

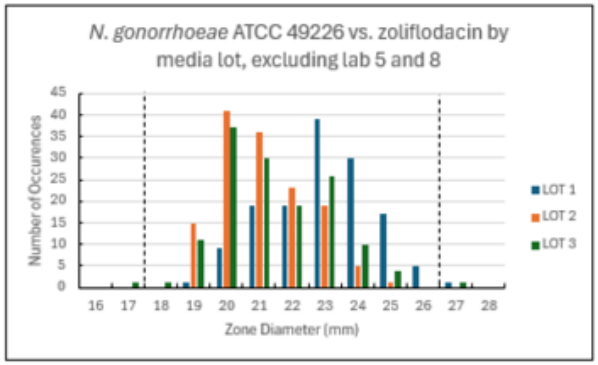
a-Lab included in analysis; identified as outlier for mode only by Rangefinder

Medium lot 1, Remel; medium lot 2, Criterion; medium lot 3, BD/BBL medium lot 3, BD/BBL

All lab median	22
Median of ranges {MR}	7
1/2/MR rounded up {R}	4
All lab median +/- R	18-26

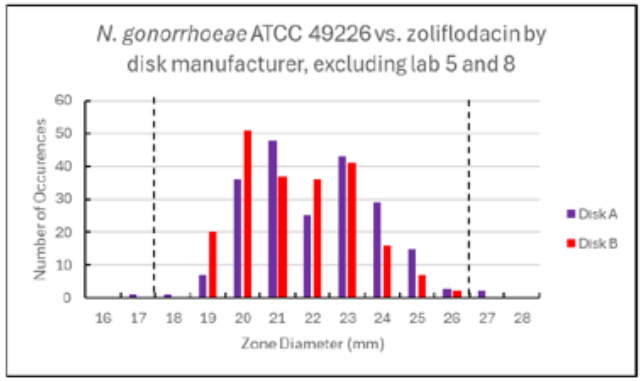
Gavan Statistic	18-26	9	99.3% (417/420)
Rangefinder	18-26	9	99.3% (417/420)
	19-26	8	99.0% (416/420)
	19-25	7	97.9% (411/420)

Figure 7. Distribution of Zoliflodacin Disk Diffusion Zones (mm) for *N. gonorrhoeae* ATCC 49226 by Medium Lot, Lab 5 and 8 excluded



Black dash line represents calculated range from Rangefinder and Gavan Statistic.
Medium lot 1, Remel; medium lot 2, Criterion; medium lot 3, BD/BBL

Figure 8. Distribution of Zoliflodacin Disk Diffusion Zones (mm) for *N. gonorrhoeae* ATCC 49226 by Disk Lot, Lab 5 and 8 excluded



Black dash line represents calculated range from Rangefinder and Gavan Statistic.

- Proposed Disk Diffusion QC Ranges

Drug Name:	Zoliflodacin 0.5 µg	**Votes:**	10/0/1/1

QC Strain	Range	% In	Median	Mm	Media	Disk	Labs	Gavan	Range Finder	Comments
Neisseria gonorrhoeae ATCC 49226	18-26	99.4%	22	9	2@21. 1@23	21, 22	1@2-. 5@21. 1@22. 2@23	18-26, 9mm, 99.4%	18-26, 9mm, 99.4%	Media and lab variabily 9mm range is similar to other size of other QC ranges for this organism
~~*Neisseria gonorrhoeae* ATCC 49226~~	~~19-26~~	~~99.1%~~	~~22~~	~~8~~						~~More stringent to address inter-lab and media related variabilty~~
~~*Neisseria gonorrhoeae* ATCC 49226~~	~~19-25~~	~~97.8%~~	~~22~~	~~7~~						~~More stringent to address inter-lab and media related variabilty~~

A motion to accept the zoliflodacin disk diffusion (0.5 µg) QC for Neisseria gonorrhoeae ATCC 49226 (18-26 mm) was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

TIER 3 MIC QC

QC Strain	Agent	Current Range	Action Recommended	Concern/Analysis	Reported
E. coli ATCC 25922	Aztreonam/ avibactam	0.03/4-0.12/4	No change with new data, shoulder at 44% and <1% out of QC. No change in range 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. Jun 2026: Added 106 results from 2024-2026 to dataset from lab 1 which initially had 280 results from 2019-2023; no change from prior analysis, shoulder at 0.12/4 at 44% and 0.5% out of QC.	21-Jun

QC Strain	Agent	Current Range	Action Recommended	Concern/Analysis	Reported
K. pneumoniae ATCC BAA-1705	Meropenem	8-64	Signal not observed at two other labs, no new data provided for review. 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. Jun 2026: no new data	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. Jun 2026: no new data	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 Jun 2026: no new data	23-Jun

TIER 3 DISK DIFFUSION QC

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.

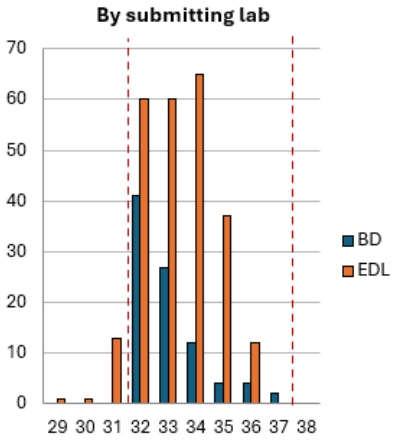
QC Strain (ATCC)	Antimicrobic	Current Range	Action Recmd	Concern	Update	Date Reported
E. coli NCTC 13353	Ceftibuten 30 µg	15-23	Continue to monitor until January 2027. Request additional data.	Zone diameters in the lower part of range and out of range	June 2025: No additional data. Jan 2026: No additional data Jun 2026: No additional data	Jan-24
E. coli ATCC 25922	Aztreonam-avibactam 30-20 µg	32-38	Request additional data. EUCAST may be moving range. Analyze with tier 2 data.	Zone diameters in the lower part of range and out of range. Concern for differences in disk manufacturer, particularly Liofilchem and Mast (also demonstrated by PsA 27853).	Jan 2026: Data received from 2 labs. Jun 2026:Additional data from EDL. EDL considering 31-37. Tier 3 QC median is 2 mm lower than Tier 2.	Jan-26
P. aeruginosa ATCC 27853	Meropenem-vabobactam 20-10 µg	29-35	Request additional data.	Differences in performance by disk manufacturer	Jan 2026: Data received from 2 labs Jun 2026:Additional data from EDL. 99.5% of tier 3 data in range. Data did not include Oxoid disk, which was an outlier in previous tier 3 data	Jan-26
S. aureus ATCC 25923	Debio 1452	20-27	Request additional data.	Differences in performance by disk and media manufacturer	Jan 2026: no data requested Jun 2026: no data requested	Jan-26
S. aureus ATCC 29213	Debio 1452		Request additional data.	Differences in performance by disk and media manufacturer	Jun 2026: no data requested	Jun-26

AZTREONAM-AVIBACTAM *E. COLI* ATCC 25922

Aztreonam-avibactam
E. coli ATCC 25922

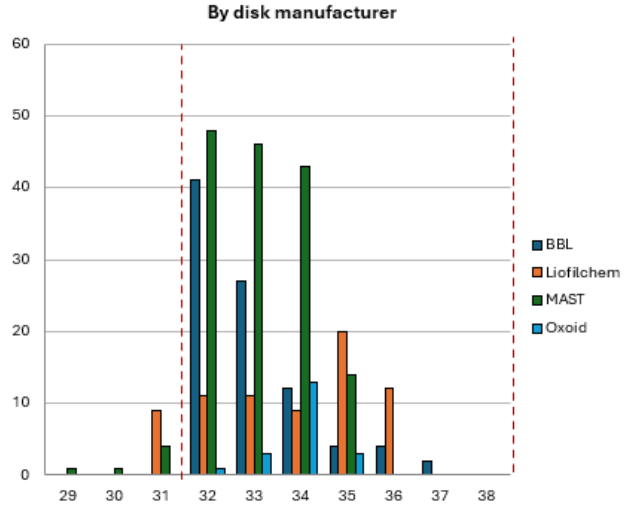
Tier 3

QC range: 32-38		Submitter		Disk				Media				
Diameter	total	BD	EDL	BBL	Liofilchem	MAST	Oxoid	BBL	Bio-Rad	Oxoid	Biomerieux	Remel
29	1		1			1			1			
30	1		1			1				1		
31	13		13		9	4		1	1	11		
32	101	41	60	41	11	48	1	58	11	31	1	
33	87	27	60	27	11	46	3	44	12	28	3	
34	77	12	65	12	9	43	13	42	9	19	5	2
35	41	4	37	4	20	14	3	18	6	2	5	10
36	16	4	12	4	12			6	3	1	2	4
37	2	2	0	2				2				
38	0											
total	339	90	249	90	72	157	20	171	43	93	16	16
Mean	32.93	32.99	33.33	32.99	33.78	33.05	33.90	32.92	33.30	32.68	34.25	35.13
SD	1.24	1.24	1.32	1.24	1.68	1.10	0.72	0.93	1.44	1.10	1.13	0.62
+2SD	35.41	35.47	35.97	35.47	37.14	35.24	35.34	34.79	36.18	34.87	36.50	36.36
-2SD	30.45	30.51	30.69	30.51	30.42	30.86	32.46	31.06	30.42	30.49	32.00	33.89
Median	33	33.00	33.00	33.00	34.00	33.00	34.00	33	33	33	34	35



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

Tier 3



		Disk			
Diameter	total	BBL	Liofilchem	MAST	Oxoid
29	1			1	
30	1			1	
31	13		9	4	
32	101	41	11	48	1
33	87	27	11	46	3
34	77	12	9	43	13
35	41	4	20	14	3
36	16	4	12		
37	2	2			
38	0				
total	339	90	72	157	20
Mean	32.93	32.99	33.78	33.05	33.90
SD	1.24	1.24	1.68	1.10	0.72
+2SD	35.41	35.47	37.14	35.24	35.34
-2SD	30.45	30.51	30.42	30.86	32.46
Median	33	33.00	34.00	33.00	34.00

- Difference between MAST/Liofilchem also seen with *P. aeruginosa* ATCC 27853

***E. coli* ATCC 25922 and aztreonam-avibactam 30-20 µg**

Agar Disk	TOTAL Lio	TOTAL Mast
31		1
32		3
33	2	8
34	8	19
35	18	9
36	12	
37		
38		

***P. aeruginosa* ATCC 27853 and aztreonam-avibactam 30-20 µg**

Agar Disk	TOTAL Lio	TOTAL Mast
24		1
25		9
26		23
27	2	7
28	16	
29	22	
30		

Data from EDL (2nd data set, Apr 2026)
5 Media manufacturer – no media effect

- Tier 2 Data – BD and MAST disks

Drug Name:	Aztreonam-Avibactam			
Table 6 information:	Solvent: NA (used commercial disks)	Diluent: NA (used commercial disks)		
Table 6C information if applicable:	Combination Tested:30/20 µg disks	Preparation: N/A		Example:
Glossary information:	Class:monobactam-β-lactamase inhibitor	Subclass: N/A	Agent Abbreviation: TBD	Route of Administration: TBD

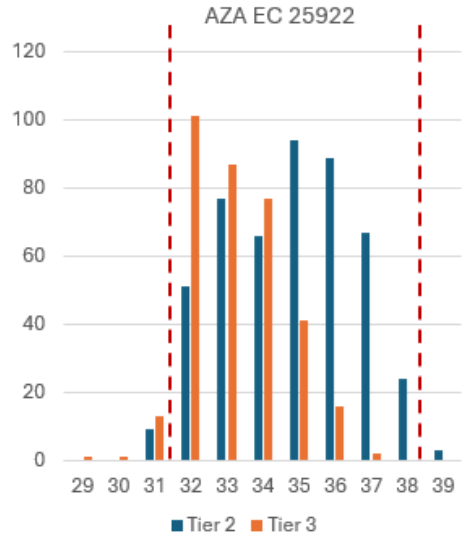
QC Strain (ATCC)	QC Range Approved mm or dil	WG Vote: Y/N/A/NP	% in Range	Mode/ Median	# mm or dilutions	Shoulder %	Footnote to add with drug/range	Variability/Comments
E. coli 25922	32-38	9/0/2/2	97.5	35	7	NA		Proposed 32-38 (97.5%, 7mm) or 31-38 (99.4%, 8mm) RF: 30-38mm (9mm)
P. aeruginosa 27853	24-30	9/0/2/2	100	27	7	NA		Original proposal 25-29 (98.5%, 5mm). Concern 5mm range too narrow RF: 21-32 (12mm)
E. coli 35218	31-38	9/0/2/2	98.5	35	8	NA		Original proposal 32-38 (96.5%, 7mm) Lab median 33-37. Media B 6% of results @ 31 RF: 31-39 (9mm)
K. pneumoniae 700603	26-32	9/0/2/2	99.2	29	7	NA	Add footnote d from Table 4A and f from 5A	RF: 26-32 (7mm)

Additional Information (eg if to be added to Troubleshooting Guide provide info for this):
Study conducted by CMI for AstraZeneca Pharmaceuticals (Report ATM-AVI-M2-026)
No significant media or disk variability observed. Some lab to lab variability.

Proposed and approved by QCWG by email vote (8 for, none against, 6 no response when minutes were finalized) after the AST Subcommittee meeting (not discussed during the AST meeting) to add footnote currently used for other avibactam combinations on the MIC and Disk QC tables. Also recommended revising the statement to remove list of drugs to avoid need to update as new compound are added.

- Tier 2 and Tier 3 Combined

	Tier 2	Tier 3	Total
28			0
29		1	1
30		1	1
31	9	13	22
32	51	101	152
33	77	87	164
34	66	77	143
35	94	41	135
36	89	16	105
37	67	2	69
38	24		24
39	3		3
total	480	339	819
Mean	34.79	33.24	34.15
SD	1.81	1.31	1.79
+2SD	38.42	35.85	37.73
-2SD	31.16	30.63	30.56
Median	35.00	33.00	34.00
% captured	97.5%	95.6%	96.7%



		Tier 3	Tier 2+3	
QC range	range	% captured	% captured	Notes
30-36	7	99.1	88.2	Tier 3: Rangefinder; Gavan
30-37	8	99.7	96.6	
30-38	9	99.7	99.8	T2+3:Rangefinder
31-37	7	99.4	96.5	T2+3:Gavan; EDL*
31-38	8	99.4	99.5	
32-38	7	95.6	96.7	Current range

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

- Quality Control Working Group Discussion and Recommendation
  - No action taken on aztreonam-avibactam *E. coli* ATCC 25922
  - Not a commonly used strain for this drug
  - Wait to see if there is more end-user data
  - See if there is a disk manufacturer differences for *K. pneumoniae* ATCC 700603
  - Discuss further with EUCAST

MEROPENEM-VABORBACTAM *P. AERUGINOSA* ATCC 27853

Meropenem-vaborbactam

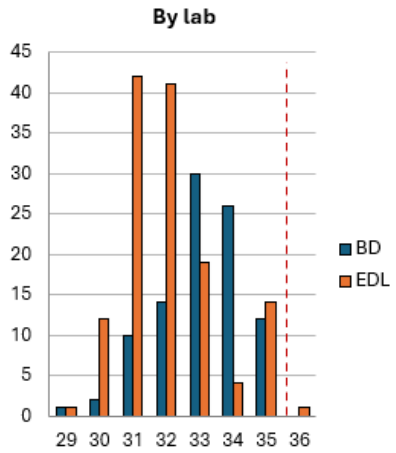
***P. aeruginosa* ATCC 27853**

QC range: 29-35

Tier 3

		Submitter		Disk				Media				
Diameter	total	BD*	EDL	BBL*	MAST	Bio-Rad	Oxoid	BBL	Oxoid	Biomerieux	Bio-rad	Remel
29	2	1	1	1	1			2				
30	14	2	12	2	12			9	2	1	1	1
31	52	10	42	10	30	12		20	5	7	12	8
32	55	14	41	14	25	16		23	17	7	3	5
33	49	30	19	30	5	12	2	37	9	1		2
34	30	26	4	26	0		4	28	2			
35	26	12	14	12	0		14	20	6			
36	1		1				1		1			
total	229	95	134	95	73	40	21	139	42	16	16	16
Mean	32.43	33.00	32.03	33.00	31.29	32.00	34.67	32.74	32.62	31.50	31.13	31.50
SD	1.47	1.31	1.44	1.31	0.87	0.78	0.73	1.51	1.43	0.73	0.50	0.82
+2SD	35.36	35.63	34.91	35.63	33.04	33.57	36.13	35.76	35.48	32.96	32.13	33.13
-2SD	29.50	30.37	29.15	30.37	29.54	30.43	33.21	29.72	29.76	30.04	30.13	29.87
Median	32.00	33.00	32.00	33.00	31.00	32.00	35.00	33.00	32.00	31.50	31.00	31.00

*BD MEV disc is investigational

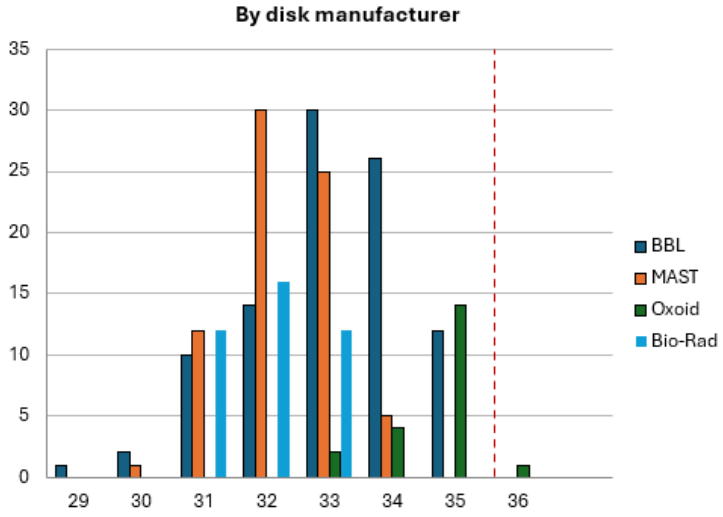


Meropenem-vaborbactam
***P. aeruginosa* ATCC 27853**
QC range: 29-35

Tier 3

		Submitter		Disk			
Diameter	total	BD*	EDL	BBL*	MAST	Bio-Rad	Oxoid
29	2	1	1	1	1		
30	14	2	12	2	12		
31	52	10	42	10	30	12	
32	55	14	41	14	25	16	
33	49	30	19	30	5	12	2
34	30	26	4	26	0		4
35	26	12	14	12	0		14
36	1		1				1
total	229	95	134	95	73	40	21
Mean	32.43	33.00	32.03	33.00	31.29	32.00	34.67
SD	1.47	1.31	1.44	1.31	0.87	0.78	0.73
+2SD	35.36	35.63	34.91	35.63	33.04	33.57	36.13
-2SD	29.50	30.37	29.15	30.37	29.54	30.43	33.21
Median	32.00	33.00	32.00	33.00	31.00	32.00	35.00

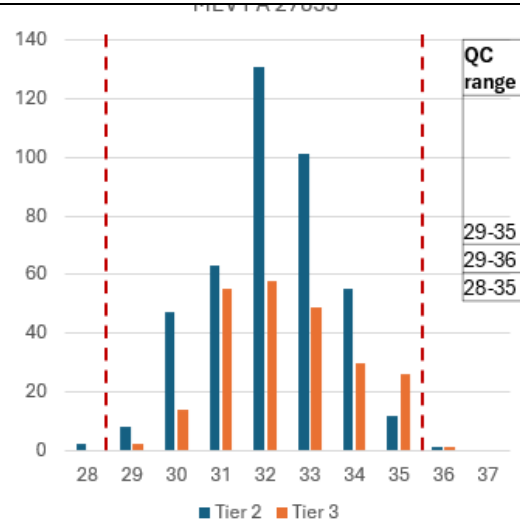
*BD MEV disc is investigational



- Tier 2 and Tier 3 Combined

	Tier 2	Tier 3*	Total
28	2		2
29	8	2	10
30	47	14	61
31	63	52	118
32	131	55	189
33	101	49	150
34	55	30	85
35	12	26	38
36	1	1	2
37			
total	420	229	655
Mean	32.15	32.43	32.25
SD	1.37	1.47	1.41
+2SD	34.90	35.36	35.07
-2SD	29.40	29.50	29.42
Median	32.00	32.00	32.00
% captured	99.3%	99.6%	99.4%

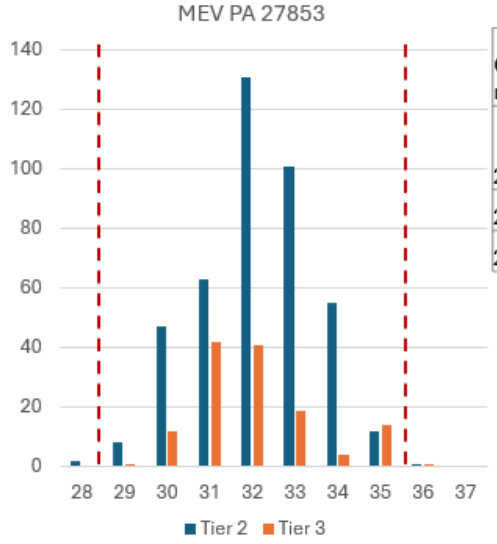
*BD MEV disc is investigational



QC range	range	% captured	Notes
29-35	7	99.4	Current range; Gavan; Rangefinder
29-36	8	99.7	
28-35	8	99.7	

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

	Tier 2	Tier 3	Total
28	2		2
29	8	1	9
30	47	12	59
31	63	42	105
32	131	41	172
33	101	19	120
34	55	4	59
35	12	14	26
36	1	1	2
37			
total	420	134	554
Mean	32.15	32.03	32.12
SD	1.37	1.44	1.39
+2SD	34.90	34.91	34.90
-2SD	29.40	29.15	29.34
Median	32.00	32.00	32.00
% captured	99.3%	99.2%	99.3%



QC range	range	% captured	Notes
29-35	7	99.3	Current range; Gavan; Rangefinder
29-36	8	99.6	
28-35	8	99.6	

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

- Quality Control Working Group Discussion and Recommendation

- No action taken on meropenem-vaborbactam *P. aeruginosa* ATCC 27853
- Tier 3 data still fit current range
- Wait for additional data
- Potential issue with inner colonies affecting zone reading
  - CLSI M02 reading guide says to measure to inner zone but this may lead to out-of-range results
- Discuss with EUCAST

BL-BLI COMBINATION QC RANGE REVIEW: MICS

- QC range differences between CLSI and EUCAST (bolded)
  - None (Note: amoxicillin/clavulanate and ampicillin/sulbactam are different but have different formulations – orange highlight).
  - No additional action needed.
- QC range differences for single agent from combination agent (yellow highlight on slides).
  - Minor differences (eg, 1 dilution shift or wider range)
  - No differences with most important QC strains (eg, recommended routine QC)
  - None are on the Tier 3 monitoring list.
- Should additional actions be taken for any differences?
  - Minor range differences could make sense with some agents due to activity of the inhibitor and/or variability due to different sample size/study results.
  - Do any warrant additional actions (eg, add to Tier 3 monitoring list)?
- No EUCAST QC ranges for the following. Discuss potential for EUCAST to add ranges when CLSI recommends as routine QC strain for agent.
  - *K. pneumoniae* ATCC BAA-1705:
    - Imipenem-relebactam and Meropenem-vaborbactam: includes range for *K. pneumoniae* ATCC BAA-2814
    - Ceftibuten-avibactam and Ceftibuten-ledaborbactam: agents not included in tables
  - *Acinetobacter baumannii* NCTC 13304:
    - Cefepime-zidebactam and Sulbactam-durlobactam: agents not included in tables
- BL-BLI Combination QC Range Review

	E.coli ATCC 25922 CLSI Range	E.coli ATCC 25922 EUCAST Range	Pseudomonas aeruginosa ATCC 27853 CLSI Range	Pseudomonas aeruginosa ATCC 27853 EUCAST Range	Staphylococcus aureus ATCC 29213 CLSI Range	Staphylococcus aureus ATCC 29213 EUCAST Range	Enterococcus faecalis ATCC 29212 CLSI Range	E. faecalis ATCC 29212 EUCAST Range
Amoxicillin	–	2-8	–	-	–	0.5-2	–	-
Amoxicillin-clavulanate CLSI (2:1) EUCAST fixed 2	2/1-8/4	2-8	–	- -	0.12/0.06-0.5/0.25	0.125-0.5	0.25/0.12-1.0/0.5	-
Ampicillin	2-8	2-8	–	-	0.5-2	0.5-2	0.5-2	0.5-2
Ampicillin-sulbactam CLSI (2:1) EUCAST fixed 4	2/1-8/4	**1-4**	–	-	–	-	–	-
Aztreonam	0.06-0.5	0.06-0.5	2-8	-	–	-	–	-
Aztreonam-avibactam	0.03/4-0.12/4	0.03-0.125	2/4-8/4	- -	–	-	–	-
Aztreonam-nacubactam (1:1)	0.06/0.06-0.25/0.25	-	2/2-8/8	-	–	-	–	-
Cefepime	0.016-0.12	0.016-0.12	0.5-4	0.5-4	1-4	-	–	-
Cefepime-enmetazobactam	0.03/8-0.12/8	0.03-0.12	0.5/8-2/8	-	–	-	–	-
Cefepime-nacubactam (1:1)	0.016/0.016-0.12/0.12	-	0.5/0.5-2/2	-	–	-	–	-
Cefepime-taniborbactam	0.03/4-0.12/4	-	0.5/4-4/4	-	–	-	–	-
Cefepime-tazobactam	0.03/8-0.12/8	-	0.5/8-4/8	-	1/8-4/8	-	–	-
Cefepime-zidebactam (1:1)	0.016-0.06	-	0.5-2	-	–	-	–	-
Zidebactam	0.06-0.25	-	1-8	-	–	-	–	-
Cefotaxime	0.03-0.12	0.03-0.12	8-32	-	1-4	-	–	-
Cefpodoxime	0.25-1	0.25-1	–	-	1-8	-	–	-
Ceftaroline	0.03-0.12	0.03-0.12	–	-	0.12-0.5	0.12-0.5	0.25-2	-
Ceftaroline-avibactam	0.03/4-0.12/4	-	–	-	0.12/4-0.5/4	-	–	-
Ceftazidime	0.06-0.5	0.06-0.5	1-4	1-4	4-16	-	–	-
Ceftazidime-avibactam	0.06/4-0.5/4	0.06/4-0.5/4	0.5/4-4/4	0.5-4	4/4-16/4	-	–	-

	E.coli ATCC 25922 CLSI Range	E.coli ATCC 25922 EUCAST Range	Pseudomonas aeruginosa ATCC 27853 CLSI Range	Pseudomonas aeruginosa ATCC 27853 EUCAST Range	Staphylococcus aureus ATCC 29213 CLSI Range	Staphylococcus aureus ATCC 29213 EUCAST Range	Enterococcus faecalis ATCC 29212 CLSI Range	Enterococcus faecalis ATCC 29212 EUCAST Range
Ceftibuten	0.12-1	0.12-1	–	-	–	-	–	-
Ceftibuten-avibactam	0.016/4-0.12/4	-	–	-	–	-	–	-
Ceftibuten-ledaborbactam	–	-	–	-	–	-	–	-
Ceftibuten-xeruborbactam	–	-	–	-	–	-	–	-
Ceftolozane-tazobactam	0.12/4-0.5/4	0.12/4-0.5/4	0.25/4-1/4	0.25-1	16/4-64/4	-	–	-
Ceftriaxone	0.03-0.12	0.03-0.12	8-64	-	1-8	-	–	-
Durlobactam	0.12-0.5	-	–	-	–	-	–	-
Imipenem	0.06-0.5	0.06-0.5	1-4	1-4	0.016-0.06	-	0.5-2	0.5-2
Imipenem-funobactam	0.06/8-0.25/8	-	0.25/8-1/8	-	–	-	–	-
Imipenem-relebactam	0.06/4-0.5/4	0.06/4-0.5/4	0.25/4-1/4	0.25-1	0.008/4-0.03/4	-	0.5/4-2/4	-
Meropenem	0.008-0.06	0.008-0.06	0.12-1	0.125-1	0.03-0.12	-	2-8	-
Meropenem-nacubactam (1:1)	0.016/0.016-0.06/0.06	-	0.12/0.12-1/1	-	–	-	–	-
Meropenem-vaborbactam	0.008/8-0.06/8	0.008/8-0.06/8	0.12/8-1/8	0.125-1	0.03/8-0.12/8	-	–	-
Meropenem-xeruborbactam	–	-	0.06/8-0.5/8	-	–	-	–	-
Nacubactam	0.5-4		64-256		–	-	–	-
Piperacillin	1-4	1-4	1-8	1-8	1-4	-	1-4	-
Piperacillin-tazobactam	1/4-8/4	1/4-8/4	1/4-8/4	1-8	0.25/4-2/4	-	1/4-4/4	-
Sulbactam	16-64		–	-	–	-	–	-
Sulbactam-durlobactam	–		–	-	–	-	–	-
Ticarcillin	4-16	4-16	8-32	8-32	2-8	-	16-64	-
Ticarcillin-clavulanate	4/2-16/2	4/2-16/2	8/2-32/2	8-32	0.5/2-2/2	-	16/2-64/2	-

MISCELLANEOUS UPDATES/FUTURE TOPICS

- Colony Counts/Inoculum Preparation
  - Practices differ by laboratory and in standards.
  - Should inoculum be adjusted for QC strains other than *E. coli* ATCC 25922?
  - Does difference in CFU/mL impact zones/MICs? – Assessment in process
  - Consider publishing data from Tier 2 QC Studies (eg. white paper, M100 appendix) – Compile for Jan 2027
  - Should CLSI recommendations be revised (M02, M07, M100, M23)
- 80% Read - Review QC Tables to determine if footnote needed – No progress
- Potentially publish mode/median -
  - Compile historical summary of Tier 2 QC Studies with mode/median by media, disk, overall results – In process

- Reassess after historical summary is available
- Potentially develop guidance for developing QC ranges – No progress
  - Qualitative (eg, screening, specialty tests)
  - Supplemental use only
  - When changing solvents

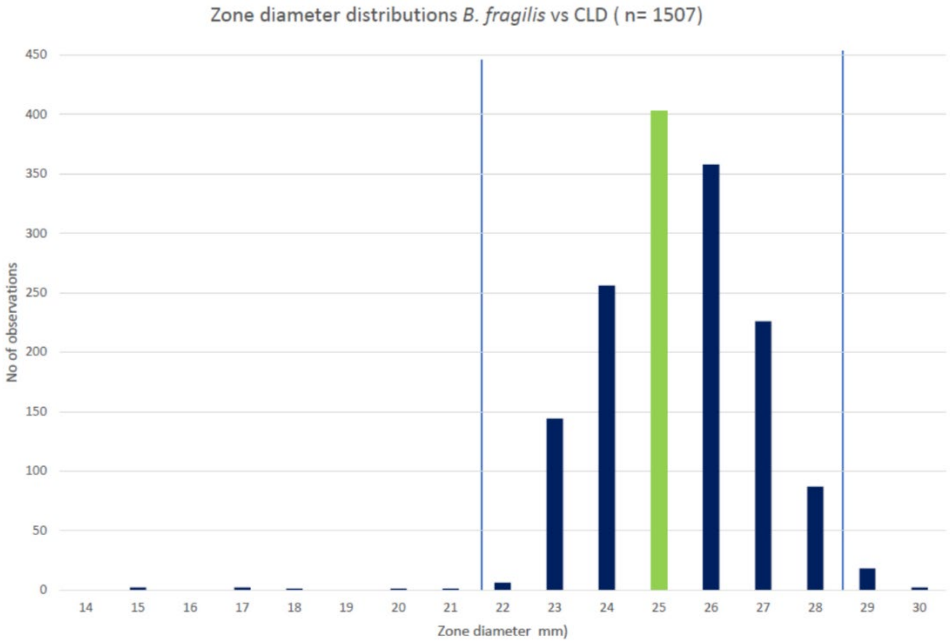
ANAEROBE TIER 2 QC

- Coordinate publication of anaerobe disk QC ranges with revisions to CLSI M11 to include new disk diffusion method (which is currently published EUCAST disk method using FAA agar).
- Anaerobes Working Group: Disk Diffusion QC Study Method
  - 3 drugs – clindamycin, meropenem, metronidazole.
  - 2 QC organisms- *B. fragilis* ATCC 25285, *C. perfringens* ATCC 13124.
  - 5 studies including Anaerobe Working Group pilot study.
  - All results are using FAA agar and current EUCAST methodology.
  - Data broken down to component parts where possible (eg, by drug/bug/media manufacturer/environment etc.)
  - Compare results with QC ranges as determined by EUCAST.
  - Analyze data according using CLSI M23 criteria (mean, median, Gavan statistic, range finder) to see if correlates for proposed CLSI QC range.
  - Study design meets requirements of CLSI M23
    - EUCAST QC study design plus additional data to improve assessment with more media and laboratories.
    - Total sample size is significantly larger than traditional Tier 2 study.
- Anaerobe Disk Diffusion QC Data Summary
  - 5 studies
  - 19 laboratories
  - 11 lots of media
  - 5 media manufacturers
  - 5 lots of disks (1 disk manufacturer - Oxoid)
  - 3 anaerobic environments

CLINDAMYCIN *BACTEROIDES FRAGILIS* ATCC 25285

- Some variability observed but no significant % out of current EUCAST/proposed CLSI range 22-28mm.
- Overall median 25, 98.21% in range

Study #	Labs	Media Mfg	Media Lots	Disk lots	Environ -ment	Total #	Median	Comments
1	2	1	3	1	1	882	25	
2	17	1	1	1	1	109	25	
3	2	1	3	1	6	311	26	
4	1	5	1	1	1	202	26	
5	3	1	1	1	1	3	24	NA, Initial pilot study



- Summary
  - EUCAST Version 16.0
    - *B. fragilis* ATCC 25285 – Clindamycin
    - Range 22-28 mm
  - Out of range from three laboratories
  - Anaerobes Working Group Recommendation
    - Motion to accept the 22-28 mm clindamycin QC range for *B. fragilis* ATCC 25285. WG Vote: 10-0-0-4.

- Quality Control Working Group Discussion and Recommendation
  - Motion to accept the clindamycin 2 µg disk with *B. fragilis* ATCC 25285 QC range 22-28 mm (same as EUCAST). WG Vote: 11-0-0-1.
  - Add footnote: Data only available from one disk manufacturer.
  - Correction: Gavan range 19-31

SC DISCUSSION (MAIN POINTS)

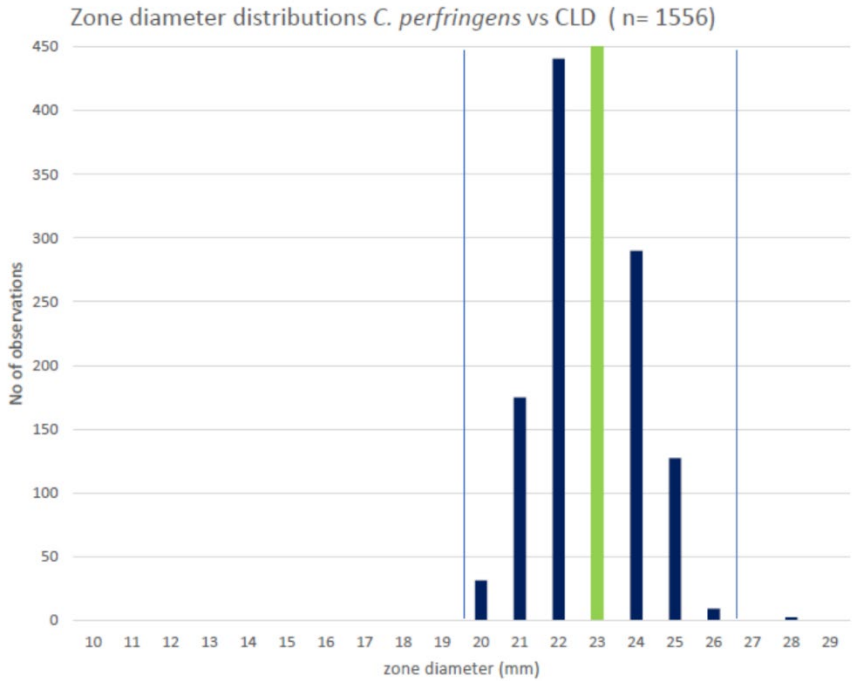
- Does the EUCAST disk diffusion method now become the CLSI disk diffusion method? The funding and resources for anaerobe disk diffusion is not out there, so the thought is to use the EUCAST method.
- The disk diffusion method is not currently in CLSI M11. It will need to be added. CLSI would not publish the QC ranges until the method is approved and published.
- The Anaerobes Working Group does not have more data. There is only data on one disk manufacturer.
- Most of the data is from EUCAST. There are three data points that the working group generated.
- The data follows the CLSI M23 process. There is laboratory and media variability.
- CLSI process does allow for one disk manufacturer and a comment is added to indicated this.

A motion to accept the clindamycin disk diffusion (2 µg) QC range for *Bacteroides fragilis* ATCC 25285 (22-28 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

CLINDAMYCIN *CLOSTRIDIUM PERFRINGENS* ATCC 13124

- Some variability observed but no significant number out of proposed range 20-26 mm.
- Overall median 23 mm, 99.87% in range

Study #	Labs	Media Mfg	Media Lots	Disk lots	Environ -ment	Total #	Median	Variability observed /Comments
1	2	1	3	1	1	879	22	
2	17	1	1	1	1	110	22	
3	2	1	3	1	6	337	23	
4	1	5	1	1	1	227	24	
5	3	1	1	1	1	3	23	NA, Initial pilot study



- Summary
  - EUCAST Version 16.0
    - *C. perfringens* ATCC 13124 – Clindamycin
    - Range 20-26 mm
  - Out of range from two laboratories
  - Anaerobes Working Group Recommendation
    - Motion to accept the 20-26 mm clindamycin QC range for *C. perfringens* ATCC 13124. WG Vote: 10-0-0-4.
- Quality Control Working Group Discussion and Recommendation
  - Motion to accept the clindamycin 2 µg disk with *C. perfringens* ATCC 13124 QC range 20-26 mm (same as EUCAST). WG Vote: 11-0-0-1.
  - Add footnote: Data only available from one disk manufacturer.
  - Correction: Range Finder 97.3% in range

SC DISCUSSION (MAIN POINTS)

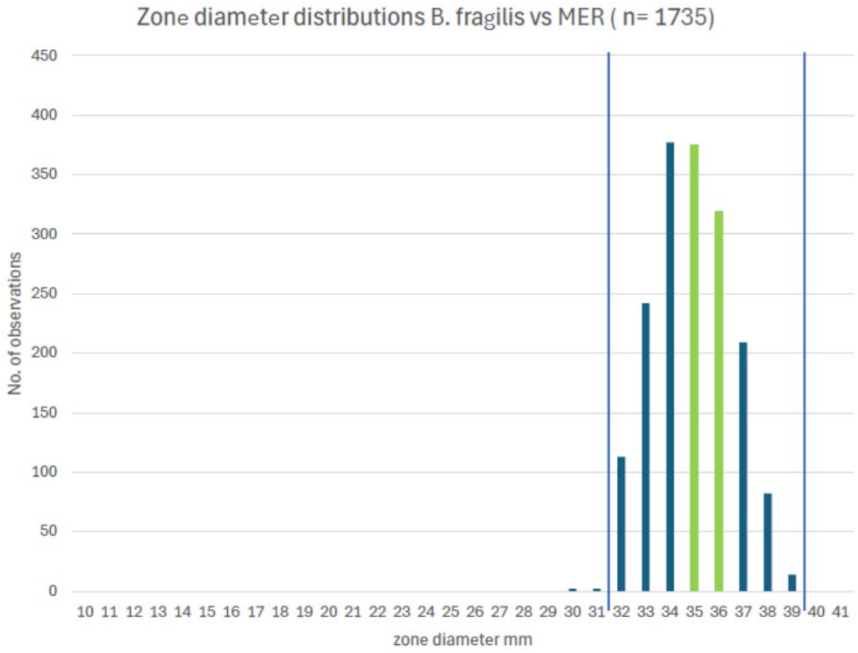
- CLSI does not have MIC breakpoints for *C. perfringens* for some of these drugs, so the breakpoints will need to be reviewed.

A motion to accept the clindamycin disk diffusion (2 µg) QC range for *Clostridium perfringens* ATCC 13124 (20-26 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update was made and seconded. Vote: 15 for, 0 against, 0 abstain, 1 absent (Pass)

MEROPENEM *BACTEROIDES FRAGILIS* ATCC 25285

- Some variability observed but no significant % out of current EUCAST/proposed CLSI range 32-39 mm.
- Overall median 34, 99.87% in range

Study #	Labs	Media Mfg	Media Lots	Disk lots	Environ-ment	Total #	Median	Variability observed /Comments
1	2	1	3	1	1	882	34	
2	17	1	1	1	1	109	34	
3	2	1	3	1	6	425	36	
4	1	5	1	1	1	316	36	
5	3	1	1	1	1	3	34	NA, Initial pilot study



- Summary

- EUCAST Version 16.0
  - *B. fragilis* ATCC 25285 – Meropenem
  - Range 32-39 mm
- Out of range from two laboratories
- Anaerobes Working Group Recommendation
  - Motion to accept the 32-39 mm meropenem QC range for *B. fragilis* ATCC 25285. WG Vote: 10-0-0-4.

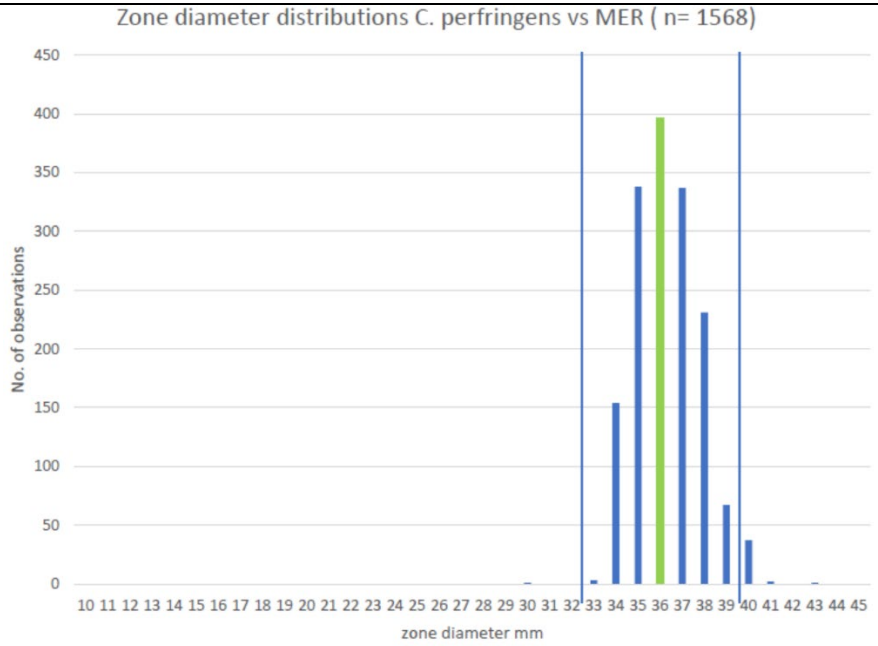
- Quality Control Working Group Discussion and Recommendation
  - Motion to accept the meropenem 10 µg disk with *B. fragilis* ATCC 25285 QC range 32-39 mm (same as EUCAST). WG Vote: 11-0-0-1.
  - Add footnote: Data only available from one disk manufacturer.

A motion to accept the meropenem disk diffusion (10 µg) QC range for *Bacteroides fragilis* ATCC 25285 (32-39 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update was made and seconded. Vote: 15 for, 0 against, 0 abstain, 1 absent (Pass)

MEROPENEM *CLOSTRIDIUM PERFRINGENS* ATCC 13124

- Current EUCAST range 33-39 mm but on watch list for reassessment since incubation in anaerobic workstation was underrepresented. Additional data provided by EUCAST to CLSI for consideration (not combined with above, see slides in agenda materials).
- Media C 27% out of range with current EUCAST range but only 3% out with new CLSI proposed range.
- Some variability observed but no significant % out of proposed CLSI range 34-40 mm.
- Overall median 36, 99.55% within proposed CLSI range, 97.39% within current EUCAST range.

Study #	Labs	Media Mfg	Media Lots	Disk lots	Environ-ment	Total #	Median	Variability observed /Comments
1	2	1	3	1	1	879	35	
2	17	1	1	1	1	109	36	
3	2	1	3	1	6	343	37	
4	1	5	1	1	1	234	37	Larger zones with Media C
5	3	1	1	1	1	3	37	NA, Initial pilot study



- Summary
  - EUCAST Version 16.0
    - *C. perfringens* ATCC 13124 –Meropenem
    - Range 33-39 mm
  - Out of range from six laboratories
  - Anaerobes Working Group Recommendation
    - Motion to accept the 34-40 mm meropenem QC range for *C. perfringens* ATCC 13124. WG Vote: 11-0-0-3.
- Quality Control Working Group Discussion and Recommendation
  - Motion to accept the meropenem 10 µg disk with *C. perfringens* ATCC 13124 QC range 34-40 mm (different from EUCAST). WG Vote: 11-0-0-1.
  - Add footnote: Data only available from one disk manufacturer.
  - EUCAST to reassess range in 2027. QCWG preferred to exclude 33 mm.

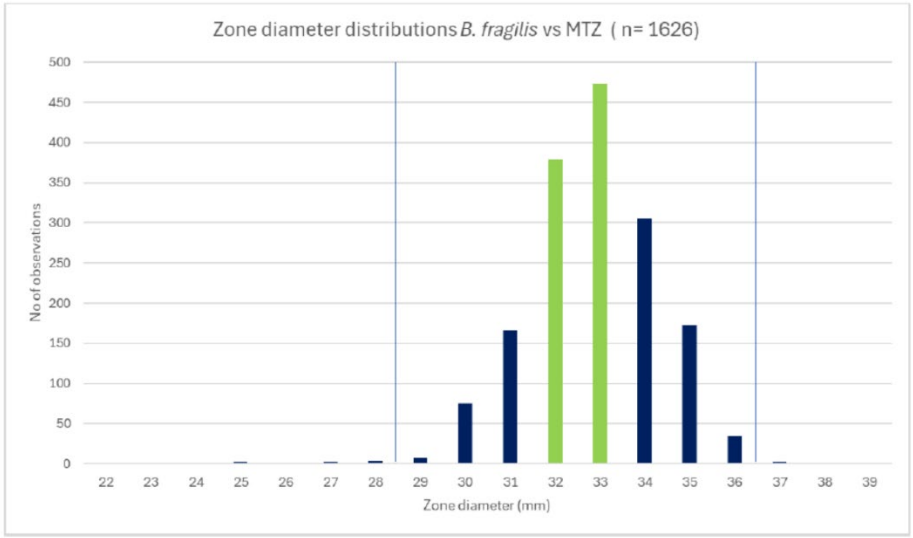
A motion to accept the meropenem disk diffusion (10 µg) QC range for *Clostridium perfringens* ATCC 13124 (34-40 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

METRONIDAZOLE *BACTEROIDES FRAGILIS* ATCC 25285

- Some variability observed but no significant number out of range

- Current EUCAST/proposed CLSI range 29-36 mm.
- Overall median 33, 99.20% in range

Study #	Labs	Media Mfg	Media Lots	Disk lots	Environ-ment	Total #	Median	Variability observed /Comments
1	2	1	3	1	1	883	33	
2	17	1	1	1	1	109	33	
3	2	1	3	1	6	370	33	
4	1	5	1	1	1	261	33	
5	3	1	1	1	1	3	35	NA, Initial pilot study



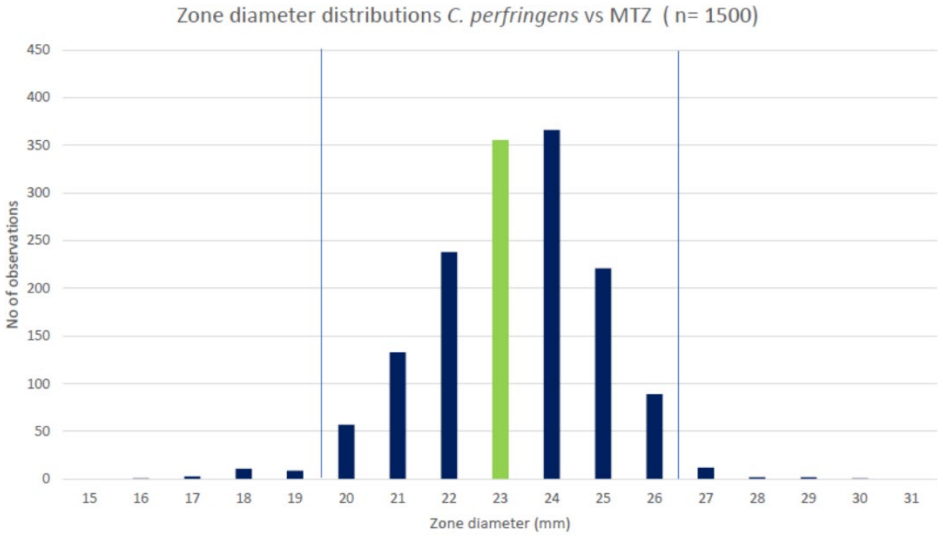
- Summary
  - EUCAST Version 16.0
    - *B. fragilis* ATCC 25285 – Metronidazole
    - Range 29-36 mm
  - Out of range from four laboratories
  - Anaerobes Working Group Recommendation
    - Motion to accept the 29-36 mm metronidazole QC range for *B. fragilis* ATCC 25285. WG Vote: 10-0-0-4.
- Quality Control Working Group Discussion and Recommendation
  - Motion to accept the metronidazole 5 µg disk with *B. fragilis* ATCC 25285 QC range 29-36 mm (same as EUCAST). WG Vote: 11-0-0-1.
  - Add footnote: Data only available from one disk manufacturer.

A motion to accept the metronidazole disk diffusion (5 µg) QC range for *Bacteroides fragilis* ATCC 25285 (29-36 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

METRONIDAZOLE *CLOSTRIDIUM PERFRINGENS* ATCC 13124

- High (10.9%) out range mm with D: Lab B gas system.
- Current EUCAST/Proposed CLSI range 20-26 mm
- Overall median 24, 97.3% in range

Study #	Labs	Media Mfg	Media Lots	Disk lots	Environ-ment	Total #	Median	Variability observed /Comments
1	2	1	3	1	1	877	24	
2	17	1	1	1	1	110	23	
3	2	1	3	1	6	310	23	D Lab B gas system 10.9% out of range high
4	1	5	1	1	1	200	22	
5	3	1	1	1	1	3	24	NA, Initial pilot study



- Summary
  - EUCAST Version 16.0
    - *C. perfringens* ATCC 13124 –Metronidazole
    - Range 20-26 mm
  - Out of range from five laboratories

- Anaerobes Working Group Recommendation
  - Motion to accept the 20-26 mm metronidazole QC range for *C. perfringens* ATCC 13124. WG Vote: 11-0-0-3.
- Quality Control Working Group Discussion and Recommendation
  - Motion to accept the metronidazole 5 µg disk with *C. perfringens* ATCC 13124 QC range 20-26 mm (same as EUCAST). WG Vote: 11-0-0-1.
  - Add footnote: Data only available from one disk manufacturer.
  - Also suggest adding reading guide and troubleshooting guide

A motion to accept the metronidazole disk diffusion (5 µg) QC range for *Clostridium perfringens* ATCC 13124 (20-26 mm) with a footnote stating, "Data only available from one disk manufacturer." and pending CLSI M11 methodology update was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

11. **ANAEROBES WORKING GROUP (D. CARPENTER AND S. COPSEY-MAWER)**

ANAEROBE DISK CORRELATION

- Data Source
  - Development of a EUCAST disk diffusion method for the susceptibility testing of rapidly growing anaerobic bacteria using Fastidious Anaerobe Agar (FAA): a development study using Bacteroides species. Bavelaar H, Justesen US, Morris TE, Anderson B, Copsey-Mawer S, Stubhaug TT, Kahlmeter G, Matuschek E. Clin Microbiol Infect. 2021 Nov;27(11):1695.e1-1695.e6. doi: 10.1016/j.cmi.2021.03.028. Epub 2021 Apr 2.PMID: 33813129
  - Data presented is CLSI Brucella Blood Agar MIC data with Fastidious Anaerobe Agar (FAA) media with disk diffusion
  - 170 *Bacteroides* species isolates
  - 10 European laboratories participated in the testing
- *Bacteroides* spp. included in testing
  - *Bacteroides caccae* (3)
  - *Bacteroides cellulosilyticus* (1)
  - *Bacteroides faeces* (2)
  - *Bacteroides fragilis* (113)
  - *Bacteroides ovatus* (9)
  - *Bacteroides thetaiotaomicron* (23)
  - *Bacteroides uniformis* (1)
  - *Bacteroides xylanisolvens* (1)
  - *Parabacteroides distasonis* (2)
  - *Parabacteroides merdae* (1)
  - *Phocaeicola dorei/vulgatus* (14)
- Data
  - Only *Bacteroides* species
  - All agar dilution data with Brucella Blood Agar (CLSI)
  - Same disk concentration and FAA agar as EUCAST
  - Interpretations
    - EUCAST Data – CLSI Interpretations
    - EUCAST Data Plus CLSI Pilot Data
    - EUCAST Data – EUCAST Interpretations
- Data was presented on clindamycin. See the SC discussion below.

SC DISCUSSION (MAIN POINTS)

- There was concern that the MIC breakpoints and disk diffusion breakpoints would be extremely different.
- Concerns that there were S/R disk diffusion breakpoints but S/I/R MIC breakpoints.
- The breakpoint errors here are for the dBETS proposed error rates
- No issue with the testing method or data source.
- Suggestion to show old and new data.

	• The dBETS error rates automatically calculated do not match the CLSI M23 error rate. • The Anaerobes Working Group was asked to reinterpret using CLSI MIC breakpoints to set the disk correlates (S/I/R) using the EUCAST disk diffusion methods and bring it forward to the Breakpoints Working Group for review. • The Anaerobes Working Group will review the EUCAST disk diffusion method and bring it forward the Methods Working Group for review. • CLSI leadership will work offline to map out a strategy for the overall anaerobe project.

12.	***STAPHYLOCCUS AUREUS* TETRACYCLINE BREAKPOINTS (J. LUTGRING)**
	Dr. Lutgring presented on the reassessment of *S. aureus* tetracycline breakpoints. The main points included:
	• Situations in Which Breakpoint Reassessment May Be Considered (CLSI M23 ED6:2023)
	  ○ Susceptible-only breakpoints exist
	  ○ New resistance emerges not currently detected
	  ○ Breakpoints initially established using organisms from several species
	  ○ New PK/PD data
	  ○ Breakpoints were set before introduction of current analytic methods
	  ○ Prevailing antimicrobial dosage regimens differ from those used to establish the breakpoints
	  ○ New data show that prior breakpoints are not optimal for common uses of the antimicrobial
	  ○ Breakpoints poorly predict clinical response
	  ○ While evaluating a newer antimicrobial in the same class, data suggests the breakpoints of older agents may be erroneous
	  ○ Discordance between MIC and disk diffusion
	  ○ Changes to CLSI-approved reference methods
	  ○ Selecting alternative breakpoints will simplify testing
	  ○ Differences between CLSI and other breakpoints
	  ○ Sponsor submits a request for determining breakpoints
	• Situations in Which Breakpoint Reassessment May Be Considered: #1
	  ○ When differences exist between breakpoints established by CLSI and those of other regulatory or standards development organizations responsible for determining breakpoints, the relevant CLSI subcommittee or working group may re-examine its breakpoints in an effort to understand the reasons for such differences and to minimize discrepancies between CLSI published breakpoints and those of other organizations whenever possible.
	  ○ MIC Breakpoints

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

CLSI M100 Breakpoints (ED36: 2026)

Tetracyclines	S	I	R
Tetracycline[1*]	≤4	8	≥16
Doxycycline[2*]	≤4	8	≥16
Minocycline[1*]	≤4	8	≥16
Tigecycline[3]	≤0.5		
Eravacycline[3]	≤0.06		

1. U.S. FDA recognizes the CLSI M100 Breakpoint
2. U.S. FDA does not recognize the CLSI M100 Breakpoint.
3. No CLSI M100 Breakpoints exist, this is a U.S. FDA Breakpoint

*Isolates that test susceptible to tetracycline are considered susceptible to doxycycline and minocycline. Isolates that test intermediate or resistant to tetracycline should be tested against doxycycline or minocycline if those results are needed for treatment.

EUCAST Breakpoint Tables v. 16.0

Tetracyclines	S	R
Tetracycline*	≤1	>1
Doxycycline*	≤1	>1
Minocycline*	≤0.5	>0.5
Tigecycline[1, 2]	≤0.5	>0.5
Eravacycline	≤0.25	>0.25

1. Resistant isolates are rare; any such results must be confirmed and sent to a reference laboratory.
2. For tigecycline BMD MIC determination, the medium must be prepared fresh on the day of use.

*Tetracycline can be used to screen for resistance in tetracycline agents. Isolates categorised as susceptible can be reported susceptible to doxycycline and minocycline. Isolates categorised as resistant should be tested for susceptibility to individual agents or reported resistant.

- Zone Diameter Breakpoints

CLSI M100 Breakpoints (ED36: 2026)

Tetracyclines	S	I	R
Tetracycline[1*] (Disk Content 30 µg)	≥19	15-18	≤14
Doxycycline[2*] (Disk Content 30 µg)	≥16	13-15	≤12
Minocycline[1*] (Disk Content 30 µg)	≥19	15-18	≤14
Tigecycline[3] (Disk Content 15 µg)	≥19		
Eravacycline[4]			

1. U.S. FDA recognizes the CLSI M100 Breakpoint
2. U.S. FDA does not recognize the CLSI M100 Breakpoint.
3. No CLSI M100 Breakpoints exist, this is a U.S. FDA Breakpoint
4. No CLSI or U.S. FDA Breakpoints exist

*Isolates that test susceptible to tetracycline are considered susceptible to doxycycline and minocycline. Isolates that test intermediate or resistant to tetracycline should be tested against doxycycline or minocycline if those results are needed for treatment.

EUCAST Breakpoint Tables v. 16.0

Tetracyclines	S	R
Tetracycline* (Disk Content 30 µg)	≥22	≤22
Doxycycline*		
Minocycline* (Disk Content 30 µg)	≥23	≤23
Tigecycline[1] (Disk Content 15 µg)	≥19	≤19
Eravacycline[2] (Disk Content 20 µg)	≥20	≤20

1. Resistant isolates are rare; any such results must be confirmed and sent to a reference laboratory.
2. For MRSA that test susceptible with disk diffusion, the results should be confirmed with an MIC test.

*Tetracycline can be used to screen for resistance in tetracycline agents. Isolates categorised as susceptible can be reported susceptible to doxycycline and minocycline. Isolates categorised as resistant should be tested for susceptibility to individual agents or reported resistant.

- Situations in Which Breakpoint Reassessment May Be Considered: #2
  - Breakpoints were set before the introduction of current analytical methods used to determine relationships among drug exposure, organism susceptibility, and clinical response.
  - Proposed CLSI M45: Tetracycline and doxycycline breakpoints have been re-evaluated based on contemporary PK/PD data
- Situations in Which Breakpoint Reassessment May Be Considered: #3
  - As new resistance mechanisms emerge (eg, new β-lactamases, multidrug resistance, carbapenem resistance) or are newly recognized (eg, by molecular methods), resistance may not be reliably detected using established breakpoints or any associated methods. The appearance of such resistance mechanisms may prompt review of breakpoints when it seems possible or when it has been reported that they may adversely affect clinical response.
  - *tetK* and *tetM*
    - *tetK*: tetracycline resistance and maybe doxycycline resistance
    - *tetM*: tetracycline, doxycycline, and minocycline resistance
  - Tetracycline Resistance Increasing
    - Tetracycline resistance has increased 16.4% (4.57% to 20.98%) in MRSA isolates between 2010 and 2023 among Veterans Health Administration outpatients in the eastern United States
  - Doxycycline Use Increasing
    - Increasing among outpatients
    - Increasing among residents in long-term care facilities (71% increase between 2013 and 2021)

- Possible Contributing Factors
  - FDA safety announcements for azithromycin and fluoroquinolones
  - Recommended in 2019 CAP Guideline
- Doxycycline Postexposure Prophylaxis and *S. aureus* Resistance to Tetracyclines
  - Luetkemeyer AF, et al. 2023: Doxycycline postexposure prophylaxis led to a reduction in *S. aureus* colonization but an increase in colonization with doxycycline-resistant *S. aureus*
  - Luetkemeyer AF, et al. 2025: In those without doxycycline-resistant *S. aureus* at baseline, doxycycline postexposure prophylaxis was associated with a statistically significant increase in the detection of doxycycline-resistant *S. aureus*
  - Soge OO, et al. 2025.: *S. aureus* colonization was less common among doxycycline postexposure prophylaxis users but colonization with tetracycline-resistant *S. aureus* was more common
  - Mittelstaedt R, et al. 2025.: Rates of tetracycline resistance higher in individuals who would be eligible for doxycycline postexposure prophylaxis than general population

- CDC Data Availability
  - Emerging Infections Program (EIP) Healthcare-Associated Infections – Community Interface Activity
    - Invasive *Staphylococcus aureus* Infection Surveillance Program
      - Currently five sites submit isolates to CDC (historically as many as nine)
  - *Staphylococcus aureus* isolates tested against tetracycline and doxycycline with reference broth microdilution (in addition to several other drugs)
  - Years: 2005-2023 with AST data (2017-2023 with WGS data)
  - Number of isolates with AST data: 17,399
    - Number of MRSA isolates: 14,934
    - Number of MSSA isolates: 2,465
  - Number of isolates with AST and WGS data: 5,350
    - Number of MRSA isolates: 3,196
    - Number of MSSA isolates: 2,154
  - Premier Healthcare Database
  - National Healthcare Safety Network AR Option
- Reassessment of Current CLSI S. aureus Tetracyclines Breakpoints
  - Breakpoints between CLSI, FDA, and EUCAST differ
  - Current breakpoints were set long ago
  - Resistance to tetracyclines among *S. aureus* isolates is increasing

SC DISCUSSION (MAIN POINTS)

- The doxycycline restrictions in children have been removed, so that can contribute to increased doxycycline use.
- This topic has massive clinical significance.

A motion to form an ad hoc working group to reassess the tetracycline *S. aureus* breakpoints was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

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

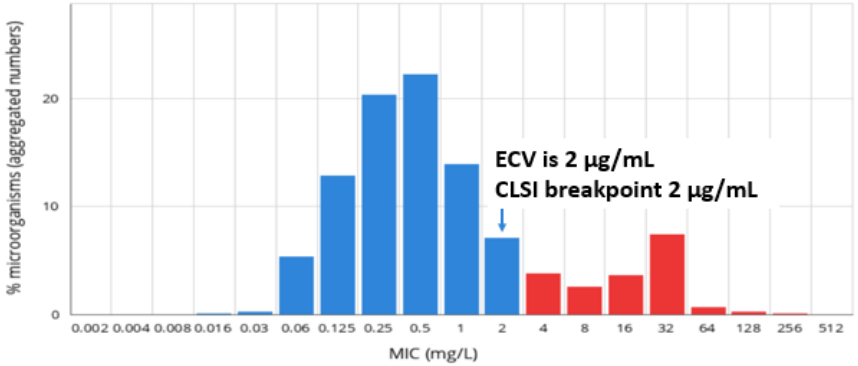
2026 JUNE AST MEETING SUMMARY MINUTES PLENARY 2: Monday, 1 June 2026 1:00 PM – 5:30 PM Central Time (US)	
#	**Description**
1.	**OPENING** Dr. Mathers opened the meeting at 1:00 PM Central Time (US).

2.	**BREAKPOINTS WORKING GROUP (N. NARAYANAN AND M. SATLIN)**
	
	BURKHOLDERIA CEPACIA COMPLEX FOLLOW UP
	• Removal of BCC breakpoints and epidemiologic cutoffs from M100 (and lack of EUCAST breakpoints) has created a gap
	• Frequent source of inquiry and concern from clinical laboratories
	  ○ Breakpoints and then ECVs for ceftazidime, meropenem, minocycline, levofloxacin, trimethoprim-sulfamethoxazole (ticarcillin/clavulanate and chloramphenicol) have been removed from the M100
	  ○ Ongoing global outbreaks linked to contaminated medical and consumer products highlight need for guidance
	  ○ Unclear treatment framework in the absence of breakpoints or validated susceptibility interpretation
	• Cefiderocol as a possible therapeutic for review
	  ○ Existing surveillance susceptibility data could support re-evaluation
	  ▪ MICs are lower here than with most other agents
	  ○ Available PK/PD data may allow reassessment of potential BCC breakpoints
	  ○ Cefiderocol availability is increasing globally, raising clinical relevance
	  ○ Parallel industry and research interest (eg, Shionogi work in *Burkholderia pseudomallei* models) suggests growing interest clinically
	• Proposal: Evaluate feasibility of cefiderocol breakpoints for BCC
	  ○ Form an ad hoc working group under the Breakpoints Working Group
	  ○ Review existing:
	  ▪ MIC distributions by species and as BCC
	  ▪ Surveillance datasets
	  ▪ Clinical outcomes (some observational data)
	  ▪ PK/PD data
	  ○ Recommendation for potential consideration/discussion in 2027
	  ○ Call for volunteers
	
	SC DISCUSSION (MAIN POINTS)
	• There is a cefiderocol methods ad hoc working group that should be included in the process.
	• Is there any consideration to look at other drugs? There is data that CLSI can look at for cefiderocol. It will be a good place to start. CLSI can consider ceftazidime-avibactam in the future.
	• Cefiderocol-xeruborbactam will be an easier method. Consider if that drug should be reviewed.
	• Reproducibility with clinical strains is critical to evaluate to avoid the issues often seen with cefiderocol testing.

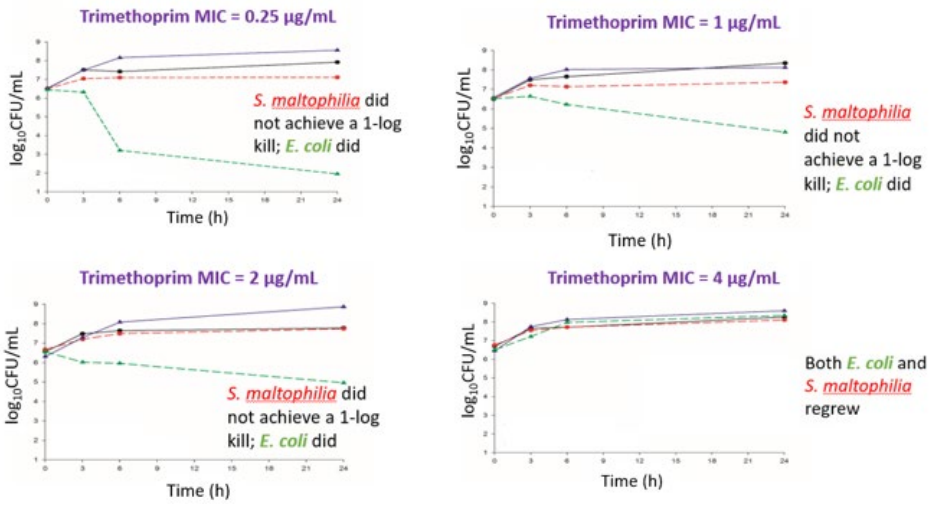
	A motion to form an ad hoc working group to review cefiderocol breakpoints for *Burkholderia cepacia* complex was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)
	AZTREONAM-AVIBACTAM MIC BREAKPOINTS FOR *STENOTROPHOMONAS MALTOPHILIA* • Background ○ Occurrence of *S. maltophilia* infections has increased markedly in recent years, especially among critically ill and immunocompromised patients.

- Treatment options remain limited due to intrinsic resistance to most antimicrobial agents commonly used to treat hospital-acquired gram-negative infections, including most recently approved β-lactamase inhibitor combinations.
- Aztreonam-avibactam (ATM-AVI) is stable against hydrolysis by both L1 (a metallo-β lactamase [MBL]) and L2 (serine carbapenemase) produced by *S. maltophilia*.

- Experience with Trimethoprim-sulfamethoxazole (TMP-SMX), Minocycline, and Levofloxacin, Cefiderocol and *S. maltophilia*
  - TMP-SMX and *S. maltophilia* MIC Distributions



    - Lasko MJ, et al. Antimicrob Agents Chemother. 2022; 66:e0216721
      - TMP-SMX human simulated dosages equivalent to 20 mg/kg/day

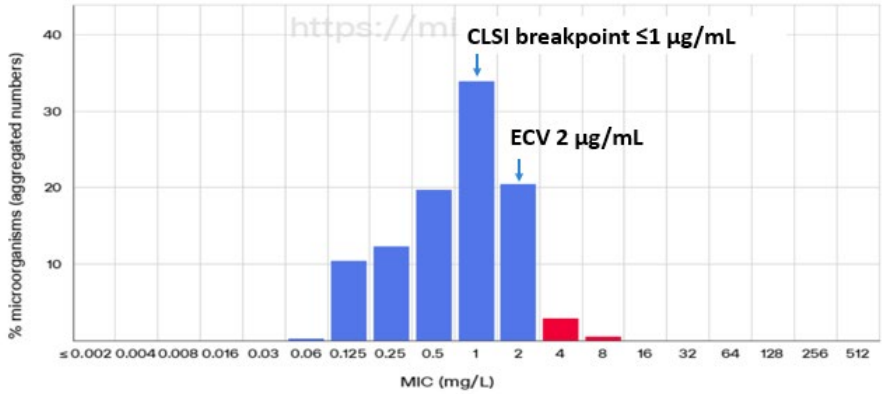


  - Results from Monte Carlo Simulations
    - Derived from Cheng AC, et al. Antimicrob Agents Chemother. 2009;53:4193-9. Chin TW, et al. Antimicrob Agents Chemother.1995;39:28-33.

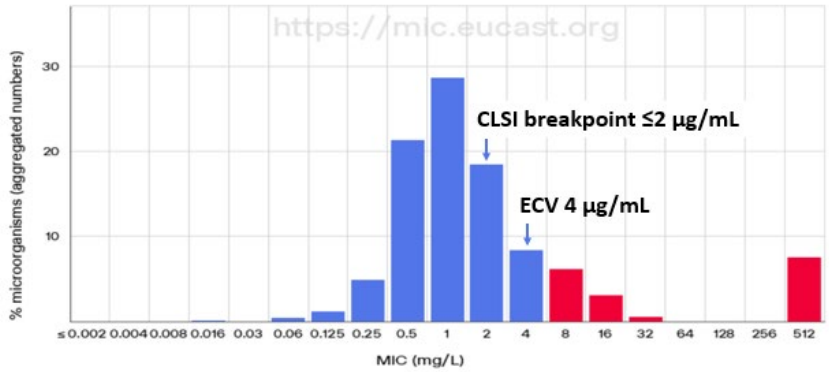
TMP-SMX 10 mg/kg/day	*S. maltophilia* stasis	*E. coli* stasis
TMP MIC 0.5 µg/mL	**96%**	100%
TMP MIC 1 µg/mL	12%	84%
TMP MIC 2 µg/mL	0%	2.5%
TMP-SMX 15 mg/kg/day	***S. maltophilia* stasis**	***E. coli* stasis**
TMP MIC 0.5 µg/mL	**100%**	100%
TMP MIC 1 µg/mL	71.1%	99.6%
TMP MIC 2 µg/mL	0.8%	39.8%

- CLSI breakpoint ≤2/38 µg/mL
- "Trimethoprim-sulfamethoxazole should not be used alone for antimicrobial therapy."

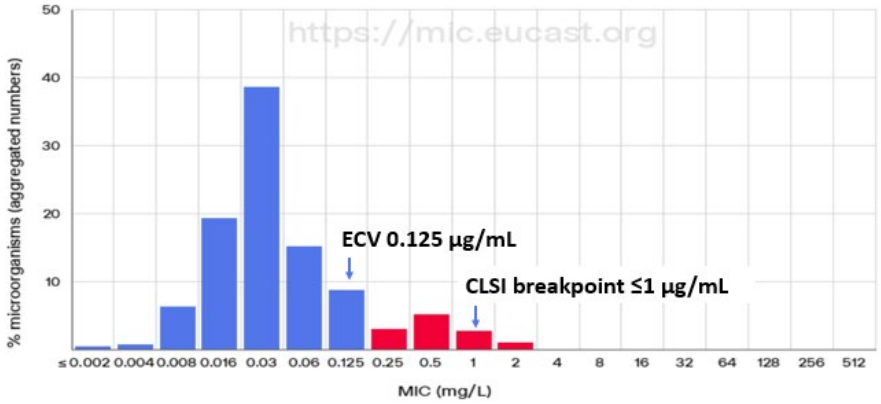
- Minocycline and *S. maltophilia* MIC Distributions



- Minocycline PK/PD Data
  - Fratoni AJ, et al. J Antimicrob Chemother. 2022;77:1052-1060.
    - Monte Carlo simulations indicate minocycline dosages of 200 mg IV every 12 hours have a >90% probability of achieving PK/PD targets associated with bacterial stasis in a neutropenic mouse thigh model for organisms with MICs of 1 µg/mL
    - 50% probability of achieving targets associated with 1-log kill
- Levofloxacin and *S. maltophilia* MIC Distributions



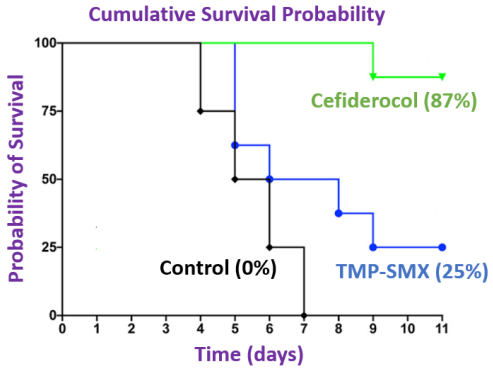
- Levofloxacin PK/PD Data
  - Fratoni AJ, et al. J Antimicrob Chemother. 2021;77:164-168.
    - Monte Carlo simulations indicate levofloxacin dosages of 750 mg IV every 24 hours have ~50% probability of achieving PK/PD targets associated with bacterial stasis in a neutropenic mouse thigh model for organisms with MICs of 2 µg/mL
    - ~30% probability of achieving targets associated with 1-log kill
- Cefiderocol and *S. maltophilia* MIC Distributions



  - Nakamura R, et al. Antimicrob Agents Chemother. 2019;63:e02031-02018
    - The % fT>MIC of cefiderocol required for 1-log10 reduction in *A. baumannii* and *P. aeruginosa* is higher than *S. maltophilia* in a murine pneumonia model
    - Using a 75% %fT>MIC target, the PTA is >90% for MICs ≤ 4 mg/L

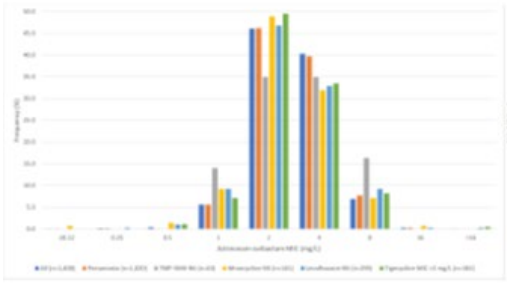
Organism	% fT>MIC Target
A. baumannii	88.1 ± 3.4%
P. aeruginosa	70.3 ± 9.0%
S. maltophilia	53.9 ± 18.1%

- Chen IH, et al. Antimicrob Agents Chemother. 2019;63(12):e01558-19.
  - Cefiderocol human-simulated exposures in a murine thigh model demonstrated ≥1-log for 100% and ≥2-log for 88% of *S. maltophilia* isolates with cefiderocol MICs ≤0.5 µg/mL
  - All isolates resistant to ceftazidime
- Cefiderocol and *S. maltophilia* in a Rabbit Model
  - Petraitis V, et al. Antimicrob Agents Chemother. 2022;66:e0061822.
    - Response of *S. maltophilia* pneumonia in neutropenic rabbits treated with:
      - No antibiotics (n=8)
      - TMP-SMX (n=8)
        - TMP MIC 0.25 µg/mL
      - Cefiderocol (n=8)
        - Cefiderocol MIC 0.03 µg/mL
    - Note: Serum thymidine levels in rabbits comparable to humans.

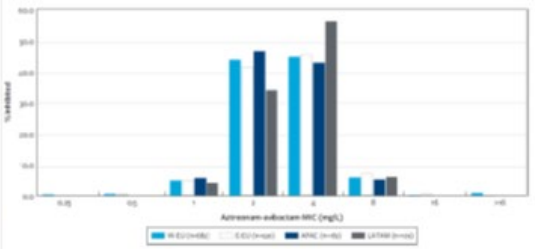


- ATM-AVI MIC Distributions for *Stenotrophomonas maltophilia* were Consistent Across Resistant Isolates and Geographies
  - Sader et al. Antimicrob Agents Chemother. 2025 Apr 2;69(4):e0012425
  - Sader et al. Int J Infect Dis. 2025 Apr;153:107803

1400 US isolates collected from 2019-2023[1]

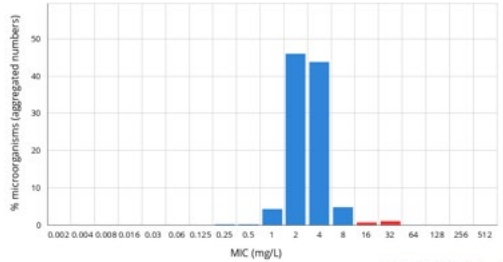


1287 isolates collected from 2018-2023 across Europe, Asia, and Latin America[2]



- MIC_{90} was consistently 4 mg/L with >99% of isolates inhibited at ≤8 mg/L.

- ATM-AVI/EUCAST *Stenotrophomonas maltophilia* International MIC Distribution – Reference Database 2025-09-30, Based on Aggregated Distributions

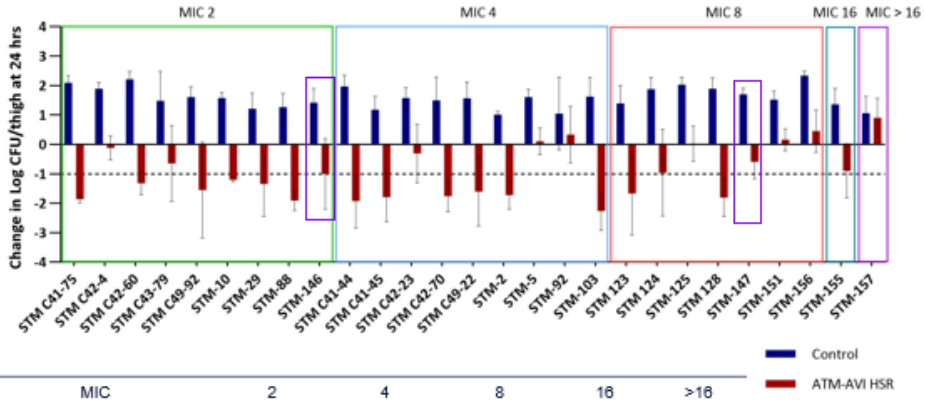


MIC
ECOFF: 8 mg/L
Wild Type organisms ≤8 mg/L

Confidence Interval: 4 – 8
805 observations (4 data sources)

MIC distributions include collated data from multiple sources, geographical areas, time periods, and can never be used to infer rates of resistance.

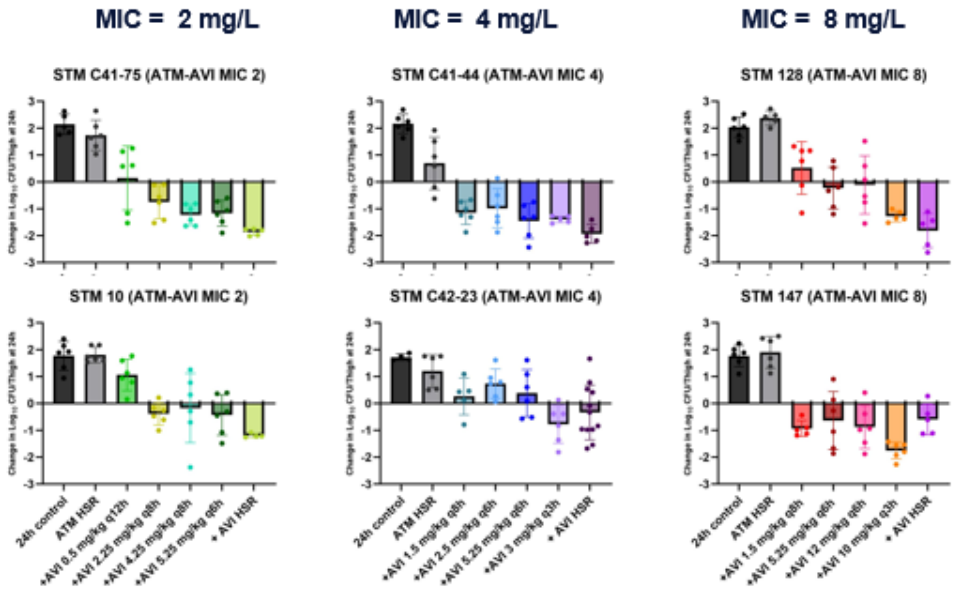
- *In Vivo* Efficacy of ATM-AVI Against *Stenotrophomonas maltophilia* in the Murine Thigh Infection Model with Human Simulated Regimen (HSR)
  - 27 *S. maltophilia* clinical isolates tested in the neutropenic mouse thigh infection model
    - ATM-AVI MICs between 2 to >16 mg/L
    - ATM MICs >64 mg/L
    - 2 isolates were from the ATM-AVI Phase 3 clinical studies
  - HSR: 1.5g ATM and 0.5g AVI q6h as a 3-hour infusion (labeled dosing regimen)
  - Conducted at Center for Anti-Infective Research & Development, Hartford Hospital, Hartford, CT
  - Investigators: Yakun Fu; Aliaa Fouad; David P Nicolau; Joseph L Kuti
- *In Vivo* Efficacy of ATM-AVI Against *Stenotrophomonas maltophilia* in Murine Thigh Infection Model – HSR



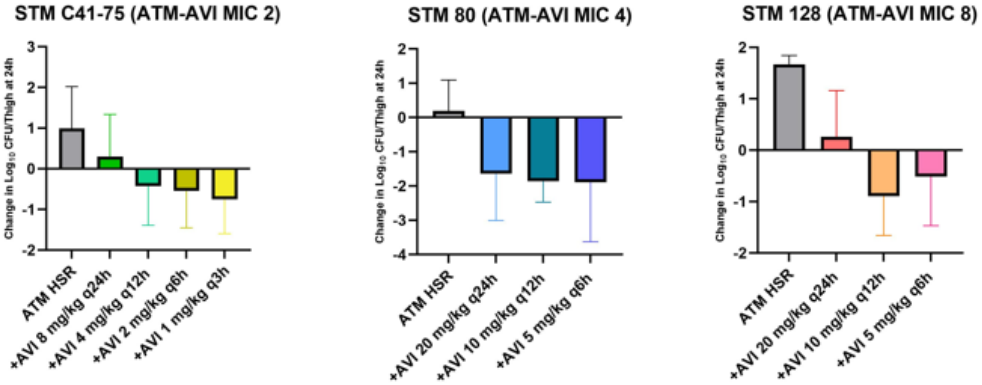
Isolates STM-146 and STM-147 were from an ATM-AVI clinical study

MIC	2	4	8	16	>16
≥1 log kill (%)	7/9 (78%)	6/9 (67%)	2/7 (29%)	0/1 (0%)	0/1 (0%)
Mean reduction in CFU (SD)	-1.22 (0.73)	-1.22 (0.78)	-0.63 (0.83)	0	0

- Additional Dose Fractionation Study to assess PK/PD target for ATM-AVI Against *S. maltophilia* in the Murine Thigh Infection Model
  - Study consisted of the following:
    - Single-dose PK studies of avibactam to establish PK across a wider dose range
    - Dose-ranging studies to assess the magnitude of exposure of avibactam associated w/efficacy
    - Dose-fractionation studies of avibactam in combination with clinical exposure (HSR) of aztreonam
    - Monte Carlo simulations to confirm PK/PD target attainment
  - 6 *S. maltophilia* clinical isolates tested
    - ATM-AVI MICs of 2, 4, or 8 mg/L (2 isolates at each MIC)
    - ATM MICs >64 mg/L
    - 1 isolate from the ATM-AVI Phase 3 clinical studies
  - Conducted at Center for Anti-Infective Research & Development, Hartford Hospital, Hartford, CT
  - PD – Dosing Ranging Results



- PD – Dose Fractionation Results



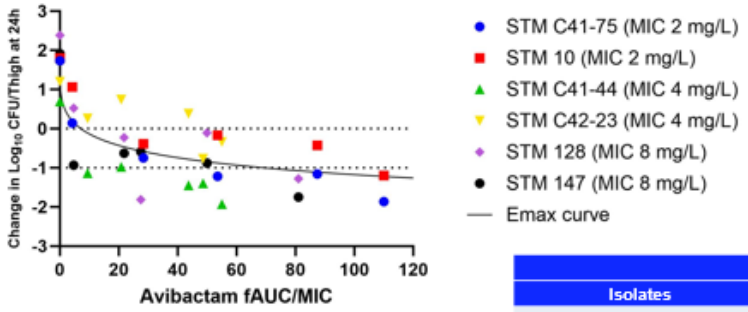
One-way ANOVA test indicated no statistically significant difference between dosing groups

- PK/PD targets from composite E_{max} curves
  - No clear distinction between PK/PD indices
  - Large difference between stasis and 1-log kill targets
  - AHWG generally favored fAUC/MIC as having more robust predictive power

- More aggressive target given lack of clinical and pneumonia model data

PK/PD index	Stasis target	1-log kill target	R^2
*f*T> C_T 2	18.37	114.12	0.48
*f*T> C_T 2.5	11.19	108.26	0.48
*f*T> C_T 3	11.16	107.98	0.48
*f*T> C_T 3.5	8.62	106.34	0.48
*f*T> C_T 4	5.51	107.63	0.48
*f*T> C_T 4.5	4.04	109.50	0.48
*f*T> C_T 5	1.14	126.56	0.48
*f*T> C_T 8	5.42	59.71	0.32
*f*T> C_T 2.5/MIC	1.39	36.47	0.47
*f*T> C_T 3.5/MIC	1.15	31.32	0.48
*f*T> C_T 4.5/MIC	0.61	29.58	0.48
*f*AUC/MIC	7.13	69.40	0.50
fC_{max}/MIC	0.69	7.53	0.47

- Summary of fAUC/MIC targets for AVI in presence of ATM HSR

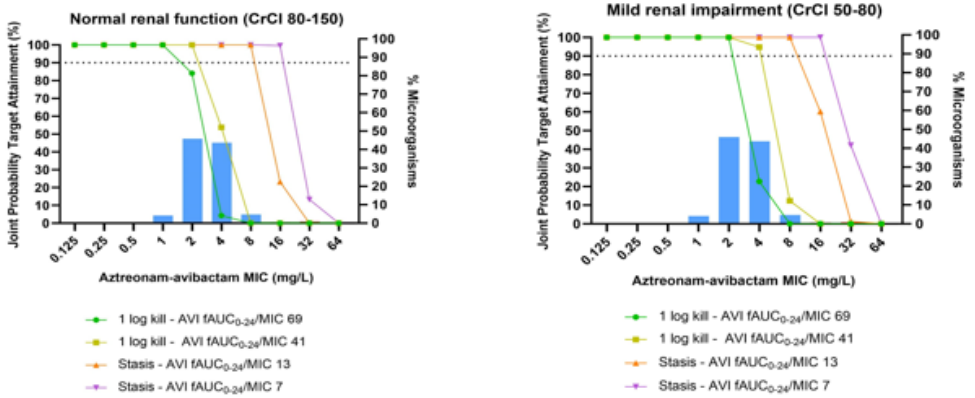


Avibactam fAUC/MIC			
Isolates	Stasis target	1-log kill target	R^2
STM C41-75 (MIC 2)	6.15	41.37	0.78
STM 10 (MIC 2)	25.43	179.35	0.65
STM C41-44 (MIC 4)	0.19	10.30	0.65
STM C42-23 (MIC 4)	37.78	92.08	0.30
STM 128 (MIC 8)	7.86	na	0.62
STM 147 (MIC 8)	0.18	34.78	0.71
Mean	12.93	71.57	0.62
Median	7.00	41.37	0.65
Composite	7.13	69.40	0.50

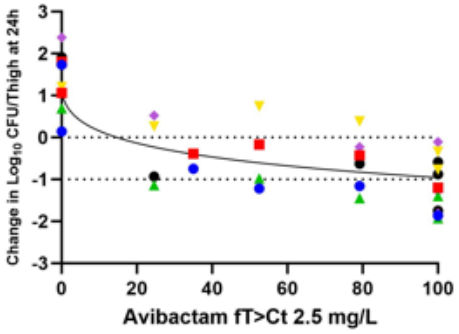
abbvie

- Joint PTA by MIC for ATM-AVI based on fAUC/MIC targets, overlaid on MIC distribution
  - AHWG focused on the 1 log kill target as it was the most consistent target predicting kill or stasis
  - PTAs support breakpoints 2 or 4 mg/L (more robust at 2 mg/L)

- Stasis targets - well-covered for MICs up to at least 8 mg/L
- 1-log kill targets – some variability depending on whether median (41) or composite (69) value is used



- Interfering Breakpoints from PK/PD Data Alone
  - Based on PK/PD alone, susceptibility breakpoint should be ≤2 µg/mL (dose fraction study) or ≤4 µg/mL (HSR study)
  - Some AHWG members did not feel comfortable setting breakpoints based on PK/PD data considering poor model fit and lack of validation in pneumonia model
- Summary of %fT>2.5 targets for AVI in presence of ATM HSR



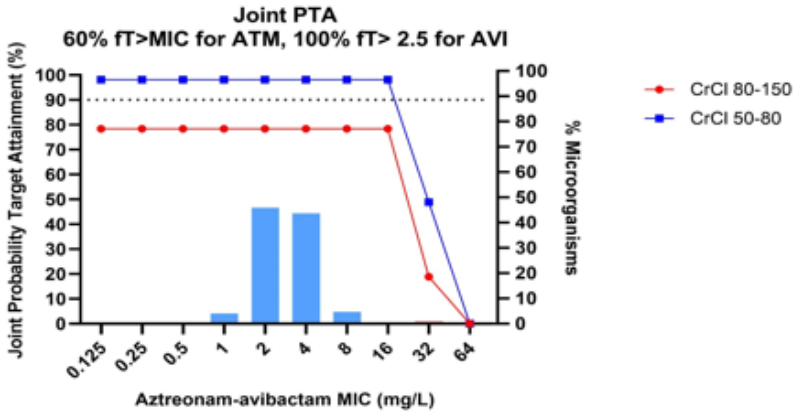
- STM C41-75 (MIC 2 mg/L)
- STM 10 (MIC 2 mg/L)
- STM C41-44 (MIC 4 mg/L)
- STM C42-23 (MIC 4 mg/L)
- STM 128 (MIC 8 mg/L)
- STM 147 (MIC 8 mg/L)
- Emax curve

Avibactam %fT>2.5			
Isolates	Stasis target	1-log kill target	R^2
STM C41-75 (MIC 2)	10.41	46.07	0.65
STM 10 (MIC 2)	25.52	134.79	0.63
STM C41-44 (MIC 4)	0.82	25.45	0.64
STM C42-23 (MIC 4)	90.30	107.81	0.35
STM 128 (MIC 8)	46.30	107.11	0.64
STM 147 (MIC 8)	0.0028	97.34	0.69
Mean	28.89	86.43	0.60
Median	17.97	102.23	0.64
Composite	11.19	108.26	0.48

abbvie

- Joint PTA by MIC for ATM-AVI based on %fT>2.5 target of 100%, overlaid on MIC distribution

- Stasis targets (not shown)- well-covered for MICs up to at least 8 mg/L
- 100% target – 80% or higher PTA up to at least 8 mg/L



- Conclusions from Murine Thigh Infection Avibactam Dose Fractionation Study
  - PK/PD index for *S. maltophilia* not clearly distinguished – both %fT>CT and fAUC/MIC show some correlation with bacterial killing
  - Regardless of the PK/PD index, stasis targets are well-covered up to an MIC of 8 mg/L (joint PTA > 90%)
  - Variability in 1-log kill targets and resulting PTA
  - These data contribute to the totality of data supportive of MIC S/I/R breakpoints of 4/8/16 mg/L

- Inferring Breakpoints from Clinical Outcomes Data Alone
  - Clinical outcomes data are insufficient to inform ATM-AVI breakpoints for *S. maltophilia*
- Proposed Interpretive Criteria for ATM-AVI and *Stenotrophomonas maltophilia*
  - Request ATM-AVI Placement in Table 1B-4 Tier 3

Pathogen	Minimum Inhibitory Concentration (mg/L)			Disk Diffusion Zone Diameters (mm)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
S. maltophilia **Proposed Breakpoints**	≤ 4/4	8/4	≥16/4	≥ 27	23-26	≤ 22

- AHWG Discussion and Recommendation
  - ECV data alone: ≥8 µg/mL
  - PK/PD data alone: ≤2 µg/mL or ≤4 µg/mL
  - Clinical data alone: not applicable

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

- Scenarios

Breakpoint Scenario	Vote	Pro	Con
No breakpoints	2 of 7	Lack of validation in pneumonia model/lungs and in patients; adheres to M23 guidance	Antibiotic options are limited for *S. maltophilia,* ATM-AVI won't be an option for regions without access to cefiderocol
2/4/8 µg/mL	0 of 7	"Safer" for patients	Cuts into wild type distribution, no clinical data that MICs of 2 µg/mL are associated with favorable outcomes
4/8/16 µg/mL	6 of 7	Harmonizes with Enterobacterales; doesn't hold ATM-AVI to a bar higher than TMP-SMX, minocycline, levofloxacin	No clinical data that MICs of 4 µg/mL are associated with favorable outcomes
8/16/32 µg/mL	2 of 7	Won't cut into wild type distribution, "some" kill or stasis still observed at 8 µg/mL in thigh infection model (HSR)	No clinical data that MICs of 8 µg/mL are associated with favorable outcomes, PTAs do not clearly support treatment of 8 mg/L, could send a message that ATM-AVI data are more robust for *S. maltophilia* than Enterobacterales

- Breakpoints Working Group Discussion and Recommendation
  - Leaning towards setting a breakpoint
  - Consideration for breakpoint as set by AHWG (4/8/16 µg/mL) but concerns about limited data
  - No data on aztreonam PK in epithelial lining fluid (lung)
  - Motion to approve ATM-AVI MIC breakpoints with comment similar to cefiderocol that states "(3) Breakpoints are based on PK/PD properties, MIC distributions, and limited clinical data." WG Vote: 6-1-1-5.
  - Opposed favored 8/16/32 to avoid cutting into WT distribution

MIC (mcg/ml)			Zone Diameter (mm)		
Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
≤4	8	≥16	≥29	23-28	≤22

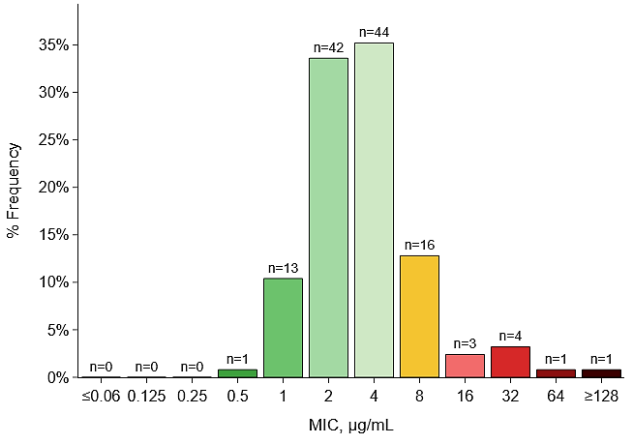
SC DISCUSSION (MAIN POINTS)

- Concern about cutting into the wild-type if a susceptible breakpoint is at 4 µg/mL and statis only will cove that.
- ATM drug is needed for places in the world who do not have access to cefiderocol.
- The data for SXT, minocycline, and levofloxacin look even worse than aztreonam-avibactam.
- CLSI needs more standards on animal models. CLSI debates minimal changes in media but does not currently consider changes in animal models.
- *S. maltophilia* is a complex, so the field needs to look at this more granular level of identification.
- Should consider setting an ECV at 8 µg/mL.
- May want to review the other *Stenotrophomonas* drugs in the future.
- Consider stating not be used as monotherapy.
- There is not good data to say that combination is better. So perhaps CLSI should not add a comment about not using as monotherapy.

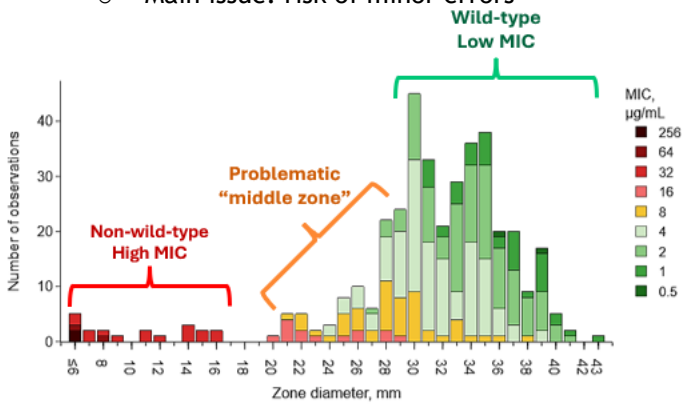
A motion to accept the aztreonam-avibactam MIC breakpoints (S ≤ 8/4, I 16/4, R ≥ 32/4 µg/mL) for *Stenotrophomonas maltophilia* with a comment stating, "Breakpoints are based on stasis PK/PD target, MIC distributions, and limited clinical data.", a comment stating to not use as monotherapy, and the sponsor dosage (2 g (1.5 g aztreonam + 0.5 g avibactam) IV q6h over 3h) was made and seconded. Vote: 14 for, 1 against, 1 abstain, 0 absent (Pass)

Against Vote Reasoning:
- The model is uncertain, an ECV would be better.

AZTREONAM-AVIBACTAM DISK DIFFUSION BREAKPOINTS FOR *STENOTROPHOMONAS MALTOPHILIA*
- Study Aim: Evaluate aztreonam-avibactam disk to MIC correlation for *Stenotrophomonas maltophilia*
- *S. maltophilia* MIC distribution
    - 125 *S. maltophilia* blood isolates
    - BMD modal MIC
    - 83% susceptible, 13% intermediate, 8% resistant
        - Based on proposed CLSI breakpoints (S<4, I=8, R>16)



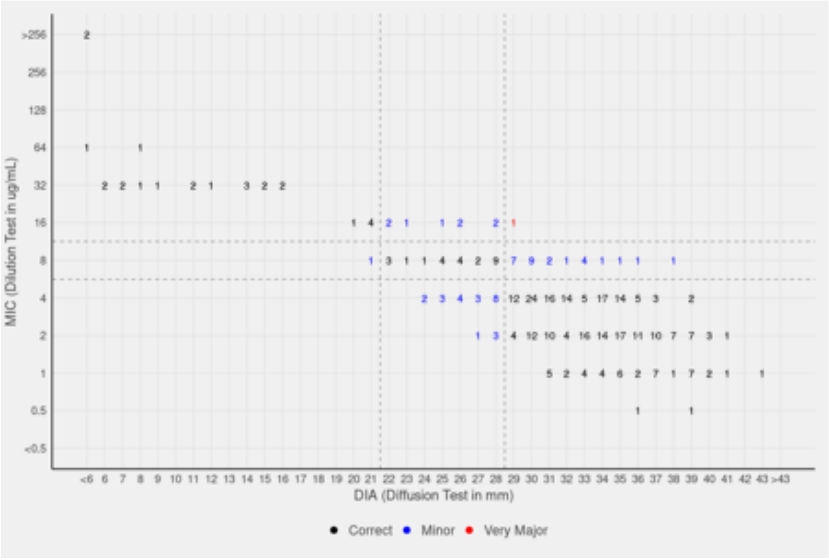
- S. maltophilia disk-to-MIC histogram (all datapoints combined)
  - Majority of *S. maltophilia* isolates have AZA MICs of 2-4 (at or near the susceptible breakpoint). Difficult to get optimal separation near the breakpoints.
  - Main issue: risk of minor errors



- dBETS best-fit disk breakpoints estimate

dBETS best-fit disk breakpoints estimate

Susceptible	Intermediate	Resistant
≥29 mm	22-28 mm	≤21 mm



MIC	No. of isolates	VME	ME	MI
≥ I + 2	20	0	0	0
I ± 1	198	1 (0.5%)	0	56 (28%)
≤ I - 2	164	0	0	4 (2.4%)
Total	382	1 (2.9%)	0	60 (15.7%)

Acceptable error rates per CLSI M23 criteria

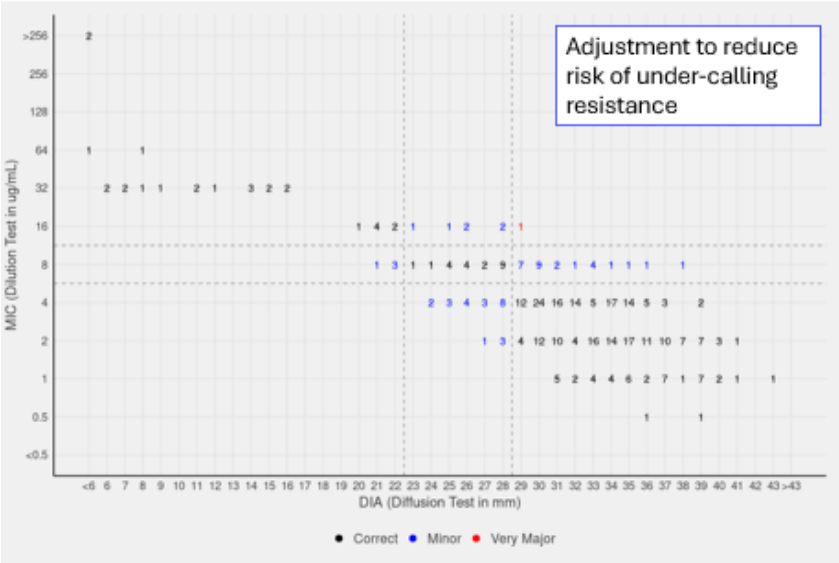
Larger intermediate zone (7mm)

MIC range	M23 Acceptable Error Rates %		
	VME	ME	MiE
≥ I+2	<2	NA	<5
I±1	<10	<10	<40
≤ I-2	NA	<2	<5

- dBETS best-fit with manual adjustment

dBETS best-fit with manual adjustment

Susceptible	Intermediate	Resistant
≥29 mm	23-28 mm	≤22 mm



MIC	No. of isolates	VME	ME	MI
≥ I + 2	20	0	0	0
I ± 1	198	1 (0.5%)	0	57 (28.8%)
≤ I - 2	164	0	0	4 (2.4%)
Total	382	1 (2.9%)	0	61 (16%)

Acceptable error rates per CLSI M23 criteria

6mm intermediate zone

MIC range	M23 Acceptable Error Rates %		
	VME	ME	MiE
≥ I+2	<2	NA	<5
I±1	<10	<10	<40
≤ I-2	NA	<2	<5

- All data vs disk A only data

All data

dBETS best fit

Susceptible	Intermediate	Resistant
≥29 mm	22-28 mm	≤21 mm

MIC	N	VME	ME	MI
≥ I + 2	20	1 (0.5%)	0	56 (28%)
I ± 1	198	0	0	4 (2.4%)
≤ I - 2	164	1 (2.9%)	0	60 (15.7%)

Disk A only

(exclusion of Disk B)

Susceptible	Intermediate	Resistant
≥27 mm	23-26 mm	≤22 mm

MIC	N	VME	ME	MI
≥ I + 2	10	0	0	0
I ± 1	99	0	0	27 (27.3%)
≤ I - 2	82	0	0	0

dBETS best fit

Manual best fit

Susceptible	Intermediate	Resistant
≥29 mm	23-28 mm	≤22 mm

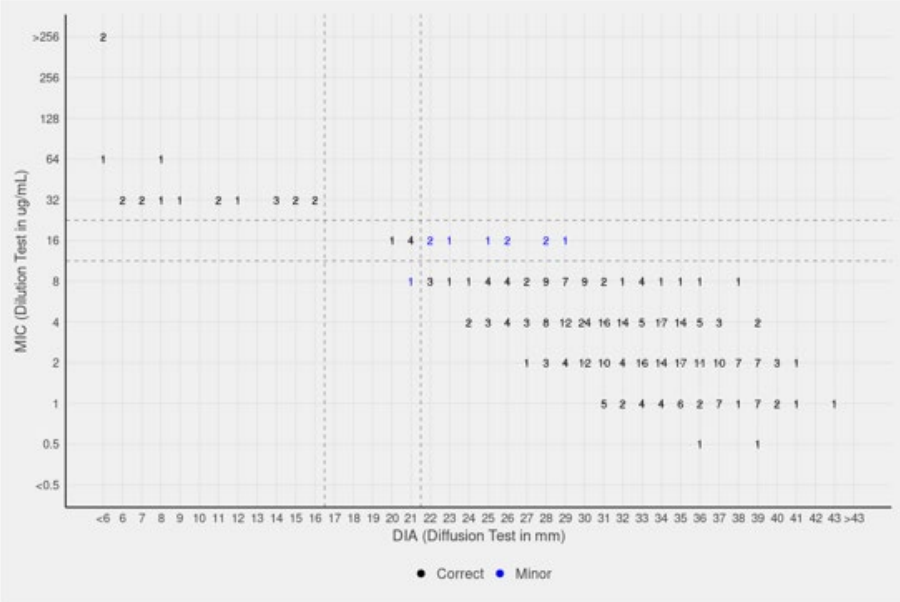
MIC	N	VME	ME	MI
≥ I + 2	20	1 (0.5%)	0	57 (28.8%)
I ± 1	198	0	0	4 (2.4%)
≤ I - 2	164	1 (2.9%)	0	61 (16%)

MIC range	M23 Acceptable Error Rates %		
	VME	ME	MiE
≥ I+2	<2	NA	<5
I±1	<10	<10	<40
≤ I-2	NA	<2	<5

- Best fit disk breakpoints with MIC breakpoints as S ≤ 8, I = 16, R ≥ 32

S	I	R
≥22mm	17-21mm	≤16mm

10 minor errors
All MI within one doubling dilution of intermediate MIC



- Breakpoints Working Group Discussion and Recommendation
  - Same motion as MIC breakpoints. One motion made to accept the MIC and disk diffusion breakpoints.
  - Motion to approve ATM-AVI MIC breakpoints with comment similar to cefiderocol that states "(3) Breakpoints are based on PK/PD properties, MIC distributions, and limited clinical data." WG Vote: 6-1-1-5.
  - Opposed favored 8/16/32 to avoid cutting into WT distribution

MIC (mcg/ml)			Zone Diameter (mm)		
Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
≤4	8	≥16	≥29	23-28	≤22

A motion to accept the aztreonam-avibactam disk diffusion breakpoints (S ≥ 22, I 17-21, R ≤ 16 mm) for *Stenotrophomonas maltophilia* was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

ORAL CEPHALOSPORINS AD HOC WORKING GROUP REPORT

- Enterobacterales Cephalexin and Cefpodoxime Background

	Cephalexin	Cefpodoxime
FDA-labeled Indications	Bone infections: *P. mirabilis* Genitourinary tract infections, including acute prostatitis: *E. coli, P. mirabilis, K. pneumoniae* 250 – 500 mg q6 – 12h x 7 – 14 d	Uncomplicated UTI: *E. coli, K. pneumoniae, P. mirabilis* 100 mg q12h x 7 d
FDA-labeled max daily dose	4 g	800 mg (400 mg q12h)
FDA STIC	None	CLSI BP recognized: ≤2/4^/≥8
PO Bioavailability	~90%	~50% (↑ w/ food)
Urine Excretion	70 – 100% within 6-8h	29 – 33% within 12h
Urine Concentrations	250 mg dose: 1,000 mcg/mL 500 mg dose: 2,200 mcg/mL 1 g dose: 5,000 mcg/mL (peak concentrations)	~20 mcg/mL at 8-12h

  - USCAST Oral Cephalosporin STIC for Enterobacterales
    - Cephalexin: 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
  - 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	

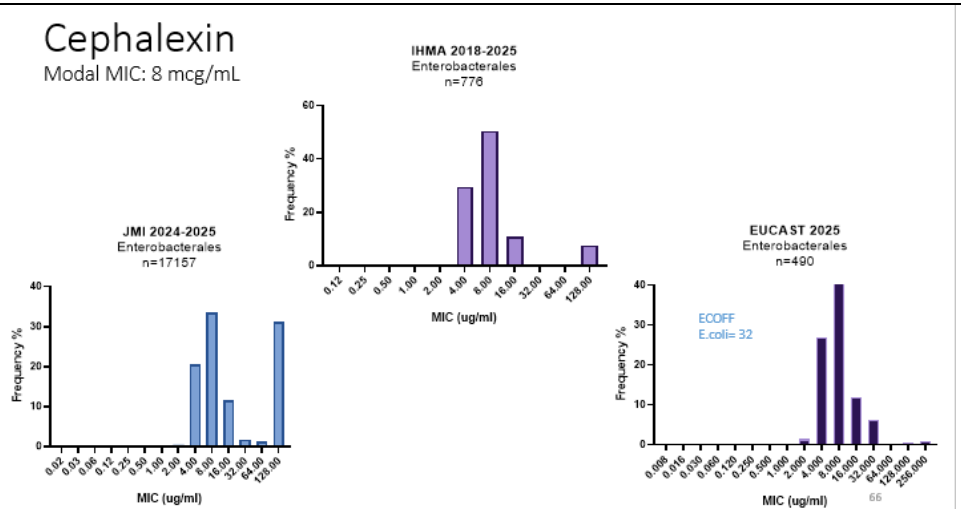
Cephalosporins	Uncomplicated UTI
Cefadroxil	0.5-1 g x 2 oral
Cefalexin	0.25-1 g x 2-3 oral
Cefixime	0.2-0.4 g x 2 oral
Cefpodoxime	0.1-0.2 g x 2 oral
Ceftibuten	
Cefuroxime oral	0.25 g x 2 oral

- CLSI Cefazolin Breakpoints for Enterobacterales
- Key Point: The cefazolin surrogate breakpoint indicates >95% categorical agreement based on an MIC ≤16 mcg/mL for BOTH agents.
- It is not based on a cefazolin MIC ≤16 mcg/mL compared to the "S" breakpoint in Table 2A for each oral cephalosporin (eg, cefpodoxime MIC ≤2 mcg/mL)
- FDA Rationale on the Use of Cefazolin Breakpoints for Oral Cephalosporins for the Treatment of Uncomplicated Urinary Tract Infections

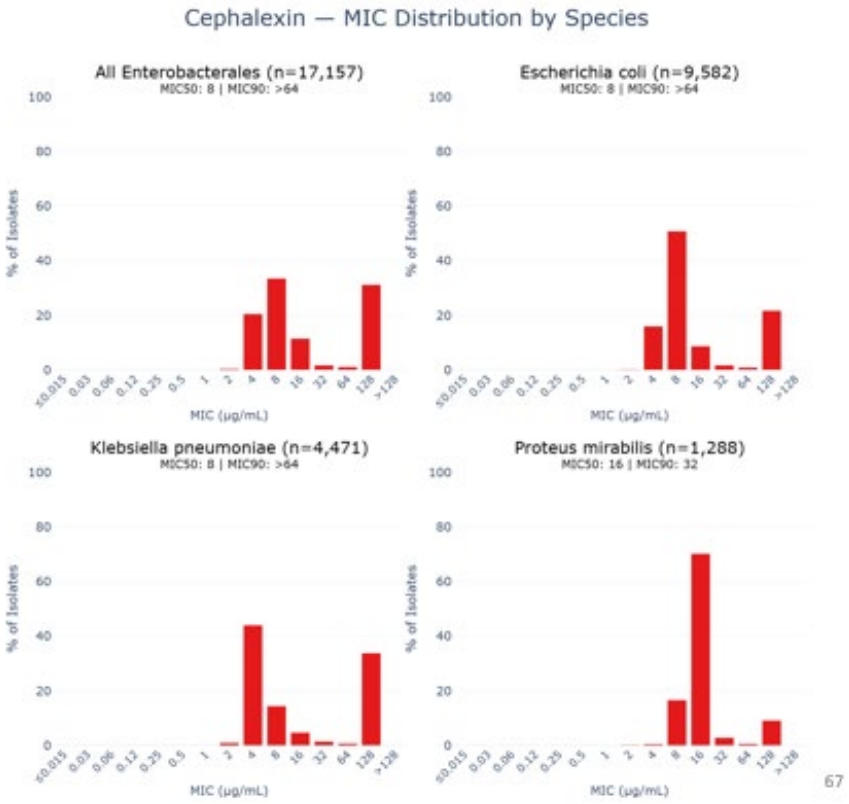
No clinical studies were provided that evaluated the efficacy of cefazolin or oral cephalosporins in the treatment of uUTI based on the proposed urine-specific breakpoint for *Enterobacterales*. The submitted materials included studies reviewing the microbiolog and clinical outcomes of uUTI when treated with oral cephalosporins without specifying the distribution of MICs of the urinary pathogens nor the association of MICs with the microbiological or clinical outcomes. No decision support logic was provided using urinary drug exposure-antibacterial response relationships nor probability of target attainment analyses in patients with uUTI.

FDA has determined that there are insufficient data at this time to support the proposed cefazolin susceptible MIC breakpoint of ≤16 mg/L as a surrogate for determining breakpoints for oral cephalosporins for the treatment of uUTIs due to *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Therefore, no changes are recommended on the FDA STIC website.

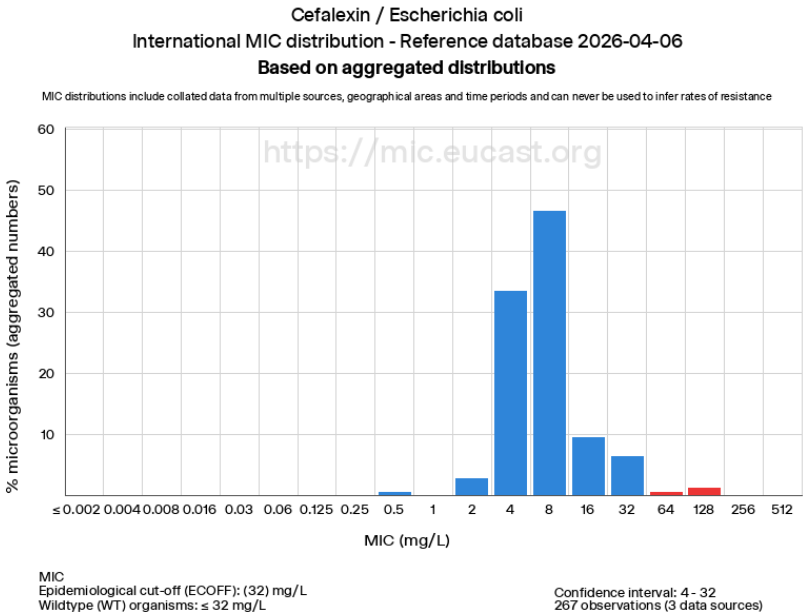
- Cephalexin



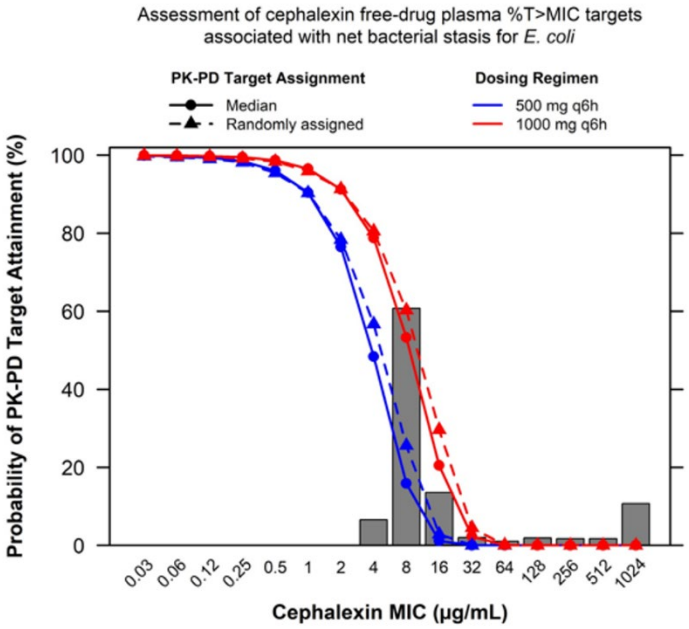
- JMI 2024-2025 (Sentry) species-level data



- EUCAST species-level data for *E. coli*. ECV: EUCAST = 32 and USCAST = 16



- PK/PD (Plasma)
  - USCAST: Probability of Target Attainment for *E. coli* in plasma
    - Target: ~60% fT>MIC (stasis)
    - MIC Distribution of *E. coli* isolates (n=1,170) collected in US in 2018 or 2021 provided by JMI

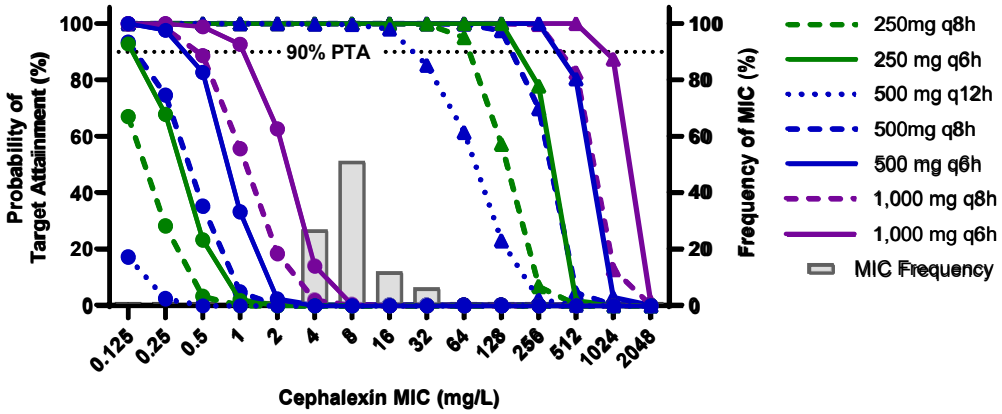


- Cattrall et al. Eur J Clin Microbiol Infect Dis 2019; 38: 2311–2321
  - For *E. coli* isolates with MICs ≤16 mcg/mL (EUCAST breakpoint), 4,000 mg every 6 hours required to achieve CFR >0.9.
- Yamada et al. Diagn Microbiol Infect Dis 2022; 103: 115662
  - PTA >90% achievable with cephalexin MICs ≤4 mg/mL
- Cephalexin Model-Informed Dosing Recommendations: A Pooled PK Analysis From Infancy to Early Adulthood (Dr. Andrew Haynes)
  - Cephalexin PTA for Enterobacterales (60% fT>MIC)
  - >90% PTA unachievable for MICs ≥8 mcg/mL even with dosing above FDA label (1,500 mg QID). Exception: neonates.

- PK/PD (Urine)
  - Population PK Modeling (Data from Zack Bulman and Nick Smith)
    - No prior oral cephalexin or cefpodoxime PopPK models for urine existed
    - A PopPK meta-analysis was performed to build a model
      - 4 PK studies with cephalexin
    - Final Cephalexin Model:
      - 1-compartment oral PK model with a separate urine compartment for renal excretion/output
      - Zero-order absorption, linear total clearance
      - Consistent with previous studies, found that >90% was excreted in urine
  - Monte Carlo Simulations
    - Simulations performed in Simulx (n=500 simulated patients)

- Doses:
  - Cephalexin: 250 mg (q6h and q8h), 500 mg (q12h and q8h and q6h), 1,000 mg (q6h and q8h)
- Used model to predict plasma and urine concentrations over 5 days against isolates with simulated MICs from 0.125-2,048 mg/L
  - Urine frequency: every 4 hour
- Used stasis targets for *E. coli* in murine thigh infection model from USCAST study
  - Applied protein binding factor for plasma (cephalexin: 15%)
- MIC distributions were obtained from EUCAST website
  - Cephalexin: 267 *E. coli*, 64 *K. pneumoniae*, 159 *P. mirabilis*

▪ Probability of Target Attainment
- Target: 60% fT>MIC (~stasis target for *E. coli* in thigh infection model)



- Assumptions and Limitations
  - Assumes target is the same in urine and thigh
  - Population PK models were built via meta-analysis of previous studies
    - Some previous studies did not report individual-level data, making it difficult to assess inter-individual variability
    - However, one study did report individual subject data
  - Urine models are largely informed by a single study that had subject level cephalexin concentrations in plasma and urine.
  - Urine concentrations have higher degree of variability than plasma due to additional sources of variability (urination frequency, urine volume) in addition to sources of variability that impact plasma concentrations (volume and clearance)

○ Clinical Summary

▪ Bacteremia from ≥50% urinary source:
- Two retrospective trials of mainly hospitalized adults status post 3-4 days IV antibiotic and median 11 days total duration

- Outcomes specific to cephalexin not parsed out (proportion of ß-lactam group 42% – 90%) and dosing varied 500 mg – 1 g q12 – q6h), but no differences in primary outcome
- One large, retrospective trial of hospitalized, older adults with unspecified IV days and median 12 days total duration concluded ¤-lactam (30% cephalexin 500 mg – 1 g q6h) inferior to FQ/TMP-SMX

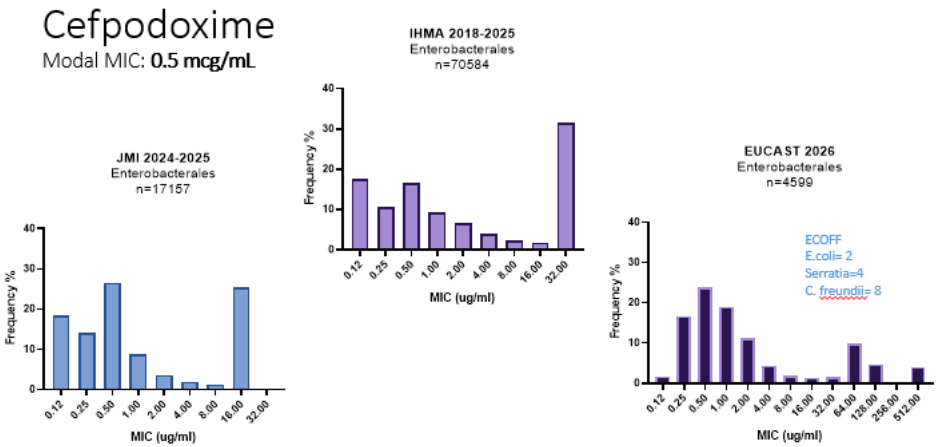
▪ Pyelonephritis without bacteremia:
- One retrospective trial of mainly adult females discharged from ED that reported superiority of cephalosporins (mainly cephalexin 500 mg three times daily) but groups were imbalanced and favored cephalosporins (eg, FQ/TMP-SMX often underdosed and lacked IV antibiotic dose, high rates of TMP-SMX resistance)
- One prospective trial of pregnant adult females with no difference in clinical success using cephalexin 1 g load, then 500 mg q6h x 14 days with no IV lead-in.

▪ UTI (cystitis):
- One retrospective trial of adults discharged from ED on either cephalexin 500 mg twice daily vs four times daily
  - No difference in clinical failure regardless of dosing

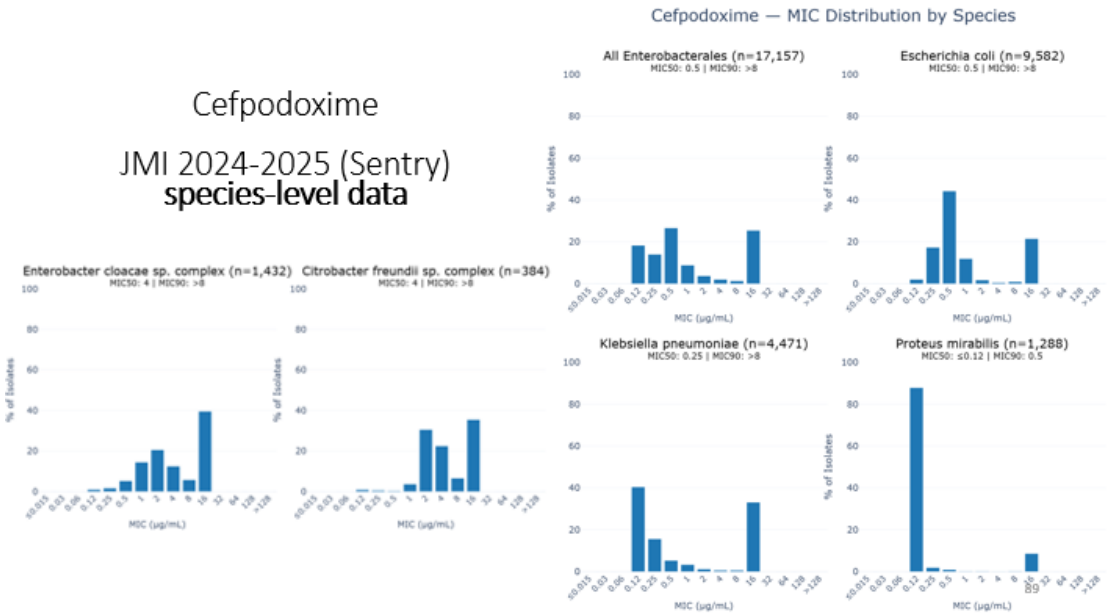
▪ No cephalexin MICs in any study
- Application of cefazolin surrogate outside of M100 recommendations noted in multiple studies

o AHWG Discussion and Recommendation
▪ Data do not support creation of a cephalexin breakpoint for systemic infections
▪ Data validate inclusion of cephalexin in the list of oral cephalosporins for which cefazolin is a surrogate for uncomplicated UTI at MIC ≤16 mcg/mL
▪ The PK/PD data obtained and reviewed by the AHWG support the creation of a cephalexin breakpoint for uncomplicated UTI at an MIC ≤32 mcg/mL based on a dose regimen of 500 mg at least q8h
▪ Is the juice worth the squeeze for clinical microbiology laboratories and AST device manufacturers?

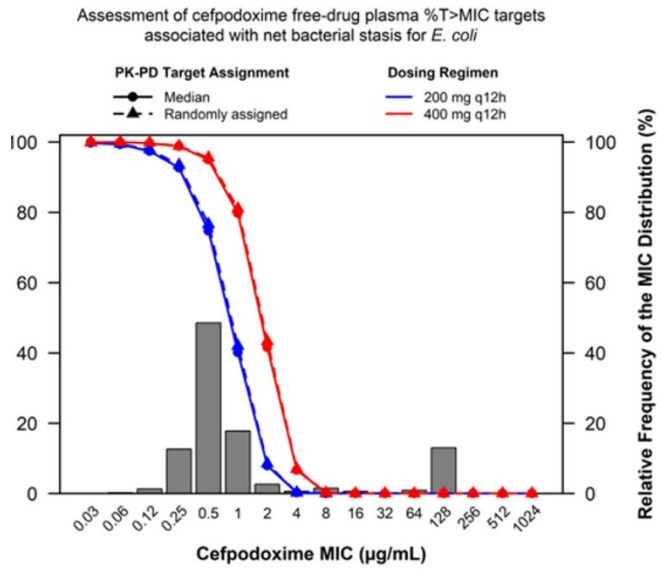
- Cefpodoxime



o JMI 2024-2025 (Sentry) species-level data

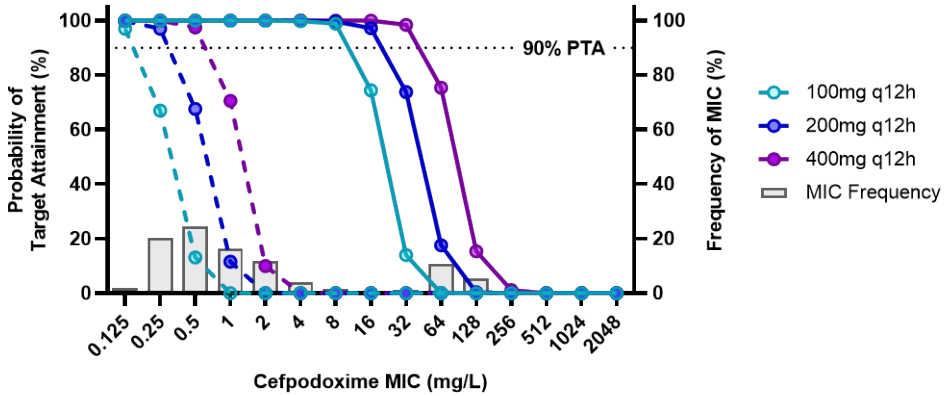


- USCAST: Probability of Target Attainment for *E. coli* in plasma
  - Target: ~50% fT>MIC (stasis)
  - MIC Distribution of *E. coli* isolates (n=1,171) collected in US in 2018 or 2021 provided by JMI



- PK/PD (Urine)
  - Population PK Modeling (Data from Zack Bulman and Nick Smith)
    - No prior cefpodoxime population PK models for urine existed
    - A PopPK meta-analysis was performed to build a model
      - 4 PK studies with cefpodoxime
    - Final Cefpodoxime Model:
      - 2-compartment model with a separate urine compartment for renal excretion/output
      - First-order absorption with lag time, linear total clearance
      - Fraction excreted in urine was fixed at 29% based on the prior studies
  - Monte Carlo Simulations
    - Simulations performed in Simulx (n=500 simulated patients)
    - Doses:
      - Cefpodoxime: 100, 200, 400 mg q12h
    - Used model to predict plasma and urine concentrations over 5 days against isolates with simulated MICs from 0.125-2,048 mg/L
      - Urine frequency: every 4 hours
    - Used stasis targets for *E. coli* in murine thigh infection model from USCAST study
      - Applied protein binding factor for plasma (cefpodoxime: 25%)
    - MIC distributions were obtained from EUCAST website
      - Cefpodoxime: 1,765 *E. coli*, 64 *K. pneumoniae*, 809 *E. cloacae*, 842 *C. freundii*
  - Probability of Target Attainment

- Target: 50% fT>MIC (~stasis target for *E. coli* in thigh infection model)



- Assumptions and Limitations
  - Assumes target is the same in urine and thigh
  - Population PK models were built via meta-analysis of previous studies
    - Some previous studies did not report individual-level data, making it difficult to assess inter-individual variability
    - However, one study did report individual subject data
  - Urine concentrations have higher degree of variability than plasma due to additional sources of variability (urination frequency, urine volume) in addition to sources of variability that impact plasma concentrations (volume and clearance)

- Clinical Summary
  - Bacteremia from ≥50% urinary source:
    - Two retrospective trials of mainly hospitalized adults status post 4-5 days IV antibiotics and median 14 days total duration
      - Unable to attribute lack of difference in primary outcome to cefpodoxime specifically as proportion of cephalosporin group was highly variable (25% – 70%)
      - Most patients across both studies (~70%) received cefpodoxime 200 mg twice daily
  - Pyelonephritis without bacteremia:
    - Three retrospective trials of mainly adult females discharged from ED status post 1 dose of IV antibiotic
      - Unable to attribute lack of difference in primary outcome to cefpodoxime specifically as proportion of cephalosporin group was highly variable (7% – 70%), as was dosing (100 – 400 mg twice daily)
      - Lin (2022) noted treatment failure higher for cefpodoxime (23.4%) vs cefuroxime (7.8%)
  - UTI (cystitis):
    - Four prospective trials of cefpodoxime 100 mg twice daily
      - No difference in bacteriological cure vs ß-lactam comparators x 7 days (~80%)
      - No difference in clinical or bacteriological cure vs TMP/SMX x 3 days (87% vs 86%)
      - Inferiority to ciprofloxacin 250 mg twice daily x 3 days with respect to clinical cure (82% vs 93%)

        - No cefpodoxime MICs in any study
            - Application of cefazolin surrogate outside of M100 recommendations noted in multiple studies
    - AHWG Discussion and Recommendation
        - Although some microbiologic distributions may support a cefpodoxime breakpoint of ≤2 mcg/mL for *E. coli*, the microbiology data for Enterobacterales as a whole are more problematic (eg, ampC producers with MIC_{50} = 2 to 4)
        - PK/PD data do not support retaining the cefpodoxime breakpoint of ≤2 mcg/mL for systemic infections due to Enterobacterales
        - Data validate inclusion of cefpodoxime in the list of oral cephalosporins for which cefazolin is a surrogate for uncomplicated UTI at MIC ≤16 mcg/mL
        - The PK/PD data obtained and reviewed by the AHWG support the creation of a cefpodoxime breakpoint for uncomplicated UTI at an MIC ≤16 mcg/mL based on a dose regimen of 200 mg q12h (data from Zack Bulman & Nick Smith)
        - Is the juice worth the squeeze for clinical microbiology laboratories and AST device manufacturers?
- CLSI Oral Cephalosporins Micro Laboratory Survey was conducted.
- What oral cephalosporins are available on AST Devices?

	Microscan Walkaway	BD Phoenix	Vitek II
Cefazolin (surrogate)	2 – 16 mcg/mL	2 – 16 mcg/mL	1 – 32 mcg/mL
Cefuroxime	4 – 16 mcg/mL	4 – 16 mcg/mL	1 – 64 mcg/mL
Loracarbef	-	-	-
Cefaclor	-	-	-
Cefdinir	-	-	-
Cefixime	-	-	-
Cefpodoxime	0.5 – 64 mcg/mL	-	0.25 – 8 mcg/mL
Cefprozil	-	-	-
Cefetamet (Inv.)	-	-	-
Ceftibuten (U, Inv.)	-	-	-

NOTE: May not be accurate for cards manufactured for use outside of US

- Breakpoints Working Group Discussion and Recommendation
    - Hesitant to pull one cephalosporin out of the document now, but not the others; could cause confusion
    - No ability to have FDA cleared test, would the FDA be ok with that to have urine breakpoints for individual drugs?
    - Start with low hanging fruit in the table; remove drugs that are not relevant
    - Additional urine PK/PD considerations
        - High urinary concentrations alone may not be sufficient
        - ß-lactams are concentration-independent killing drugs, which means the rate and extent of kill plateaus at a low multiple of the MIC. What matters is whether the drug remains active under actual urinary tract conditions
        - MIC values are measured under standardized conditions, not in urine
        - *In vivo* activity can shift with urine pH, protein binding, ionic composition, and metabolites

- Bacterial growth kinetics in urine differ from standard media, which can alter apparent susceptibility and the relevance of PK/PD targets
- Feedback on cephalexin
  - Agree that reviewed data support a urine only breakpoint for cephalexin
  - Agree that a urine only breakpoint may be clinically useful
  - Agree that reviewed data support inclusion of cephalexin in cefazolin uUTI surrogate
  - Agree that data do not support creation of a systemic breakpoint for cephalexin
- Feedback on cefpodoxime
  - Agree with removal of current systemic breakpoint for cefpodoxime
  - Based on the PK/PD and MIC distribution, any attempt to set systemic breakpoints will split the wild type distribution
  - Concern that only removing/updating cefpodoxime now would imply endorsement of other legacy breakpoints in the table
    - Option 1: remove all drugs now and add back as supported based on individual review
    - Option 2: leave table as is until individual drug decisions made and update as a whole later
    - Option 3: remove cefpodoxime but leave other drugs with comment similar to "legacy breakpoint that is not based on modern PK/PD or clinical outcomes data / under review"
  - Agree that reviewed data support urine only breakpoint for cefpodoxime
  - Agree that a urine only breakpoint would be clinically useful, especially for isolates with cefazolin MICs >16 that are ceftriaxone susceptible
  - A urine only cefpodoxime breakpoint of ≤16 mcg/mL may be difficult to implement with automated AST devices due to calling ranges (eg, Vitek 0.25 – 8 mcg/mL)
  - Caution advised given heavy reliance on *E. coli* exposure response for application to Enterobacterales as a whole in the absence of significant clinical data
  - Agree that reviewed data support inclusion of cefpodoxime in cefazolin uUTI surrogate
- Feedback on cefazolin surrogate
  - Consider updating cefazolin surrogate comment to reflect what has been assessed (and add in additional drugs as evaluated if supported): "Breakpoints for cefazolin when used as a surrogate test are for uncomplicated urinary tract infections due to *E. coli, K. pneumoniae,* and *P. mirabilis* and are based on microbiologic and PK/PD data to predict results for the oral agents cephalexin (at a dose of at least 500 mg every 8 hours) and cefpodoxime (at a dose of 200 mg every 12 hours). This surrogate should not be used to infer susceptibility of oral cephalosporins for systemic infections."
  - A modification of the above could refer for specific testing of cefpodoxime (and potentially other agents) if cefazolin screens >16 mg/L, and we could set urine only breakpoints agent by agent specifically for that, if desired
- General Feedback
  - Remove cefetamet (Inv.) and ceftibuten (U, Inv.) from M100 and put them wherever investigational breakpoints end up going.
  - Survey data suggest next oral cephalosporins to prioritize for review would be cefuroxime, cefixime, cefdinir
  - Consider adding a new clarifying comment to cephems (parenteral) cefazolin: "Systemic breakpoints for cefazolin should not be used as a surrogate for inferring susceptibility of oral cephalosporins for systemic infections."
- Request to AST Subcommittee – What is the path forward?
  - Straw poll but not official vote on the fate of cephalexin and cefpodoxime
  - Next drugs to focus on?
  - Next meeting: give update on next drugs but only take votes when all drugs are review and ready to be addressed?

- Pro: all oral cephalosporins addressed at once so no indirect "endorsement" for certain drugs vs ones removed
- Con: marathon session to review all drugs – TIME would be difficult to maintain with competing agenda items

SC DISCUSSION (MAIN POINTS)

- The cefazolin surrogate works with cephalexin for systemic infections. Up to the ECV at a dose of 1gq6 this does appear to work. This is an uncomplicated UTI drug up to the ECV and can use cefazolin surrogate testing to get there.
- Is this well known in the ambulatory community? Do ambulatory providers know about the performance?
- In general, oral cephalosporins are being misused from an antimicrobial stewardship perspective.
- There are a couple of large academic medical centers that transition to oral cephalosporins for Gram-negative bacteremia.
- Taking away the oral cephalosporins would likely cause issues for providers.
- Providers are not actually testing oral cephalosporins, but they are using it. Most people are using these drugs for step down therapy, and the breakpoints are not designed for step down therapy.
- The cephalexin and cefpodoxime original breakpoints were based on scattergrams.
- It is important that it is not being stated to not use the drugs for stepdown therapy.
- Perhaps there should be an IDSA guideline to educate providers or CLSI could do a rationale document. Providers want to know if they can discharge a patient on an oral cephalosporin.
- Perhaps the AHWG could spend time on more practical guidelines rather than review each additional cephalosporin.
- Would a cefpodoxime urine breakpoint be more helpful? CLSI could look at cefuroxime and cefixime.
- Is a systemic surrogate something that would work? This would be for *E. coli* and *Klebsiella*, but nothing with an AmpC.
- The comment that was removed stating that the urine breakpoints are only for uncomplicated UTI would have been helpful for laboratories.
- The literature indicates that up to the ECV the oral cephalosporins work.
- Focus on cephalexin for setting a systemic breakpoint. The cephalexin and cefpodoxime are the best options.
- Try to come up with a way for oral cephalosporins to step down out to the ECV is worth the effort. Prioritize cefixime, cefuroxime, cephalexin, and cefpodoxime. Plan early on how to get education out. Find out what the real gaps are and how to inform step down therapy. This is like what was tried to do with amoxicillin/clavulanate.
- Amoxicillin/clavulanate was different in that there were some studies using it as step down. Amoxicillin/clavulanate data also had MICs.
- There is some clinical stepdown data with oral cephalosporins such as babies with cephalexin. Many studies used cefazolin surrogate testing data.
- If there is no robust clinical data, then CLSI should be eliminating the drugs without data.
- There is not a clinical failure signal.
- Cefadroxil did not perform well with the surrogate testing.
- The AHWG should put together a publication on what they have investigated.
- Plan education to help fill in the gaps.

NACUBACTAM AD HOC WORKING GROUP REPORT

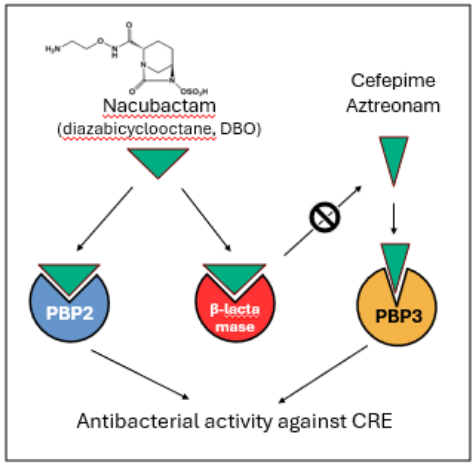
- Mechanism of Action of Nacubactam (NAC)

1. β-Lactamase Inhibition

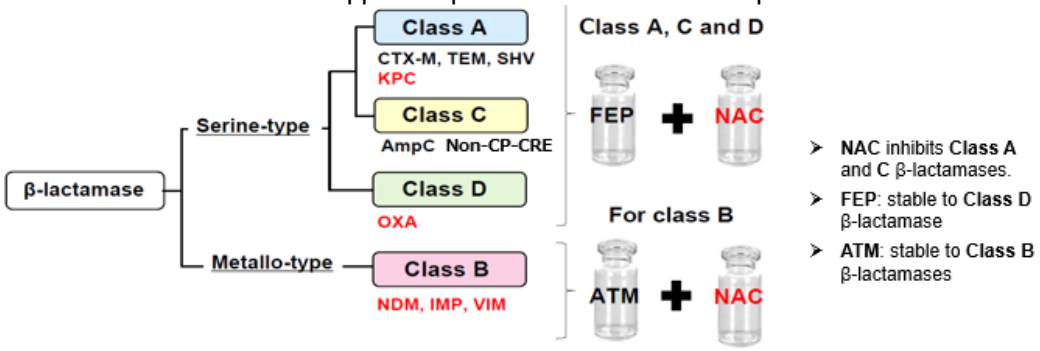
- Class A and C β-lactamases (Some class D)

2. β-lactam "enhancer"

- Directly inhibits PBP2 of Enterobacterales →Enhances PBP3-active β-lactams through PBP2 inhibition

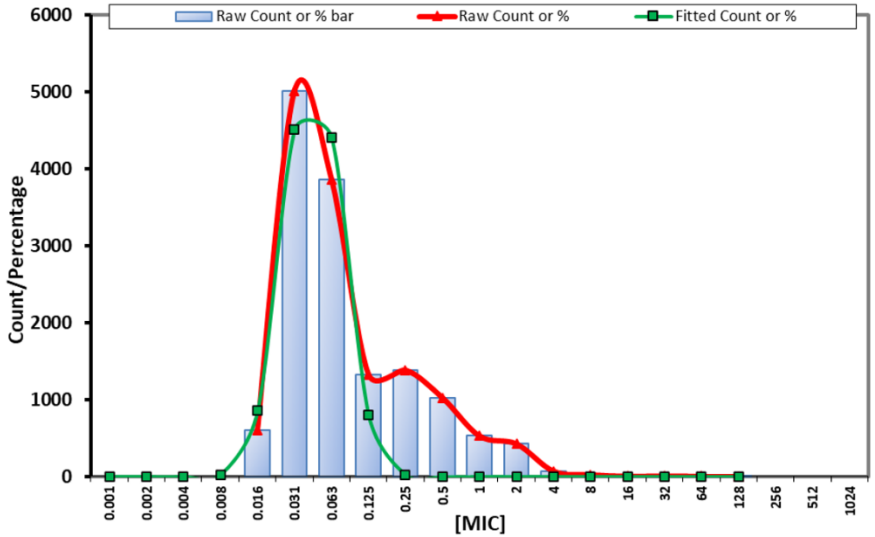


- Concept of Stand-Alone Development
  - Nacubactam (NAC) will be supplied in a single vial and used in combination with commercially available cefepime or aztreonam in clinical settings.
  - This stand-alone approach provides two treatment options for CRE with a new single agent, tailored to regional epidemiology.



- Rationale for 1:1 Ratio
  - As the plasma concentration profiles of FEP and NAC, as well as ATM and NAC, are similar when administered at the same doses in humans, a 1:1 ratio was selected for MIC determination.
  - The 1:1 ratio for MIC testing was based on an anticipated clinical dose of 1g of aztreonam or cefepime plus 1g of nacubactam in phase III studies. However, after discussion with the EMA, the phase III clinical studies were performed with 2g of aztreonam or cefepime plus 1g of nacubactam.
  - Consistency of MIC values was confirmed across concentration ratios, including 1:1 and 2:1.
- MIC Distribution Plot for FEP/NAC (1:1) Used to Calculate ECVs against all Enterobacterales combined (n=14,334)
  - The ECVs for both combinations were either 0.12 µg/mL or 0.25 µg/mL, depending on the percentage of the population analyzed.

- As per the CLSI M23 guideline, ECVs of 0.12 µg/mL obtained for FEP/NAC focusing on 97.5% of the captured population.



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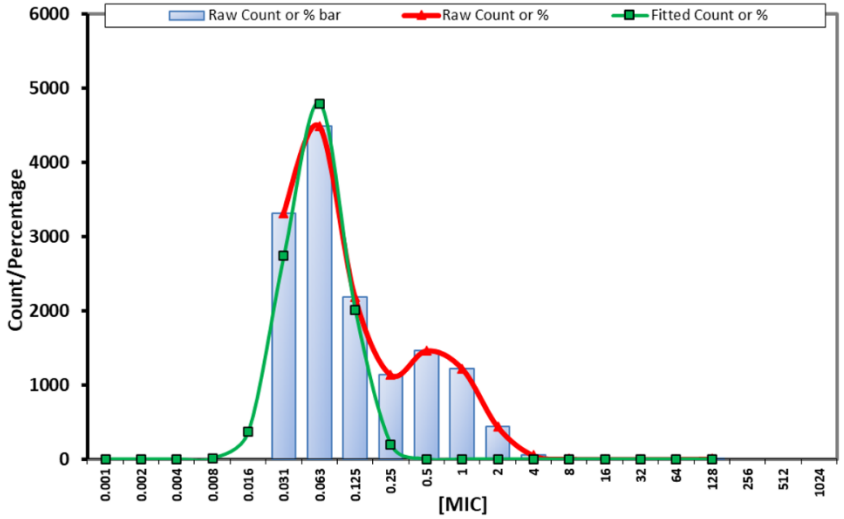
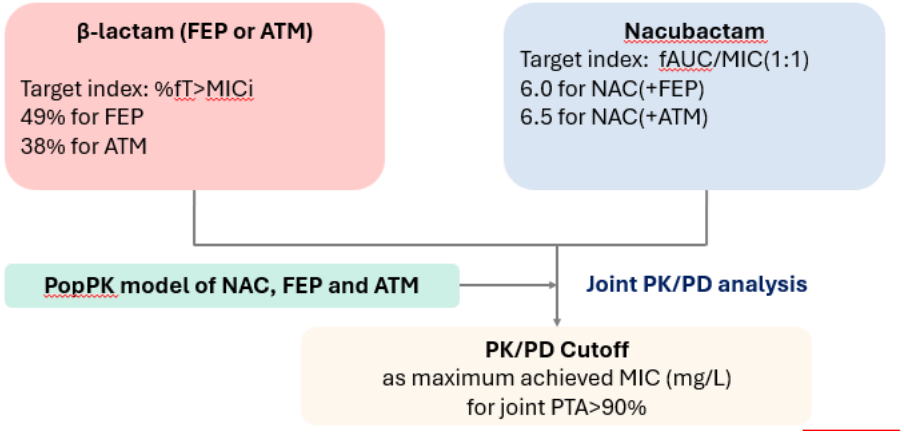
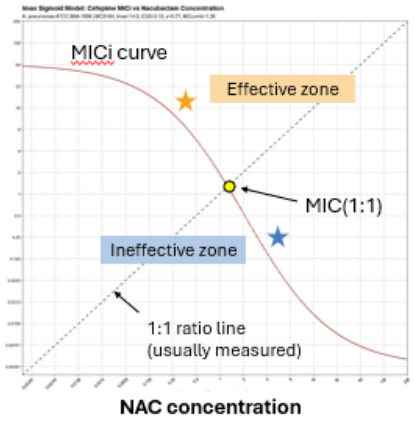


- PK/PD Summary



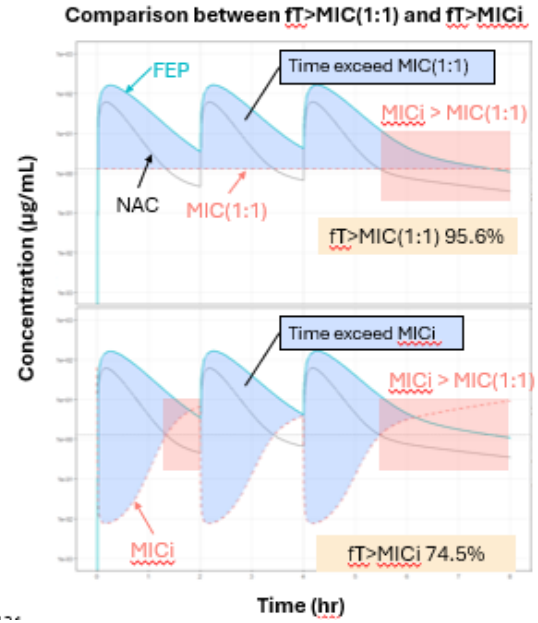
- High correlation of fT>MICi compared to conventional index
  - PK/PD of FEP/NAC was evaluated in a lung infection model against *K. pneumoniae* with MIC(1:1) of 0.5 mg/L (MSC22182) and 1 mg/L (MSC22098). (Both strains have an MIC of 128 mg/L against FEP alone.)
  - The analysis revealed that fT>MICi is a better indicator of efficacy than the conventional fT>MIC(1:1); the fT>MICi required for FEP(+NAC) to achieve a 4-log kill was approximately 30%.
- Concept of instantaneous MIC (MICi)
  - The concept of "instantaneous MIC (MICi)" was proposed by Bhagunde et al. in 2012 to explain ß-lactamase inhibitor (BLI) and the pharmacological activity of ß-lactams (BL), and was applied to NAC by Igarashi et al. in 2023.
  - In this concept, the MIC - the minimum inhibitory concentration of BL - is not a single concentration but fluctuates dynamically depending on the concentration of BLI.
- Checkerboard assay
  - Checkerboard assay can capture the MICi curve profile. MICi of FEP/ATM are dynamically changed by the NAC.
  - Also, the MICi curve may be influenced by various bacterial factors, such as bacterial species, resistance mechanisms, and the expression levels of resistance genes.
  - This assay provides valuable data regarding the drug and the bacterial strain.
- Relationship of MICi and conventional MIC (MIC1:1)
  - Plotted the MIC curve (MICi) and the 1:1 fixed ratio MIC line.
  - The intersection point represents the conventional MIC value tested clinically (referred to as MIC(1:1)(one-to-one)).
  - MIC(1:1) is merely a single point representing the MIC of BL.
  - Indeed, MIC(1:1) certainly indicates the concentration required for efficacy, it is not necessary for "both" drugs (BL and BLI) to exceed this value. This conservative approach fails to capture the synergistic effect of NAC and FEP/ATM.

(e.g.,) FEP/NAC
against *K. pneumoniae* ATCC BAA-1898

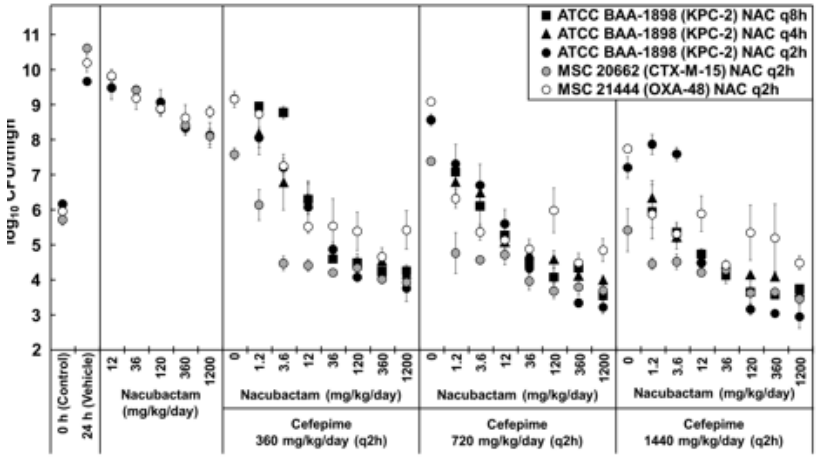


MIC0; 64 mg/L, Imax; 14.0 μg/mL, IC50; 2.10 mg/L, γ; 1.26

- fT>MIC(1:1) vs fT>MICi *in vivo*
  - Based on the PK parameters of NAC and FEP in neutropenic mice, a three-dose simulation was conducted to compare fT>MIC(1:1) and fT>MICi.
  - In the intervals highlighted in pink, NAC is below MIC(1:1), and MICi is above MIC(1:1); therefore, a high BL concentration is required for fT>MICi. On the other hand, fT>MIC (1:1) is determined based solely on MIC(1:1) and the BL concentration.
  - In this example, since the NAC concentration consistently remains lower than the BL, in the region where NAC<MIC (1:1), fT>MIC (1:1) will always be greater than fT>MICi.



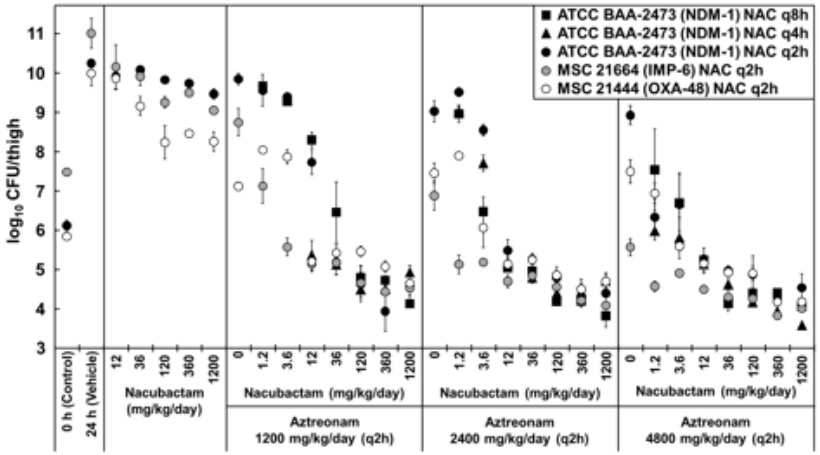
- Murine Dose-ranging Study of FEP/NAC against Murine Thigh Infection Model: Enterobacterales



Strain	BL	FEP/NAC MIC (1:1)
K. pneumoniae ATCC BAA-1898	KPC-2	1
K. pneumoniae MSC 21444	OXA-48	2
E. coli MSC20622	CTX-M-15	1

FEP/NAC showed dose-dependent bacterial reduction in a murine thigh infection model.

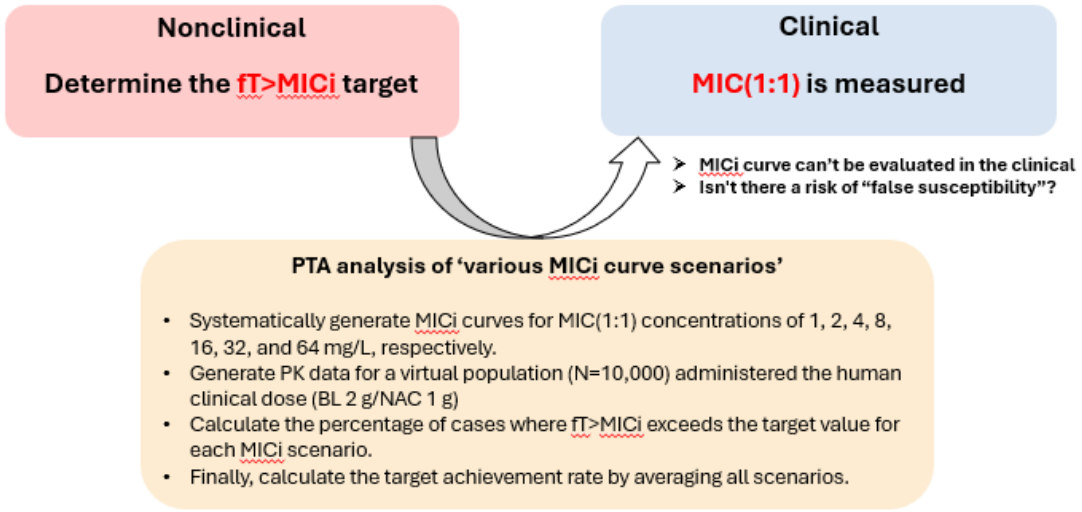
- Murine Dose-ranging Study of ATM/NAC against Murine Thigh Infection Model: Enterobacterales



Strain	BL	ATM/NAC MIC (1:1)
K. pneumoniae ATCC BAA-2473	NDM-1	1
K. pneumoniae MSC 21644	IMP-6 CTX-M-2	0.016
K. pneumoniae MSC 21444	OXA-48	1

ATM/NAC showed dose-dependent bacterial reduction in a murine thigh infection model.

- Target value of fT>MICi of FEP/ATM with NAC
  - The target values for fT>MICi for FEP and ATM in the presence of NAC were 49% and 38%, respectively.
  - These values are lower than the typical target values for ß-lactams.
- Relationship between fT>MICi and MIC(1:1)



- Robustness of fT>MICi

- As a result, the PTA achievement rate was unaffected by any of the MICi curve scenarios in this dose regimen*.
- These results suggest that the risk of "false susceptibility" is essentially low when breakpoints are set based on PTA analysis using target values for fT>MICi.
- Since these PTA analysis results are not based on the PPK model developed after Phase 3, the analysis must be performed again after the nonclinical targets have been redefined.

- AHWG Non-clinical PK/PD Studies Discussion with Sponsor
  - Not performed using human simulated regimens (HSR, sponsor proceeding with HSR studies)
  - Isolate MICs ranged from 0.016-2 mg/L, requested evaluation of MICs at proposed breakpoints, and with NDM/PBP2/PBP3 mutations where possible (sponsor to evaluate additional isolates with MICs of 8 mg/L in the animal model)
  - Additional sensitivity analyses including a range of MICi curves and PK/PD targets (sponsor to model based on PK model developed using Phase 3 study data and PK/PD targets from HSR studies and higher MIC isolates)
- Target index "fAUC/MIC" of NAC (+FEP)
  - The PK/PD parameter of NAC most strongly correlated with *in vivo* efficacy was fAUC/MIC(1:1). However, the correlation was poor for fT>MIC(1:1) and fT>Ct (1 μg/mL).
  - Most interestingly, for fT>Ct, the correlation increased as the Ct value decreased (eg, 0.0625 μg/mL). This seems to indicate that, NAC increases ß-lactam susceptibility at concentrations far below the MIC *in vivo* (sub-MIC).
  - Since the concentration threshold at which NAC can induce a synergistic effect is lower than the MIC and is likely to vary by bacterial species, it was considered appropriate to use fAUC/MIC as an index, rather than using a fixed concentration (Ct) as the threshold.
  - Note that since MICi indicate changes in the MIC of BL resulting from increased susceptibility due to BLI, there is no concept of MICi in NAC
- Target index "fAUC/MIC" of NAC (+ATM)
  - For NAC(+ATM), the situation is the same as for NAC (+FEP).
  - The parameter most strongly correlated with *in vivo* efficacy was fAUC/MIC.
- NAC target values in one-compartment (chemostat) model
  - In the *in vitro* one-compartment model, there was a good correlation between efficacy.
  - The fAUC/MIC(1:1) values of NAC required to achieve a 1-log kill were 5.1 and 10.2 in the presence of FEP and ATM, respectively.
- PK/PD target values (Enterobacterales)
  - PK/PD Target Values from *in vitro* (One-compartment model) and *in vivo* (Mouse thigh infection model) Dose-ranging studies are listed..
  - The target values for nacubactam were consistent *in vitro* and *in vivo*.
  - The PK/PD target values for FEP or ATM were determined in the mouse study. These values were consistent with general ß-lactam PK/PD target values.

Drug	PK/PD parameter	Study type	Stasis	1-log reduction	2-log reduction
Nacubactam (+Cefepime)	fAUC/MIC(1:1)	In vitro	3.1	5.1	9.0
		Mouse	1.1	6.0	NA
Nacubactam (+Aztreonam)		In vitro	6.4	10.2	21.8
		Mouse	0.7	6.5	NA
Cefepime [1)]	fT%>MICi	Mouse	30	49	NA
Aztreonam [2)]			22	38	NA

- Two global Phase 3 trials
  - Integral-1 (OP0595-5) study (cUTI/AP patients)
    - Randomized, double-blind, multi-site global study
    - Enrolled 614 patients with carbapenem-susceptible cUTI/AP (randomized 2:1:1)
    - Confirmed superiority of FEP/NAC and non-inferiority of ATM/NAC compared to imipenem/cilastatin
    - No safety concerns were identified
    - Efficacy in m-MITT
      - The difference between groups in the Primary Endpoint is attributable to differences in microbiological effects
  - Integral-2 (OP0595-6) study (CRE infection)
    - Randomized, single-blind, multi-site global study
    - Enrolled 126 patients with carbapenem-resistant cUTI/AP, cIAI and HABP/VABP
    - Assessed efficacy and safety of FEP/NAC and ATM/NAC across all infection types due to CRE
    - Primary Endpoint: Overall Success (mCRE-MITT population)
      - Overall success rates were numerically higher in FEP/NAC and ATM/NAC, compared with BAT.
      - This trend remained even when compared to the BAT group based on Ceftazidime/Avibactam or Cefiderocol.
    - Overall Success Rates by Carbapenemase Type
      - Overall success rates by carbapenemase type were also higher with FEP/NAC and ATM/NAC across all Ambler classes.
  - Clinical Cutoff Determination for FEP/NAC (2g/1g)
    - The clinical and microbiological outcomes of FEP/NAC were confirmed against baseline pathogens with MICs of up to 4 μg/mL.

m-MITT +patients with CRE in MITT population		MIC (µg/ml)										
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	64
∫Integral-1	Number	10	76	69	26	27	19	8	6	3		
	Clinical Cure	90.0%	94.7%	92.8%	88.5%	92.6%	84.2%	87.5%	100.0%	100.0%		
	Microbiological Cure	80.0%	88.2%	87.0%	92.3%	92.6%	84.2%	50.0%	83.3%	100.0%		
mCRE-MITT population		**MIC (µg/ml)**										
		0.015	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**64**
∫Integral-2	Number						2	14	8	3		1
	Clinical Cure						50.0%	78.6%	62.5%	66.7%		100%
	Microbiological Cure						50.0%	57.1%	62.5%	33.3%		0.0%
mCRE-MITT population, excluding "indeterminate" cases *		**MIC (µg/ml)**										
		0.015	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**64**
∫Integral-2	Number						2	12	6	2		1
	Clinical Cure						50.0%	75.0%	66.7%	50.0%		100%
	Microbiological Cure						50.0%	66.7%	66.7%	50.0%		0.0%

- Clinical Cutoff Determination for ATM/NAC (2g/1g)
  - The clinical and microbiological outcomes of ATM/NAC were confirmed against baseline pathogens with MICs of up to 2 µg/mL.

m-MITT +patients with CRE in MITT population		MIC (µg/ml)								
		<=0.03	0.06	0.12	0.25	0.5	1	2	4	8
∫Integral-1	Number	17	50	13	8	17	16			
	Clinical Cure	94.1%	92.0%	100.0%	100.0%	94.1%	87.5%			
	Microbiological Cure	88.2%	74.0%	84.6%	62.5%	76.5%	68.8%			
mCRE-MITT population		MIC (µg/ml)								
		<=0.03	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
∫Integral-2	Number	2	1	1		4	9	10		
	Clinical Cure	50.0%	0.0%	100.0%		25.0%	66.7%	40.0%		
	Microbiological Cure	50.0%	0.0%	100.0%		25.0%	88.9%	40.0%		
mCRE-MITT population, excluding "indeterminate" cases *		MIC (µg/ml)								
		<=0.03	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
∫Integral-2	Number	1	1	1		3	9	6		
	Clinical Cure	100.0%	0.0%	100.0%		33.3%	85.7%	66.7%		
	Microbiological Cure	100.0%	0.0%	100.0%		33.3%	85.7%	66.7%		

- AHWG Discussion and Recommendation
  - Major concern – will a standard MIC tell us what we need it to tell us?
    - Part of the mechanism of action of nacubactam is as a ß-lactam enhancer, potentiating killing through inhibition of multiple PBP targets (ie, NAC->PBP2 and FEP/ATM->PBP3)
    - PBP2 agents used as monotherapy have been associated with rapid emergence of resistance *in vitro*
    - Isolates may display low MICs, but it is unclear with just an MIC value if this is the "enhancer" effect or direct activity of nacubactam alone (ie, PBP2 monotherapy)
  - What is needed?
    - AHWG asked for HSR data to support use in isolates with MICs of 8 mg/L

- AHWG also asked to include isolates with different NDM variants, mutations in PBP2 and PBP3 for the HSR study, where possible
- This would provide supporting evidence for activity against a challenge set of isolates, and inform PK/PD targets to more confidently set a breakpoint

- Sponsor Additional Study Plan (Human-Simulated Regimen (HSR) Study)
  - While the proposed epidemiological, nonclinical PK/PD and clinical (Enterobacterales) cutoffs all support a susceptible MIC breakpoint of 8 µg/mL, it is necessary to enhance the robustness of the proposal through *in vivo* studies using strains with MICs around the breakpoint.
  - Murine human-simulated regimen (HSR) studies using multiple strains with various susceptibilities to ATM-NAC and FEP-NAC will be conducted, including the strains with a MIC of 8 µg/mL.
  - The study is expected to conclude in November 2026 and will be presented in CLSI January 2027 meeting.
- Next Steps
  - Proposed Epidemiological cut-off values, Nonclinical PK/PD cutoff, and Clinical cutoff (Enterobacterales)

Antimicrobial Agent	Epidemiological cutoff values	Nonclinical PK/PD cutoff	Clinical cutoff
Cefepime-nacubactam	0.12 µg/mL	8 µg/mL	4 µg/mL
Aztreonam-nacubactam	0.12 µg/mL	8 µg/mL	2 µg/mL

  - Proposed tentative MIC breakpoints for Enterobacterales

Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL			
	S	SDD	I	R
Cefepime-nacubactam	≤ 8/8	-	16/16	≥ 32
Aztreonam-nacubactam	≤ 8/8	-	16/16	≥ 32

  - Regulatory approval for Nacubactam in Japan is expected to be obtained by December 2026.
  - Proposed indications include various infections caused by Gram-negative bacteria showing resistance to carbapenem antibiotics.
  - Dosage 1 g of nacubactam with either cefepime 2 g or aztreonam 2 g, three times a day every 8h (1h infusion).
  - Stock solution preparation details already included in CLSI M100.
  - Abbreviations already agreed and included in CLSI M100: cefepime-nacubactam (FNC), aztreonam-nacubactam (ANC).
  - Full breakpoints of FNC and ANC for both MIC and disk method will be presented for a vote at the January 2027 meeting.
- Breakpoints Working Group Discussion and Recommendation
  - Confusion about the instantaneous MICs. Answered that this is done clinically a lot when thinking about *Enterococcus* with ampicillin and ceftriaxone.
  - Cefepime prolonged infusions are common but looks like only evaluated a 1h infusion. Sponsor says the prolonged 3h infusion has not been evaluated
  - Not convinced that the MICi is the end-all, be-all; may need to think more about how to do this testing.

SC DISCUSSION (MAIN POINTS)

- Is there something that could be measured in a laboratory?
- The MIC is an old concept and going to see more often that the MIC may not be able to predict the new drugs with new mechanisms.

- The pharmaceutical companies need to help take on the challenge of what a laboratory test will be to predict efficacy.
- For many BL/BLI in the past have not looked at the BLI alone, so this has gone above and beyond to understand what is going on.

GEPOTIDACIN MIC BREAKPOINTS FOR ENTEROBACTERALES

- Introduction (uUTI)
  - Novel, bactericidal, first in class triazaacenaphthylene antibacterial
  - Discovered at GSK
  - Chemically and mechanistically distinct from fluoroquinolones
    - Inhibits bacterial DNA replication by a distinct binding site, a unique mechanism of action, and a well balanced inhibition (most pathogens) of 2 enzymes (DNA gyrase and topoisomerase IV)
  - Is active against most isolates of target uUTI uropathogens, such as *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Citrobacter freundii* complex, *Staphylococcus saprophyticus* and *Enterococcus faecalis*, including those resistant to marketed antibacterials.
  - No target-specific cross-resistance with other classes of antibacterials
  - Oral formulation
  - Developed in partnership with DTRA and BARDA
  - Approved by the FDA on March 25, 2025 for the oral treatment of females with uUTI.
    - Also approved in the UK by the Medicines & Healthcare products Regulatory Agency on August 27, 2025 and by the FDA in December 2025 for the treatment of uncomplicated urogenital gonorrhea (being discussed in a separate slide set)
- FDA indication for uncomplicated UTI
  - Treatment of female patients ≥12 years of age with uncomplicated urinary tract infections (uUTI) caused by susceptible organisms
  - Oral formulation: 750 mg tablets
  - Dosage:
    - 1,500 mg (two 750 mg tablets) orally, twice daily (approximately 12 hours apart) for 5 days
    - Administer after a meal to reduce gastrointestinal intolerance
  - List 1 organisms:
    - Gram-positives: *Enterococcus faecalis, Staphylococcus saprophyticus*
    - Gram-negatives: *Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii* complex
  - List 2 organisms:
    - *Citrobacter koseri, Klebsiella aerogenes, Klebsiella oxytoca/Raoultella ornithinolytica, Morganella morganii, Proteus mirabilis, Providencia rettgeri*
- FDA Breakpoints

Gepotidacin – Oral Products

	Minimum Inhibitory Concentrations (mcg/mL)			Disk Diffusion (zone diameter in mm)		
Pathogen	S	I	R	S	I	R
Enterobacterales[a, b]	≤ 16	32	≥ 64	≥ 12	8-11	≤ 7
Staphylococcus saprophyticus[b]	≤0.25	-	-	≥ 23	-	-
Enterococcus faecalis[b]	≤4	-	-	≥ 14	-	-
Neisseria gonorrhoeae[c]	≤ 1	2	≥ 4	≥ 28	23-27	≤ 22

S = susceptible; I = intermediate; R = resistant

[a] Clinical efficacy was shown for *Escherichia coli*, *Klebsiella pneumoniae*, and *Citrobacter freundii* complex.

[b] Susceptibility interpretive criteria are based on a dose of 1500 mg twice daily.

[c] Susceptibility interpretive criteria are based on a dose of 3000 mg followed by a second dose of 3000 mg approximately 12 hours later.

- *In vitro* activity: List 1 uropathogens
  - ESBL-producing *E. coli*: 99.4% susceptible to gepotidacin (MIC ≤16)
  - ESBL-producing *K. pneumoniae*: 84.3% susceptible to gepotidacin
  - FQ-resistant *E. coli*: 99.7% susceptible to gepotidacin
  - FQ-resistant *K. pneumoniae*: 79.7% susceptible to gepotidacin

Organism*	Number of Isolates (n)	Gepotidacin MIC (µg/mL)		% Susceptible[a] (MIC ≤16)
		MIC range	MIC90	
Gram negative				
E. coli	8,495	≤0.03 to 32	4	99.9
K. pneumoniae	4,044	0.12 to >64	16	93.5
Citrobacter spp.**	250	0.5 to 128	8	98.8
Gram positive				
S. saprophyticus	774	≤0.03 to 0.5	0.12	99.9
E. faecalis	500	0.25 to 64	4	99.6

- *In vitro* activity: List 2 uropathogens
  - ≥ 90% of uropathogens on list 2 had an *in vitro* gepotidacin MIC less than or equal to the gepotidacin susceptible breakpoint for Enterobacterales (≤16 µg/mL)

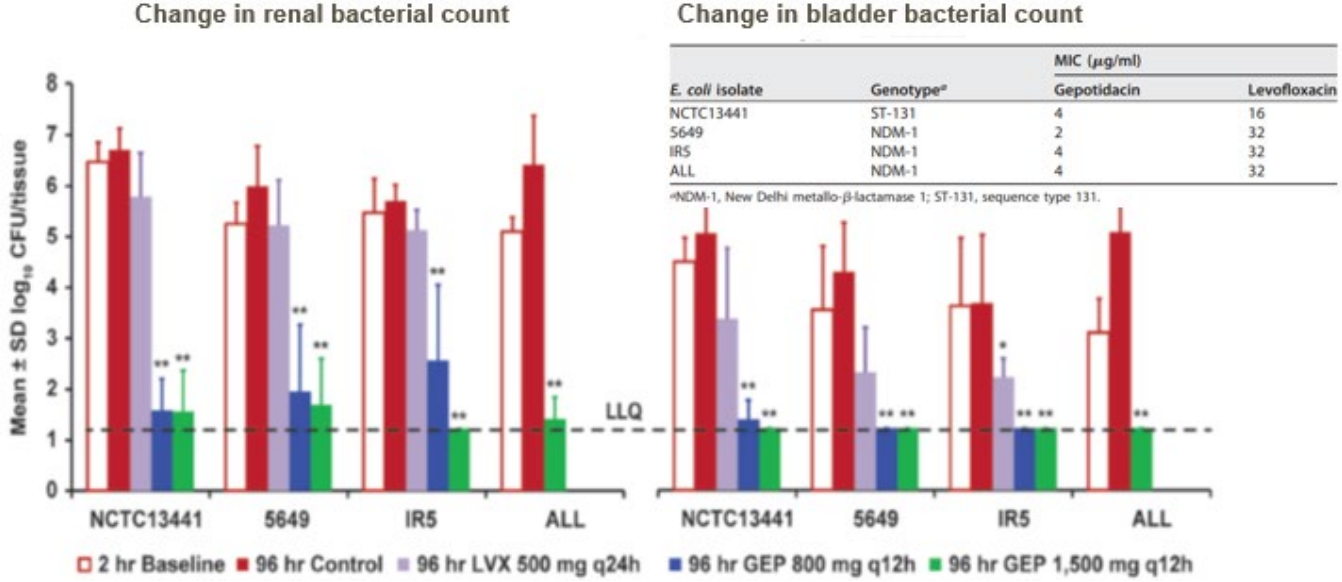
Organism[a]	Number of Isolates (n)	Gepotidacin MIC (µg/mL)		
		MIC range	MIC50	MIC90
K. oxytoca/ R. ornithinolytica	250	0.5-64	4	4
K. aerogenes	250	0.06-64	4	8
M. morganii	40	1-16	4	8
P. mirabilis	250	0.25-128	8	16
P. rettgeri	250	0.5->128	8	16

- *In vitro* AST test conditions/QC
  - Susceptibility testing variables that were shown to impact gepotidacin MIC results by ≥2 dilutions were pH and high inoculum concentration for all species tested
  - The effect of urine on gepotidacin MIC results: 1 - 2 dilutions higher and was not inclusive of all strains
    - For *E. coli* and *S. saprophyticus*, there was a trend for higher gepotidacin MIC results with the addition of increasing amounts of urine
  - Equivalency of dried MIC panels with CLSI reference method
    - Sensititre dried plates compared with CLSI frozen plate reference method for testing of 200 isolates
    - Essential agreement and reproducibility rates were both >99% (Zollos ICAAC 2014)

QC strain	CLSI approved QC range for gepotidacin	
	MIC (µg/mL)[a]	Zone diameter (mm)
E. coli ATCC 25922	1 to 4	18 to 26
E. faecalis ATCC 29212	1 to 4	NA
S. aureus ATCC 29213	0.12 to 1	NA
S. aureus ATCC 25923	NA	23 to 29

- Low potential for development of resistance and lack of target-specific cross-resistance (uUTI)
  - Gepotidacin's well-balanced inhibition (for most uUTI uropathogens) of two different Type II topoisomerase enzymes, DNA gyrase and topoisomerase IV leads to a low propensity for development of resistance due to the need for gepotidacin target-specific mutations in both enzymes to significantly impact gepotidacin susceptibility.
  - Gepotidacin target-specific mutations in a single enzyme may not significantly impact gepotidacin activity
  - Also illustrates the lack of target specific cross-resistance between fluoroquinolones and gepotidacin, as key gepotidacin target mutations in *E. coli* and *K. pneumoniae* do not result in fluoroquinolone resistance
- One possible case of development of resistance from EAGLE-2 and EAGLE-3 clinical trials
  - From initial screening 58/1,045 (5.6%) patients in EAGLE-2 and EAGLE-3 showed ≥4-fold increases in gepotidacin MIC between baseline and post-baseline isolates
  - Only 7 had confirmed MICs that were genetically related based on MLST/PFGE
  - After further genetic analysis (number of single nucleotide polymorphisms, SNPs within a given timeframe) only 1 case was considered potential development of resistance
- *In vivo* efficacy

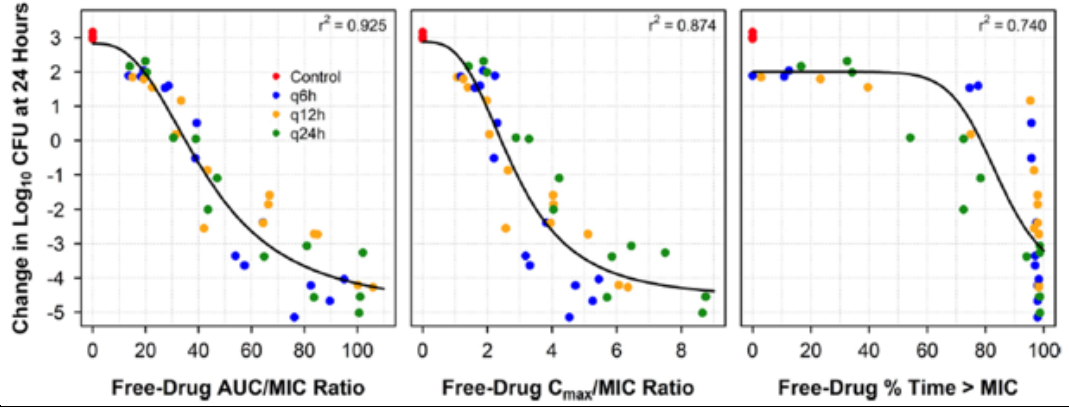
- Hoover AAC 2019
- Humanized dosing of gepotidacin against *E. coli* in an immunocompetent rat pyelonephritis model



E. coli isolate	Genotype[a]	MIC (μg/ml) Gepotidacin	Levofloxacin
NCTC13441	ST-131	4	16
5649	NDM-1	2	32
IR5	NDM-1	4	32
ALL	NDM-1	4	32

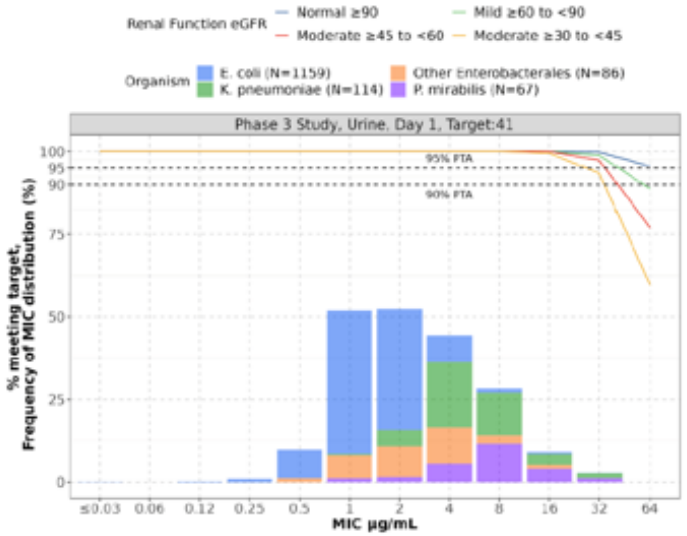
[a]NDM-1, New Delhi metallo-β-lactamase 1; ST-131, sequence type 131.

- fAUC/MIC is the PK/PD index associated with gepotidacin efficacy
  - VanScoy AAC 2021
  - Relationships between change in log10 CFU/mL from baseline at 24 h and gepotidacin PK/PD indices: data from dose fractionation studies for *E. coli* NCTC 13441 in a 1-compartment *in vitro* infection model
  - Similar findings were seen from dose fractionation studies in a neutropenic thigh infection models against 6 strains of *S. pneumoniae* and 6 strains of *S. aureus* (Bulik AAC 2017)



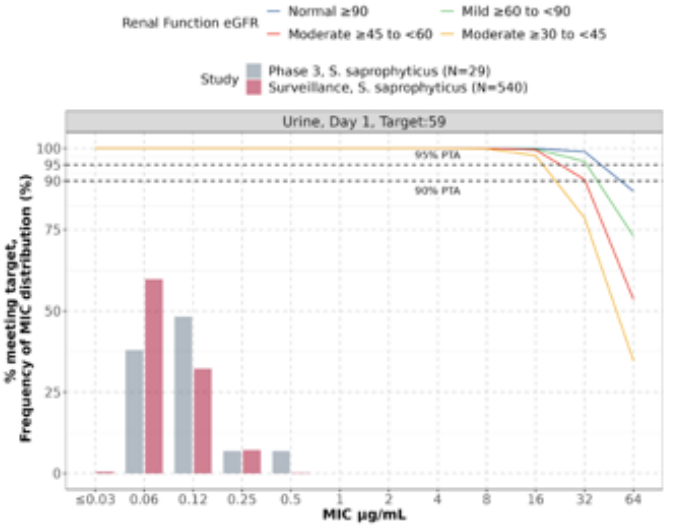
- PK/PD magnitude for *E. coli* – *in vitro* PK/PD model
  - Mean fAUC/MIC for 1 log CFU reduction in (n = 4) *E. coli* isolates was 41
- PK/PD magnitude for Enterobacterales – *in vivo* PK/PD model
  - Median fAUC/MIC ratio of 13 corresponds to a 1-log drop in *in vivo* model
  - Median fAUC/MIC ratio of 13 corresponds to a 1-log drop in a neutropenic thigh infection model and represents a conservative approach to cover both organisms and is associated with:
    - 1-log reduction, excluding strains with <1 log growth in the model; and
    - 1-log reduction for strains with ≥1 log growth and 2-log reduction for strains with <1 log growth
  - A randomly assigned target was not applied to the *in vivo* PK/PD target as the conservative *in vitro* target encompasses the variability seen in the *in vivo* model and the variability in the *in vitro* PK/PD target was low
- PK/PD magnitude for staphylococci – *in vivo* PK/PD model
  - Median fAUC/MIC for 1 log CFU reduction in *S. aureus* was 59
  - In the absence of an *in vivo* PK/PD model for *S. saprophyticus*, conservative nonclinical PK/PD targets for gepotidacin for *S. saprophyticus* were based on PK/PD relationships derived using data for *S. aureus* from a neutropenic murine thigh infection model
- Pharmacokinetic parameters and key characteristics
  - No food effect on PK, and should be taken with food to improve GI tolerability
  - Low plasma protein binding –with limited CNS distribution
  - Eliminated via urine and fecal excretion
    - Urine gepotidacin exposure ~300 times that of free plasma
    - Metabolism is a minor elimination pathway and involves CYP3A4 and strong CYP3A4 inhibition increases gepotidacin plasma Cmax
  - Rapidly reversible acetylcholine esterase inhibitor *in vitro*, with an IC50 value slightly above the mean Cmax in uUTI patients
- Urine PK of Gepotidacin uUTI dose (1500 mg oral twice daily dose for 5 days)
  - Gepotidacin urine exposures in patients exceed optimal *in vitro* targets for efficacy and prevention of resistance
  - In a Phase 2a study, the steady-state (day 4) minimum urine concentration from participants with uUTI following oral gepotidacin at 1500 mg twice daily for 5 days (total of 10 doses) was a AUC_{24} of 4,512 µg·h/mL
  - Thus, the minimum urine AUC24 for the 1,500 mg oral twice daily dose determined in participants with uUTI exceeded the *in vitro* nonclinical efficacy and resistance suppression target by ~27-fold and ~4-fold, respectively, for *E. coli* isolates with gepotidacin MICs ≤4 µg/mL
- High probability of target attainment (PTA) in urine for gepotidacin MICs ≤32 µg/mL for Enterobacterales and ≤16 µg/mL for *S. saprophyticus*

Target attainment in urine for Enterobacterales



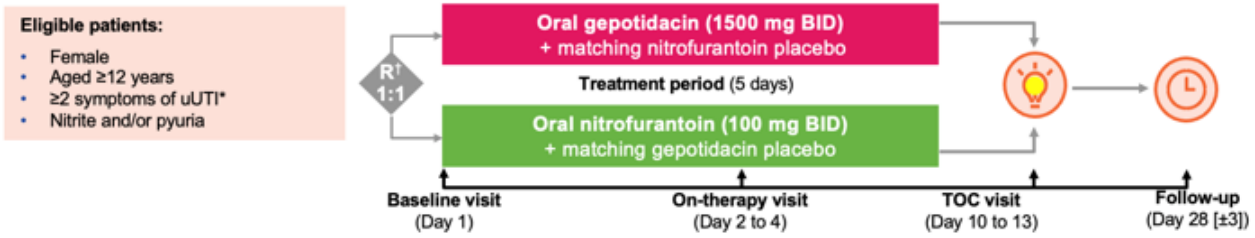
Note: Day 1 urine probabilities of PK/PD target attainment by gepotidacin MIC and renal impairment for the 1500 mg BID x 5 uUTI gepotidacin dosing regimen based on PK/PD target of 41 for Enterobacterales overlaid upon gepotidacin MIC distributions for 1426 Enterobacterales (from the EAGLE-2 and EAGLE-3 Phase 3 uUTI studies).

Target attainment in urine for *S. saprophyticus*

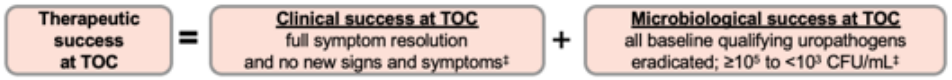


Note: Day 1 urine probabilities of PK/PD target attainment by gepotidacin MIC and renal impairment for the 1500 mg BID x 5 uUTI gepotidacin dosing regimen based on the PK/PD target of 59 for *S. saprophyticus* overlaid upon gepotidacin MIC distributions for 569 *S. saprophyticus* (from EAGLE-2 and EAGLE-3 Phase 3 uUTI studies and surveillance)

- EAGLE-2 and EAGLE-3 were global, Phase 3 double-blind, non-inferiority (-10% margin) randomized controlled trials (uUTI)



Primary endpoint analysis: Therapeutic response (success or failure) at TOC visit in the micro-ITT NTF-S (IA Set)

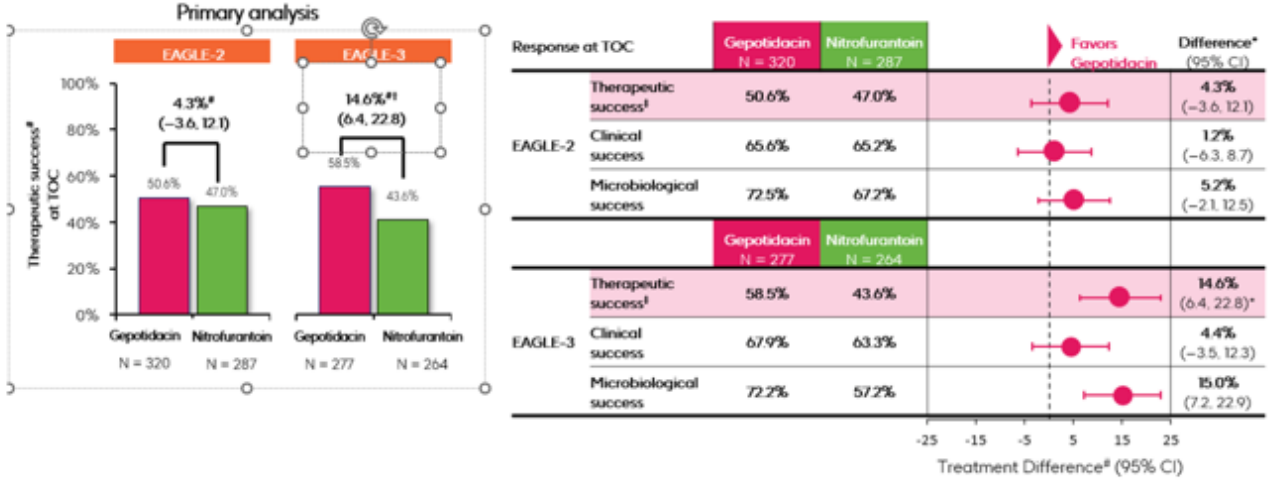


Note: patients who had missing outcome assessments or who received any potentially confounding systemic antimicrobial before the TOC assessment, were considered to not have attained therapeutic success

- Gepotidacin *in vitro* activity against baseline uropathogens for pooled EAGLE-2/3 trials (micro-ITT)

Uropathogen	Number of isolates	Gepotidacin MIC (µg/mL)			
		Range	MIC50	MIC90	%S
Escherichia coli	1,159	≤0.03-32	1	4	>99.9
Klebsiella pneumoniae	114	1-32	4	16	96.5
Proteus mirabilis	67	1-32	8	16	95.5
Citrobacter freundii complex	19	0.5-8	2	8	100
Morganella morganii	13	1-16	4	16	100
Staphylococcus saprophyticus	29	0.06-0.5	0.12	0.25	93.1
Enterococcus faecalis	21	0.5-4	1	2	100

- Gepotidacin demonstrated non-inferiority to nitrofurantoin across EAGLE-2 and EAGLE-3, and superiority in EAGLE-3

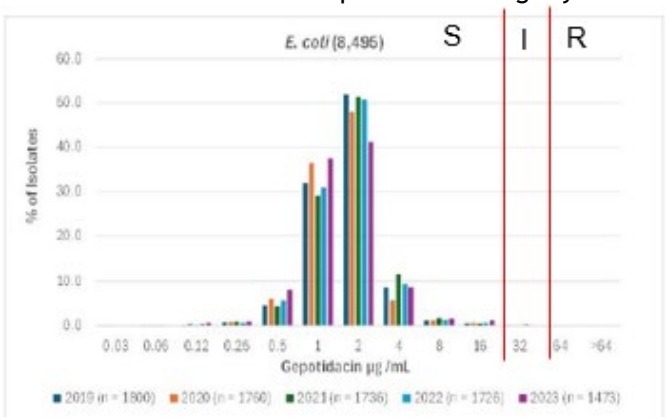


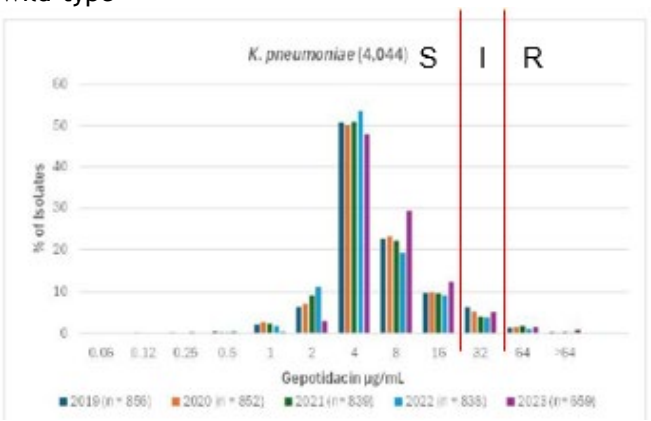
Across EAGLE-2 and EAGLE-3, few patients in either treatment arm (<7%) used additional systemic antibiotics for uUTI before and including the TOC visit.

- Therapeutic, clinical and microbiological success at time of care by baseline qualifying uropathogen for pooled EAGLE-2 and 3 (micro-ITT Population)

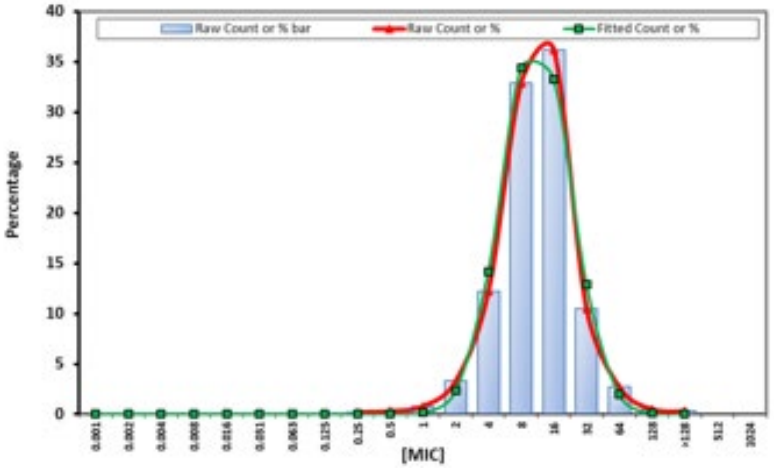
Baseline qualifying uropathogen	Clinical success		Microbiological success		Therapeutic success	
	Gepotidacin n/N1 (%) N=732	Nitrofurantoin n/N1 (%) N=689	Gepotidacin n/N1 (%) N=732	Nitrofurantoin n/N1 (%) N=689	Gepotidacin n/N1 (%) N=732	Nitrofurantoin n/N1 (%) N=689
E. coli	402/591 (68.0)	356/556 (64.0)	431/598 (72.1)	339/561 (60.4)	322/591 (54.5)	241/556 (43.3)
K. pneumoniae	27/54 (50.0)	29/57 (50.9)	38/56 (67.9)	27/58 (46.6)	21/54 (38.9)	16/57 (28.1)
P. mirabilis	27/34 (79.4)	16/33 (48.5)	32/34 (94.1)	21/33 (63.6)	24/34 (70.6)	9/33 (27.3)
C. freundii complex	9/13 (69.2)	2/6 (33.3)	12/13 (92.3)	6/6 (100)	9/13 (69.2)	2/6 (33.3)
S. saprophyticus	9/15 (60.0)	13/14 (92.9)	12/15 (80.0)	12/14 (85.7)	9/15 (60.0)	11/14 (78.6)
E. faecalis	8/14 (57.1)	3/7 (42.9)	12/14 (85.7)	6/7 (85.7)	8/14 (57.1)	2/7 (28.6)

- Gepotidacin: uUTI breakpoint considerations for Enterobacterales
  - Epidemiological cutoff values (ECV)
    - Gepotidacin ECVs calculated using aggregates (N) of 5-6 independent distributions (per species) of broth microdilution MICs (µg/mL)
    - The proposed susceptible gepotidacin breakpoint for Enterobacterales (≤16 µg/mL) is 1-dilution lower than the ECV for *K. pneumoniae* and *P. mirabilis*
    - For some species, the gepotidacin MIC distributions in the ECV study, trended higher than the MIC distributions from the gepotidacin uropathogen global surveillance and Phase 3 uUTI clinical studies
    - The % of *K. pneumoniae* isolates inhibited at gepotidacin MICs of ≤16 µg/mL from each of the 6 laboratories in the ECV study was lower (~76 to 90%) than that (~94 to 97%) from surveillance and the Phase 3 uUTI clinical studies.
  - Proposed gepotidacin breakpoint is one dilution lower than the *Klebsiella pneumoniae* ECV
    - The breakpoint would slightly cut into wild-type

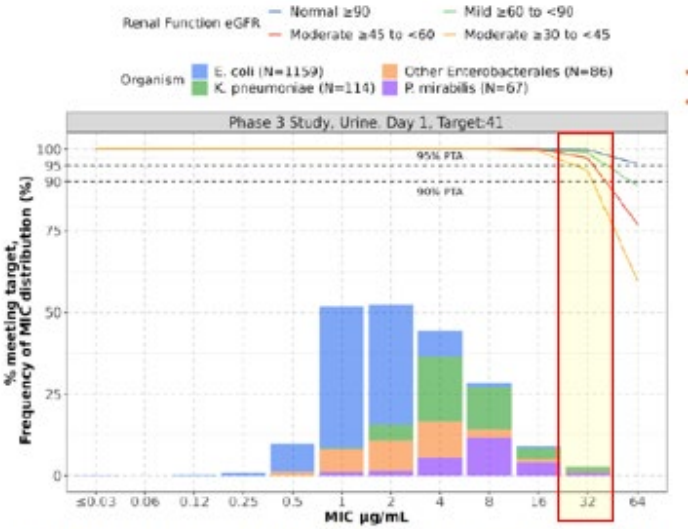




  - Proposed gepotidacin breakpoint is one dilution lower than the *Proteus mirabilis* ECV
    - Proposed urine gepotidacin/Enterobacterales breakpoints: S ≤16; I: 32; R: ≥64 (µg/mL)
    - Gepotidacin MIC distribution (aggregated/summed to 500%) for N=599 *P. mirabilis* isolates and corresponding ECOFFinder data
    - The breakpoint would cut into wild-type



- Non-clinical PK/PD breakpoint
  - >90% PTA at MIC of 32 µg/mL for gepotidacin and Enterobacterales
  - Using a conservative 1-log kill target from *in vitro* model that was higher than target from murine thigh infection model



Note: Day 1 urine probabilities of PK/PD target attainment by gepotidacin MIC and renal impairment for the 1500 mg BID x 5 uUTI gepotidacin dosing regimen based on PK/PD target of 41 for Enterobacterales overlaid upon gepotidacin MIC distributions for 1426 Enterobacterales (from the EAGLE-2 and EAGLE-3 Phase 3 uUTI studies).

- Clinical Exposure Response (CER)
  - The results of the PK/PD analyses for efficacy across efficacy endpoints and analysis populations failed to demonstrate statistically significant and biologically plausible univariable CER relationships for the evaluation of fAUC/MIC in plasma.
  - No significant exposure-response relationship was identified across microbiological, clinical and therapeutic response for Enterobacterales.

- MIC breakpoint proposal for Enterobacterales
  - Susceptible/intermediate/resistant: ≤16/32/≥64 µg/mL
  - Supported by the clinical cutoff, similar success rates up to and including a MIC of ≤16 µg/mL and a strong PK/PD package (nonclinical cutoff of ≤32 µg/mL).
  - While the ECV for *K. pneumoniae* is higher than the proposed breakpoint, the observation of higher distributions in the ECV study in comparison with the distributions from the gepotidacin uropathogen global surveillance study and/or EAGLE-2 and EAGLE-3, and the totality of the nonclinical and clinical data supports the proposed breakpoints.
- AHWG Discussion and Recommendation

M23 Criteria	Breakpoint (µg/mL)
ECV	*E. coli*: 8; ***K. pneumoniae*: 32**; *C. freundii*: 16; *K. aerogenes*: 8; ***P. mirabilis*: 32**
Non-Clinical PK/PD cutoff	≤32
Clinical exposure response cutoff	Not available
Clinical Cutoff	≤16

  - Proposal: MIC breakpoints for gepotidacin and Enterobacterales (µg/mL)
    - Susceptible: ≤16
    - Intermediate: 32
    - Resistant: ≥64
    - With (U) and same footnote as nitrofurantoin: "Report on organisms isolated from the urinary tract"
  - Motion to accept the proposed gepotidacin MIC breakpoints for Enterobacterales with the comment. AHWG Vote: 6-0-0-0.
  - Comments:
    - "Although I would prefer breakpoint of 32 µg/mL for *K. pneumoniae*"
    - "*K. pneumoniae* makes me a bit wary but disk performance seems ok although ≤32 might be better"
- Breakpoints Working Group Discussion and Recommendation
  - Concern about setting breakpoint below ECV for *Klebsiella pneumoniae* and *Proteus mirabilis*
    - EUCAST: susceptible breakpoint only for *E. coli* and at *E. coli* ECV of ≤8 µg/mL
      - EUCAST not convinced by data for other organisms
    - May consider gepotidacin for isolates that are resistant to other agents, may be more likely to be *Klebsiella* and *Proteus*
    - Maybe misclassifying some wild-type isolates as intermediate is not such a negative for this drug: conservative approach
  - PK/PD modeling: thigh infection model not indicative of target in urine
    - MIC values 1-2 dilutions higher when using urine
    - PK/PD breakpoints based on conservative *in vitro* target (not animal target) which was much higher (AUC/MIC of 41 instead of 8-13)

- Motion to accept the gepotidacin MIC breakpoints (S ≤ 16, I 32, R ≥ 64 µg/mL) for Enterobacterales with a footnote stating, "Report on organisms isolated from the urinary tract." WG Vote: 8-0-1-4.

SC DISCUSSION (MAIN POINTS)

- The MIC breakpoints are for all Enterobacterales. Will gepotidacin work for *P. mirabilis*? Yes, the clinical data indicates that it does work.
- There are very few *Proteus* isolates at the higher MICs in the study. If the ECV is 64 µg/mL, then it would be good to see more clinical data.
- EUCAST has an ECOFF of 64 µg/mL, but the CLSI ECV was 32 µg/mL.
- STIC website calls out specific uropathogens. Suggestion was made for CLSI to add a comment as well. The consensus was not to add.

A motion to accept the gepotidacin MIC breakpoints (S ≤ 16, I 32, R ≥ 64 µg/mL) for Enterobacterales with a U designation to report only on organisms isolated from the urinary tract and the FDA approved dose (1,500 mg PO q12h) was made and seconded. Vote: 15 for, 1 against, 0 abstain, 0 absent (Pass)

Against Vote Reasoning:

- There is not enough data for *Klebsiella* and *Proteus*.

GEPOTIDACIN DISK DIFFUSION BREAKPOINTS FOR ENTEROBACTERALES

- Proposal: S: ≥12; I: 8-11; R: ≤7 mm
  - MIC breakpoints: S: ≤16; I: 32; R: ≥64 µg/mL
  - 12,537 isolates from surveillance studies: *E. coli* (n=8495) and *K. pneumoniae* (n=4042)

MIC(µg/mL)																																										
≥128	8	1	1																																							
64	72	6	7	7	18	8	2	4																																		
32	31	2	18	29	69	64	73	47	39	25	8	1			1	1																										
16			1	10	29	69	123	147	202	133	92	52	27	16	9																												
8					2	5	25	62	173	337	441	403	343	165	77	40	10	4	1	0	0	2																				
4								4	19	180	651	1094	1226	1053	622	372	174	80	37	14	5	1																				
2									2	8	53	321	919	1533	1619	1318	794	453	225	113	45	22	8	13	6	2																
1											14	73	309	702	1104	1246	1078	750	483	268	156	68	25	14	4	1	1	1		1												
0.5												2	10	41	99	195	321	330	308	224	138	65	42	24	14	5	7	4														
0.25														2	7	7	16	36	59	64	45	25	17	16	15	3	3		1												
0.12																1	2	3	6	4	6	11	9	5	8	4	4	2	1	2										
0.06																						2	3	5	2	4	1	2	2	1										
≤0.03																									4	2	2	1	1									2		
	≤6	7	8	9	10	11	12	13	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		
Disk Zone Diameter (mm)																																								

MIC Range	Number	Very Major (%)	Major (%)	Minor (%)
≥I + 2	10	0 (0.0)	N/A	1 (10.0)
I + 1 to I - 1	1,442	6 (0.4)	0 (0.0)	377 (26.1)
≤ I – 2	23,621	N/A	0 (0.0)	7 (0.03)
Total	25,073	6 (0.02)	0 (0.0)	385 (1.5)

- *Klebsiella pneumoniae* only
  - Proposal: S: ≥12; I: 8-11; R: ≤7 mm
  - MIC breakpoints: S: ≤16; I: 32; R: ≥64 µg/mL
  - 3580 *K. pneumoniae* isolates from surveillance study (2019-2022) and RCT

MIC (µg/mL)	≤5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	≥36
≥128																																
64		69	1	2	5	16	7	2	4																							
32		28		10	21	55	48	63	42	40	23	9	1			1	1															
16				1	4	18	37	80	105	161	105	73	47	26	16	9																
8						1	1	8	30	114	201	313	340	311	147	71	35	9	1													
4									1	10	108	420	810	923	750	403	161	59	12	4	1											
2											1	11	61	106	129	122	88	49	25	12	6											
1												1	2	2	8	15	29	26	22	24	13	4	4									
0.5																		2	5	3	6	2										
0.25																							3	2	1							
0.12																									1		1					
≤0.03																																

Disk Zone Diamater (mm)

MIC Range	Number	Very Major (%)	Major (%)	Minor (%)
≥I + 2	0	0 (0.0)	N/A	0 (0.0)
I + 1 to I - 1	1130	6 (0.53)	0 (0.0)	298 (26.37)
≤ I – 2	6030	N/A	0 (0.0)	2 (0.03)
Total	7160	6 (0.08)	0 (0.0)	300 (4.19)

- AHWG Discussion and Recommendation
  - Proposal: Disk diffusion breakpoints for gepotidacin and Enterobacterales (mm)
    - Susceptible: ≥12
    - Intermediate: 8-11
    - Resistant: ≤7
  - Motion to accept the proposed gepotidacin disk diffusion breakpoints for Enterobacterales. AHWG Vote: 5-1-0-0.
  - Comments:
    - "I don't like all the *K. pneumoniae* errors AND the fact that the R zone is 1 mm from disk edge."
- Breakpoints Working Group Discussion and Recommendation
  - Motion to accept the gepotidacin disk diffusion breakpoints (S ≥ 12, I 8-11, R ≤ 7 mm) for Enterobacterales. WG Vote: 8-0-1-4.

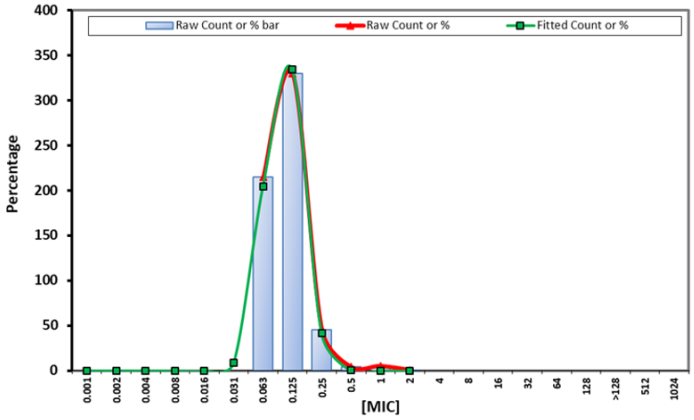
SC DISCUSSION (MAIN POINTS)

- Was the disk potency optimized for gonorrhea first then the Enterobacterales. The 10 μg disk was optimized for the Enterobacterales first.
- These are the same disk diffusion breakpoints as the FDA breakpoints.

A motion to accept the gepotidacin disk diffusion breakpoints (S ≥ 12, I 8-11, R ≤ 7 mm) for Enterobacterales was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

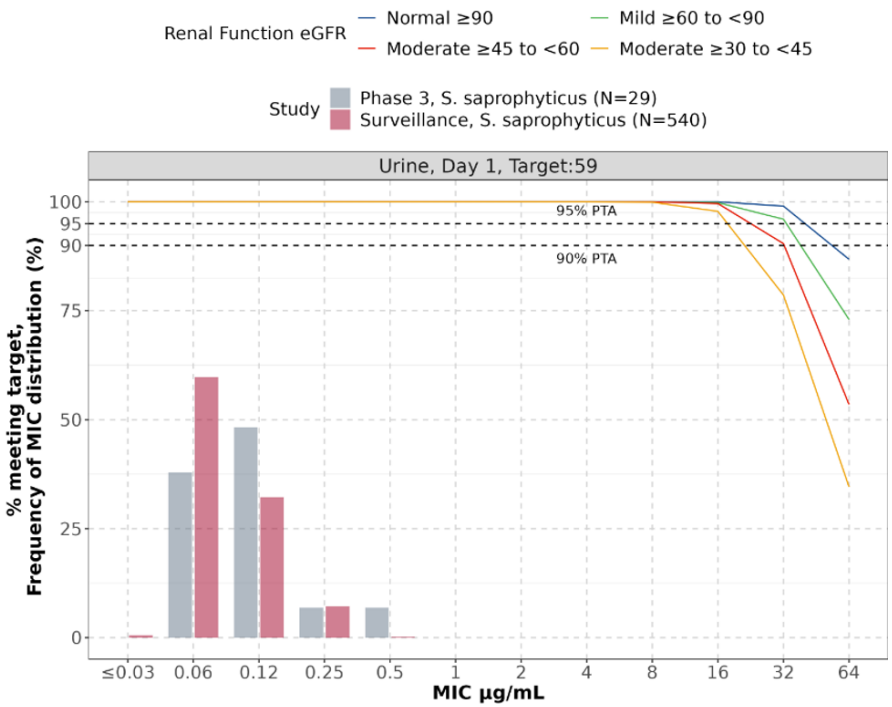
GEPOTIDACIN MIC BREAKPOINTS FOR *STAPHYLOCOCCUS SAPROPHYTICUS*

- ECV: 0.25 μg/mL
  - Same as EUCAST



Selected Values	Exact	R'd-up	%Obs>	%@ECOFF
ECOFF 95.0%	0.1335	0.25	1.6%	7.0%
ECOFF 97.5%	0.1505	0.25	1.6%	7.0%
ECOFF 99.0%	0.1730	0.25	1.6%	7.0%
ECOFF 99.5%	0.1902	0.25	1.6%	7.0%
ECOFF 99.9%	0.2312	0.25	1.6%	7.0%

- Non-clinical PK/PD breakpoint *S. saprophyticus*
  - Target attainment in urine
  - Uses 1-log kill target from neutropenic thigh infection model for *S. aureus* (AUC/MIC = 59)
  - Supports non-clinical PK/PD breakpoint of ≤16 μg/mL



- Clinical urine MIC breakpoint *S. saprophyticus*

Baseline gepotidacin MIC (µg/mL)	Clinical Success (participant-level)	Microbiological Success (uropathogen-level)	Therapeutic Success (participant-level)
≤0.03			
0.06	5/10 (50.0%)	7/10 (70.0%)	5/10 (50.0%)
0.12	3/4 (75.0%)	4/4 (100%)	3/4 (75.0%)
0.25	1/1 (100%)	1/1 (100%)	1/1 (100%)
0.5			
1			
Total	9/15 (60.0%)	12/15 (80.0%)	9/15 (60.0%)
Note: Microbiological response is uropathogen-level response. Clinical and therapeutic responses are participant-level responses.			

- MIC breakpoint proposal for *S. saprophyticus*

Uropathogen	ECV 97.5% (µg/mL)	Nonclinical PK/PD cutoff (µg/mL)	Clinical cutoff (µg/mL)	CER cutoff	Proposed breakpoints (µg/mL)		
					MIC (µg/mL)		
					S	I	R
S. saprophyticus	0.25	≤16	≤0.25	ND	≤0.25	-	-
S = susceptible; I = intermediate; R = resistant; NA=not applicable; ND = not determined or was not able to be identified							

- Supported by the generally similar success across all gepotidacin MICs and that isolates with higher gepotidacin MICs from nonclinical and clinical studies is rare.
- It should be noted that the overall number of isolates in EAGLE-2 and EAGLE-3 and the number of isolates at each gepotidacin MIC was low, but this species is not 1 of the most common uropathogens, a conservative target derived from a more virulent/pathogenic organism (*S. aureus*) was applied and the gepotidacin MIC range was narrow.
- While the proposed breakpoint is lower than the nonclinical cutoff (≤16 µg/mL), it is consistent with the clinical cutoff and ECV (both ≤0.25 µg/mL)

- AHWG Discussion and Recommendation

M23 Criteria	Breakpoint (µg/mL)
ECV	0.25
Non-Clinical PK/PD cutoff	≤16
Clinical exposure response cutoff	Not available
Clinical Cutoff	≤0.25

  - Proposal: MIC breakpoints for gepotidacin and *S. saprophyticus* (µg/mL)
    - Susceptible: ≤0.25
    - Comment in Table 2C: "Routine testing of urine isolates of *S. saprophyticus* is not advised, because infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute, uncomplicated UTIs."
  - Motion to accept the proposed gepotidacin MIC breakpoints for *S. saprophyticus* with the comment. AHWG Vote: 6-0-0-0.
- Breakpoints Working Group Discussion and Recommendation
  - Motion to accept the gepotidacin MIC breakpoints (S ≤ 0.25 µg/mL) for *S. saprophyticus* with a footnote stating, "Report on organisms isolated from the urinary tract." WG Vote: 8-0-1-4.

SC DISCUSSION (MAIN POINTS)

- There is a comment already in table that states susceptibility testing of *S. saprophyticus* is typically not necessary.

A motion to accept the gepotidacin MIC breakpoints (S ≤ 0.25 µg/mL) for *Staphylococcus saprophyticus* with a U designation to report only on organisms isolated from the urinary tract was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

GEPOTIDACIN DISK DIFFUSION BREAKPOINTS FOR *STAPHYLOCOCCUS SAPROPHYTICUS*

- Proposal: S: ≥23 mm
  - MIC breakpoints: S: ≤0.25 µg/mL

Gepotidacin MICs and disk diffusion zone diameters (2 disk manufacturers disks combined) for *S. saprophyticus* (n=580)[a] tested in in pooled EAGLE-2 and EAGLE-3 and in the gepotidacin uropathogen global surveillance study during 2019 to 2021

Scattergram

MIC (µg/mL)	≤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	≥48
≥8																													
4																													
2																													
1																													
0.5									**3**	**1**					**1**	**1**													
0.25												2	4	3	2	1													
0.12						2	1	4	9	21	36	42	38	35	27	26	22	12	15	6	2								
0.06							1	2	5	11	37	59	115	158	156	132	69	33	29	9	3	6	1	2	2	3		1	
≤0.03													1	1	2	2	1	1		1	1								

Disk Zone Diameter (mm)

a. Disk diffusion testing was performed using disks from 2 different manufacturers. The n-values in the figure and table are doubled for a combined analysis.
Bold red values represent the very major errors as defined by CLSI M23
Gray shading indicates the boundaries of the I+1 to I-1 subset.

Discrepancy rates

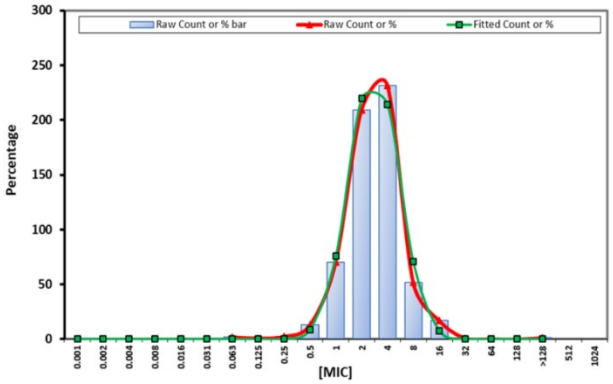
MIC Range	Number	Very Major (%)	Major (%)
≥NSUS + 1	0	0 (0.0)	N/A
NSUS + S	18	6 (33.3)	0 (0.0)
≤S - 1	1142	N/A	0 (0.0)
Total	1160	6 (0.5)	0 (0.0)

- AHWG Discussion and Recommendation
  - Proposal: Disk diffusion breakpoints for gepotidacin and *S. saprophyticus* (mm)
    - Susceptible: ≥23
  - Motion to accept the proposed gepotidacin disk diffusion breakpoints for Enterobacterales. AHWG Vote: 5-1-0-0.
  - Comments:
    - Test shows little ability to distinguish NS from S, high VME, and not necessary since AST not recommended for clinical use
- Breakpoints Working Group Discussion and Recommendation
  - Motion to accept the gepotidacin disk diffusion breakpoints (S ≥ 23 mm) for *S. saprophyticus*. WG Vote: 8-0-1-4.

A motion to accept the gepotidacin disk diffusion breakpoints (S ≥ 23 mm) for *Staphylococcus saprophyticus* was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

GEPOTIDACIN MIC BREAKPOINTS FOR *ENTEROCOCCUS FAECALIS*

- ECV: 8 µg/mL
  - Same as EUCAST
  - Isolates from Phase 3 clinical trials
  - 5.1% of isolates with MIC of 8 µg/mL



Selected Values	Exact	R'd-up	%Obs>	%@ECOFF
ECOFF 95.0%	5.5833	8	3.3%	11.8%
ECOFF 97.5%	6.8174	8	3.3%	11.8%
ECOFF 99.0%	8.5991	16	0.4%	1.3%
ECOFF 99.5%	10.0719	16	0.4%	1.3%
ECOFF 99.9%	13.9535	16	0.4%	1.3%

- Clinical urine MIC breakpoint *E. faecalis*

Baseline gepotidacin MIC (µg/mL)	Clinical Success (participant-level)	Microbiological Success (uropathogen-level)	Therapeutic Success (participant-level)
≤0.03			
0.06			
0.12			
0.25			
0.5	2/2 (100%)	2/2 (100.0%)	2/2 (100.0%)
1	2/6 (33.3%)	5/6 (83.3%)	2/6 (33.3%)
2	2/4 (50.0%)	3/4 (75.0%)	2/4 (50.0%)
4	2/2 (100%)	2/2 (100%)	2/2 (100%)
8			
Total	**8/14 (57.1%)**	**12/14 (85.7%)**	**8/14 (57.1%)**

- AHWG Discussion and Recommendation

M23 Criteria	Breakpoint (µg/mL)
ECV	8
Non-Clinical PK/PD cutoff	Not available
Clinical exposure response cutoff	Not available
Clinical Cutoff	≤4

  - Proposal: MIC breakpoints for gepotidacin and *E. faecalis* (µg/mL)
    - Susceptible: ≤4
  - Motion to accept the proposed gepotidacin MIC breakpoints for *E. faecalis* with the comment. AHWG Vote: 6-0-0-0.
  - Comments:
    - If gepotidacin works for uUTI due to *E. faecalis*, should work for organisms out to WT distribution. Would prefer susceptible-only breakpoint at 8 or no breakpoint.
    - Cautious about this one—based only on ECV and clinical trial with 14 patients, no PK/PD
    - EUCAST susceptible breakpoint is ≤8 µg/mL (this breakpoint would differ with them)
- Breakpoints Working Group Discussion and Recommendation
  - Concern about setting breakpoint below ECV for *E. faecalis*
  - Clinical data limited to 14 patients and no supportive PK/PD data
    - Supportive PK/PD data are uncommon with enterococci because of challenges with animal models
    - *S. saprophyticus* also has limited PK/PD data (but MIC distributions much lower)
  - EUCAST has a susceptible only breakpoint of ≤8 µg/mL (ECV)
  - Clinical need for breakpoint given lack of resistance with *E. faecalis*?
  - *E. faecalis* is a List 1 FDA organism for gepotidacin
  - Motion to accept the gepotidacin MIC breakpoints (S ≤ 4 µg/mL) for *E. faecalis* with a footnote stating, "Report on organisms isolated from the urinary tract." WG Vote: 5-3-2-3.
  - Motion to accept the gepotidacin MIC breakpoints (S ≤ 8 µg/mL) for *E. faecalis*. WG Vote: 3-5-2-3.

SC DISCUSSION (MAIN POINTS)

- *Enterococcus* is a different organism, so it may not be appropriate to infer that gepotidacin will work. There is not a large clinical need for this organism/drug combination.
- Setting the breakpoint at the ECV makes sense except there are no data at the ECV.
- FDA is drawing a line where their clinical experience ends and cut into the ECV. There are no data that says there is something different between a 4 and 8 µg/mL MIC.

A motion to accept the gepotidacin MIC breakpoints (S ≤ 8 µg/mL) for *Enterococcus faecalis* with a footnote stating, "Report on organisms isolated from the urinary tract." was made and seconded. Vote: 5 for, 11 against, 0 abstain, 0 absent (Does Not Pass)

Against Vote Reasoning:

- There are limited clinical data.
- Want to align with FDA.

- Concern with split vote by the Breakpoints Working Group.

SC DISCUSSION (MAIN POINTS)

- The susceptibility testing manufacturers came to the FDA the same time as the drug manufacturer. Concerns about disagreeing with the FDA breakpoint.
- Risk of harm is low. It is uncomplicated UTIs.
- For patients who cannot receive ß-lactams, gepotidacin could be helpful.
- The MIC distributions from additional studies that went to the FDA were lower than what was presented at CLSI.
- Were lower MICs from the lyophilized panels or are from frozen BMD? The lower MICs were across both media. There was no bias.

A motion to accept the gepotidacin MIC breakpoints (S ≤ 4 µg/mL) for *Enterococcus faecalis* with a footnote stating, "Report on organisms isolated from the urinary tract." was made and seconded. Vote: 7 for, 9 against, 0 abstain, 0 absent (Does Not Pass)

Against Vote Reasoning:

- Concerns with setting the breakpoint in the wild-type.
- Need more data.

GEPOTIDACIN TABLE 1 PLACEMENTS FOR ENTEROBACTERALES AND *STAPHYLOCOCCUS SAPROPHYTICUS*

- Enterobacterales
  - Sponsor request: Tier 2 (urine only)
  - AHWG Discussion and Recommendation
    - Motion to place gepotidacin in Tier 2 in Table 1 for Enterobacterales. AHWG Vote: 4-2-0-0.
    - Comments:
      - Belongs in Tier 3
      - Goal to preserve new drug, belongs with fosfomycin
  - Breakpoints Working Group Discussion and Recommendation
    - Motion to place gepotidacin in Tier 2 in Table 1 for Enterobacterales. WG Vote: 8-1-1-3.
    - Reason for No: belongs in Tier 3 with fosfomycin
- *S. saprophyticus*
  - Sponsor request: Tier 2 (urine only)
  - AHWG Discussion and Recommendation
    - Motion to place gepotidacin in Tier 2 in Table 1 for *S. saprophyticus*. AHWG Vote: 4-2-0-0.
    - Comments:
      - Belongs in Tier 3
      - Belongs in Tier 4 since routine testing of *S. saprophyticus* not advised
  - Breakpoints Working Group Discussion and Recommendation
    - Motion to place gepotidacin in Tier 4 in Table 1 for *S. saprophyticus* with comment. WG Vote: 9-0-1-3.
    - Comment in Table 2C: "Routine testing of urine isolates of *S. saprophyticus* is not advised, because infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute, uncomplicated UTIs"

SC DISCUSSION (MAIN POINTS)

- It is a lot to ask laboratories to test gepotidacin on all isolates; therefore, it should be in Tier 3.
- Tier 1 and Tier 2 drugs are tested upfront. Tier 1 drugs are reported upfront. Tier 2 drugs are cascade reporting. Gepotidacin is a new agent and susceptibly testing is not easily available.
- Will be in its own row and not cascade off nitrofurantoin in Table 1.

A motion to place gepotidacin in Table 1 Tier 3 for Enterobacterales was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

A motion to place gepotidacin in Table 1 Tier 4 for *Staphylococcus* was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

GEPOTIDACIN MIC BREAKPOINTS FOR *NEISSERIA GONORRHOEAE*

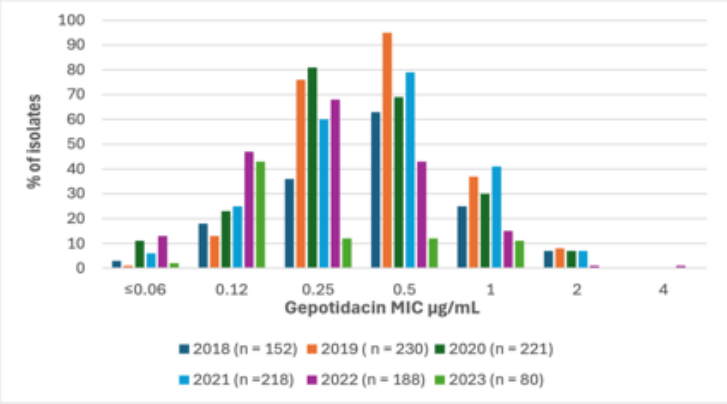
- FDA indication for *Neisseria gonorrhoeae*
  - Patients ≥12 years of age who have limited or no alternative options for uncomplicated urogenital gonorrhea caused by susceptible strains
  - Approval of this indication based on limited clinical safety data
  - Dosage:
    - 3,000 mg (four 750 mg tablets) orally, followed by a second dose of 3,000 mg approximately 12 hours later
    - Administer after a meal to reduce gastrointestinal intolerance
- FDA Breakpoints

Gepotidacin – Oral Products

	Minimum Inhibitory Concentrations (mcg/mL)			Disk Diffusion (zone diameter in mm)		
Pathogen	S	I	R	S	I	R
Enterobacterales[a, b]	≤ 16	32	≥ 64	≥ 12	8-11	≤ 7
Staphylococcus saprophyticus[b]	≤0.25	-	-	≥ 23	-	-
Enterococcus faecalis[b]	≤4	-	-	≥ 14	-	-
Neisseria gonorrhoeae[c]	≤ 1	2	≥ 4	≥ 28	23-27	≤ 22

- Spectrum of Activity
  - Activity of gepotidacin against *N. gonorrhoeae*

Gepotidacin MIC distribution against 1,089 *N. gonorrhoeae* isolates collected from global surveillance studies (2018 to 2023)



- *In vitro* activity against resistant *Neisseria gonorrhoeae*

Drug-resistant Phenotypes[a]	Number of isolates	Gepotidacin MIC (µg/mL)			% Susceptible[b]
		MIC range	MIC50	MIC90	
All isolates	1089	≤0.06 to 4	0.5	1	97.2
Azithromycin-nonsusceptible	43	0.12 to 4	0.5	2	88.4
Ciprofloxacin-resistant	542	≤0.06 to 4	0.5	1	94.6
Penicillin-resistant	192	≤0.06 to 2	0.5	1	94.8
Tetracycline-resistant	241	0.12 to 4	0.5	1	93.4

- Quality control range, MIC test method and disk content for *N. gonorrhoeae*

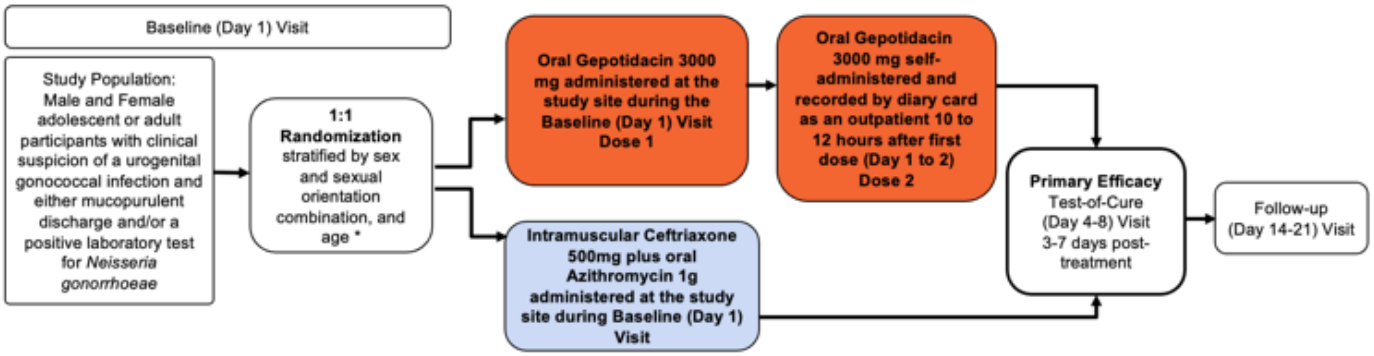
QC strain	CLSI approved QC range	
	MIC (µg/mL)[a]	Zone diameter (mm)
N. gonorrhoeae ATCC 49226	0.25 to 1	32 to 40

a. Range approved for agar dilution only
Source: CLSI M100

  - Quality Control ranges for gepotidacin
    - QC during susceptibility testing of surveillance isolates
      - All (100%) gepotidacin agar dilution MICs and 99.2% of disk diffusion zone diameter values against *N. gonorrhoeae* ATCC 49226 were within the CLSI approved MIC QC ranges for gepotidacin
    - QC during susceptibility testing of EAGLE-1 clinical study isolates
      - All (100%) of gepotidacin QC results (agar dilution MICs and disk diffusion zone diameters) were within the reference ranges established by CLSI
  - Agar dilution comparison to broth microdilution

- Equivalency was not established (essential agreement rate <95%) between broth microdilution and agar dilution for *N. gonorrhoeae*
- Disk content
  - 10 µg disk concentration was found to be also appropriate for disk diffusion testing of *N. gonorrhoeae*

- Potential mechanisms of resistance and cross-resistance (*N. gonorrhoeae*)
  - Mechanisms that may impact gepotidacin activity are as follows:
    - Gepotidacin-specific alterations of DNA gyrase (*gyrA, gyrB*) and/or topoisomerase IV (*parC, parE*) gene targets
      - As shown through studies with isogenic mutants in *N. gonorrhoeae*, the following amino acids may be important for gepotidacin activity: GyrA D90, A92 and ParC D86 (based on *N. gonorrhoeae* numbering)
      - Due to gepotidacin's well balanced targeting of 2 enzymes (for most pathogens), target-specific mutations in a single enzyme may not significantly impact gepotidacin activity
    - Efflux
  - Target-specific cross-resistance to other classes of antibacterials has not been identified
    - Isolates resistant to other drugs may be susceptible to gepotidacin
    - A direct relationship between gepotidacin and fluoroquinolone-susceptibility has not been established
- Phase 2 urogenital uGC clinical trial (BTZ116576) development of resistance findings
  - Single oral doses of 1500 mg and 3000 mg gepotidacin were ≥95% efficacious for bacterial eradication of *N. gonorrhoeae* in adults with urogenital uGC
    - 3 participants (1 in the 1500 mg and 2 in the 3000 mg treatment groups) were microbiological failures at time of care for urogenital *N. gonorrhoeae*
    - All 3 microbiological failures had urogenital *N. gonorrhoeae* baseline isolates with a gepotidacin MIC value of 1 µg/mL and carried a pre-existing ParC D86N mutation
    - An individual mutation in ParC D86N had minimal effect on gepotidacin susceptibility alone, but led to reduced susceptibility when in combination with GyrA A92T.
  - These data are consistent with well balanced inhibition of 2 enzymes for gepotidacin: target-specific mutations in both enzymes are required to significantly impact gepotidacin susceptibility
- *In vitro* data supporting gepotidacin's low potential for development of resistance
  - Spontaneous frequency of resistance *in vitro* at 10XMIC ranged from 10^{-9} to 10^{-10}
  - A 7 day *in vitro* hollow fiber infection model was used to characterize the relationship between gepotidacin exposure and on-therapy *N. gonorrhoeae* resistance amplification
    - Gepotidacin dose regimens of ≥ 4.5 g per 24 hours (equating to fAUC/MIC values ≥46*) were able to prevent the amplification of resistant subpopulations
    - Included the 3g regimen administered twice (with the second dose administered either 8 or 12 hours post treatment initiation)
    - This optimized dose was used in EAGLE-1, and for which no development of resistance was seen
- PK/PD data
  - No validated PK/PD models at the time of development
  - No PK/PD targets for stasis, 1-log kill
- High-level Overview of EAGLE-1 Study Design



- Primary endpoint: Culture-confirmed bacterial eradication (ie, microbiological success) of *N. gonorrhoeae* from the urogenital body site at time of care visit
- Primary efficacy analysis population: microbiological-ITT (micro-ITT) ceftriaxone-susceptible
  - All participants randomly assigned to study treatment who received at least 1 dose of study treatment
  - Had confirmed *N. gonorrhoeae* isolated that was ceftriaxone-susceptible (based on CLSI breakpoints) from baseline culture of their urogenital specimen.
- Other populations and endpoints are defined in Ross Lancet 2025

- EAGLE-1 Primary Endpoint – Microbiological Response at time of care at the Urogenital body site: Primary Analysis (Micro-ITT Population)
  - Primary Study Objective Met: Non-inferiority was declared (Superiority was not met)

	Gepotidacin 2x3000mg (N=202)	Ceftriaxone 500mg plus azithromycin 1g (N=204)
Microbiological Success		
n (%)	187 (92.6%)	186 (91.2%)
95% CI	(88.0%, 95.8%)	(86.4%, 94.7%)
Treatment Difference (95% CI) *	–0.1% (–5.6%, 5.5%)	
1-sided p-value for superiority	0.5072	

Non-inferiority is met as the lower limit of the 2-sided 95% CI is above the non-inferiority margin of -10%

Superiority was not met as the lower limit of the 2-sided 95% CI is not above 0%. p-value ≥ 0.025.

* Difference: Gepotidacin – Ceftriaxone plus azithromycin. Miettinen-Nurminen (MN Score) method adjusting for treatment and actual strata of sex and sexual orientation (men who have sex with men, men who have sex with women, or female).

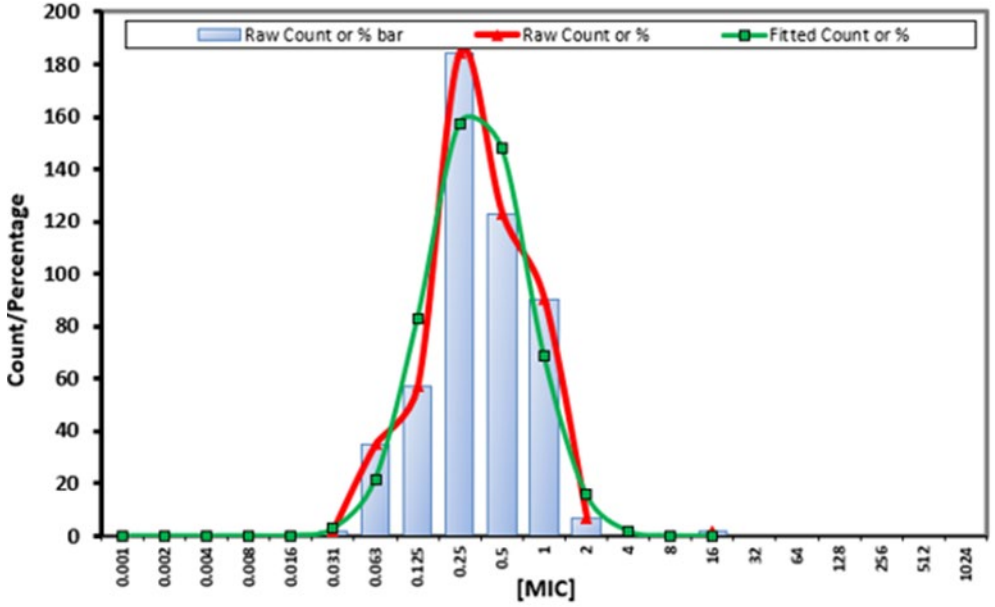
None of the microbiological failures were due to bacterial persistence.
All microbiological failures were due to unable to determine

- Microbiological success of baseline urogenital *N. gonorrhoeae* at time of care by phenotypic and genotypic subcategories – micro-ITT population
  - No urogenital or rectal isolates available at test-of-cure to assess for increases in MIC values

- 2 pharyngeal culture-positive isolates at test-of-cure (neither had ≥4-fold increase in gepotidacin MICs)

Subcategory	GEP 2 x 3000 mg N=202	CRO 500 mg + AZM 1 g N=204	Difference in % for success rate (95% CI)[a]
Overall	187/202 (92.6%)	186/204 (91.2%)	-0.1% (-5.6%, 5.5%)
Azithromycin-nonsusceptible	17/17 (100%)	19/20 (95.0%)	5.0% (-14.3%, 24.0%)
Ciprofloxacin-resistant	103/110 (93.6%)	101/109 (92.7%)	1.0% (-6.2%, 8.3%)
Penicillin-resistant	35/36 (97.2%)	30/33 (90.9%)	6.3% (-6.4%, 21.4%)
Tetracycline-resistant	42/49 (85.7%)	47/53 (88.7%)	-3.0% (-17.1%, 10.6%)
Ciprofloxacin-resistant and penicillin-resistant and tetracycline-resistant	23/24 (95.8%)	21/23 (91.3%)	4.5% (-13.1%, 23.6%)
QRDR mutations	111/119 (93.3%)	112/121 (92.6%)	0.7% (-6.2%, 7.7%)
GyrA mutations	109/117 (93.2%)	111/119 (93.3%)	-0.1% (-7.1%, 6.8%)
S91F, D95A	101/107 (94.4%)	99/106 (93.4%)	1.0% (-6.0%, 8.2%)
S91F, D95G	5/6 (83.3%)	10/11 (90.9%)	-7.6% (-50.6%, 26.9%)
ParC mutations	95/101 (94.1%)	99/107 (92.5%)	1.5% (-5.9%, 9.0%)
D86N	22/24 (91.7%)	20/23 (87.0%)	4.7% (-15.3%, 25.6%)
S87R	68/72 (94.4%)	69/74 (93.2%)	1.2% (-7.6%, 10.1%)
Combination QRDR mutations			
GyrA S91F, D95A and ParC D86N	20/22 (90.9%)	16/19 (84.2%)	6.7% (-15.3%, 30.5%)
GyrA S91F, D95A and ParC S87R	64/67 (95.5%)	65/69 (94.2%)	1.3% (-7.4%, 10.2%)

- Gepotidacin: Breakpoint considerations for *Neisseria gonorrhoeae*
  - ECV
    - ECV: 2 µg/mL
    - Same as EUCAST



Selected Values	Exact	R'd-up	%Obs>	%@ECOFF
ECOFF 95.0%	0.8804	1	1.7%	13.8%
ECOFF 97.5%	1.1337	2	0.3%	3.2%
ECOFF 99.0%	1.5210	2	0.3%	3.2%
ECOFF 99.5%	1.8581	2	0.3%	3.2%
ECOFF 99.9%	2.8074	4	0.3%	0.4%

- No non-clinical PK/PD cutoff
  - No pre-clinical PK/PD targets
  - Hollow fiber infection model showed AUC/MIC ≥46 prevented emergence of resistance and sterilized the model (≥5 log decrease in bacterial growth from baseline)
  - AUC/MIC target of ≥48 was associated with 100% success in Phase 2 study with single dose (2 microbiologic failures with AUC/MIC of ≤24)
  - No microbiologic failures due to bacterial persistence in Phase 3 trial with two 3 g doses
- Clinical cutoffs

Summary of gepotidacin microbiological success at TOC by baseline urogenital gepotidacin MIC for *N. gonorrhoeae* in EAGLE-1 (micro-ITT population)

Baseline gepotidacin MIC (µg/mL)	Microbiological success
≤0.06	13/13 (100%)
0.12	55/58 (95%)
0.25	71/80 (89%)
0.5	37/39 (95%)
1	10/11 (91%)
2	2/2 (100%)
4	
Total	188/203 (93%)
MIC range (µg/mL)	≤0.06 to 2
MIC50 (µg/mL)	0.25
MIC90 (µg/mL)	0.5

Note: The denominators for each cell are based on the micro-ITT Population and include participants with Baseline *N. gonorrhoeae* isolated from the urogenital body site and with the Baseline MIC result. The numerators include all participants with eradication (success) at the urogenital body site and with the Baseline MIC result. A participant with more than 1 *N. gonorrhoeae* isolate is counted more than once for microbiological response. MICs are based on the agar dilution MICs.

- MIC breakpoint proposal for *Neisseria gonorrhoeae*
  - Susceptible/intermediate/resistant: ≤1/2/≥4 µg/mL
  - Supported by the clinical cutoff and consistent success rates up to and including a MIC of 1 µg/mL.
  - While the ECV is higher than the proposed breakpoint, the observation of a low percentage of isolates that would be considered not susceptible, and also the FDA's determination of a lower ECV further confirms that the totality of the nonclinical and clinical data supports the proposed breakpoints
- AHWG Discussion and Recommendation
  - The AHWG did not agree with the FDA assessment that the ECV was 0.5 µg/mL
  - Instead, the AHWG thought the ECV was 1 or 2 µg/mL
  - Agreed with sponsor on lack of a non-clinical PK/PD or clinical exposure response breakpoint
  - Agreed with sponsor on clinical cutoff of ≤1 µg/mL
- Breakpoints Working Group Discussion and Recommendation
  - Discussion about small % of isolates with MIC values of 2 µg/mL that might test non-susceptible even though they are within wild-type, but only 2 patients in clinical trial with MIC values of 2 µg/mL and no PK/PD supportive data
    - Need for intermediate to account for technical variability
  - Concern about emergence of resistance if patients don't take their second dose, especially given GI side effects
    - Adherence in real world likely lower than in RCTs
  - Motion to accept the gepotidacin MIC breakpoints (S ≤ 1, I 2, R ≥ 4 µg/mL) for N. *gonorrhoeae* from urogenital sources only. WG Vote: 9-0-1-3.
    - Matches sponsor proposal and FDA breakpoints

SC DISCUSSION (MAIN POINTS)

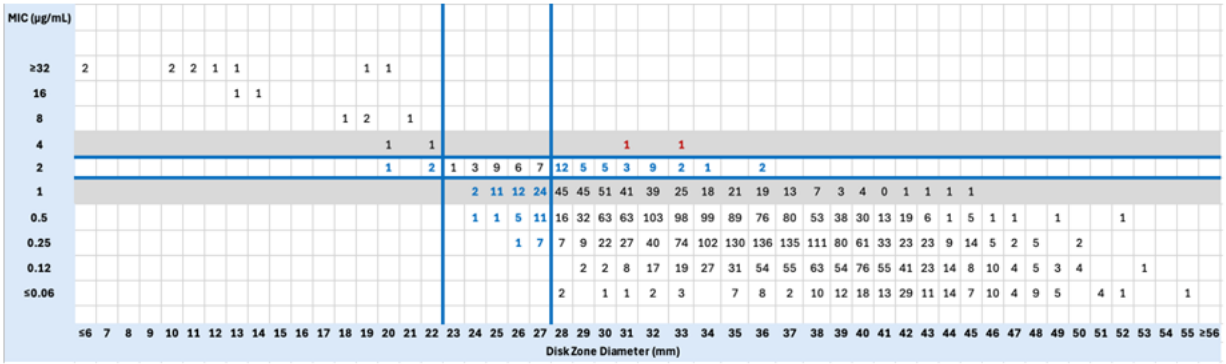
- *N. gonorrhoeae* is difficult to culture, did the trial work to maximize culture recovery? Yes, they used *N. gonorrhoeae* appropriate media.
- The data shown here were for culture confirmed cases, but they have data that gepotidacin works well for patients with PCR positive results as well.
- FDA dosage is 3,000 mg PO x 1, followed by a second 3,000 mg PO dose 12 hours later.

A motion to accept the gepotidacin MIC breakpoints (S ≤ 1, I 2, R ≥ 4 µg/mL) for *Neisseria gonorrhoeae* for urogenital isolates only was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

GEPOTIDACIN DISK DIFFUSION BREAKPOINTS FOR *NEISSERIA GONORRHOEAE*

- Proposal: S ≥ 28, I 23-27, R ≤ 22 mm
  - MIC breakpoints: S ≤ 1, I 2, R ≥ 4 µg/mL

Gepotidacin MICs and disk diffusion zone diameters (disk from 2 manufacturers combined) for 1,593[a] *N. gonorrhoeae* (all body sites) from EAGLE-1 (n=493), gepotidacin gonococcal global surveillance studies (n=1,089) during 2018-2023, 2 ceftriaxone-nonsusceptible isolates from CDC and 9 gepotidacin-resistant isolates from nonclinical studies



a. Disk diffusion testing was performed using disks from 2 different manufacturers. The n-values in the figure and table are doubled for a combined analysis.
Bold red values represent very major errors and blue values represent the minor errors as defined by CLSI M23
Gray shading indicates the boundaries of the I+1 to I-1 subset.

Discrepancy rates

MIC Range	Total #	VME # (%)	Major # (%)	Minor # (%)
≥I + 2	16	0 (0.0%)	NA (NA)	0 (0.0%)
I + 1 to I - 1	456	2 (0.4%)	0 (0.0%)	91 (20.0%)
≤ I – 2	2,714	NA (NA)	0 (0.0%)	26 (1.0%)
Total	3,186	2 (0.1%)	0 (0.0%)	117 (3.7%)

- Breakpoints Working Group Discussion and Recommendation
  - Motion to approve the gepotidacin disk diffusion breakpoints (S ≥ 28, I 23-27, R ≤ 22 mm) for *Neisseria gonorrhoeae*. WG Vote: 9-0-1-3.

A motion to accept the gepotidacin disk diffusion breakpoints (S ≥ 28, I 23-27, R ≤ 22 mm) for *Neisseria gonorrhoeae* was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

GEPOTIDACIN TABLE 1 PLACEMENT FOR *NEISSERIA GONORRHOEAE*

- Sponsor request: Tier 1
- AHWG Recommendation: Tier 1
- Breakpoints Working Group Discussion and Recommendation
  - All agents in Tier 1, even second-line agents.
  - Motion to place gepotidacin in Tier 1 in Table 1 for *Neisseria gonorrhoeae*. WG Vote: 8-1-1-3.
  - Dr. Simner requested to reevaluate *Neisseria gonorrhoeae* Table 1.

SC DISCUSSION (MAIN POINTS)

- Tier 4 is where CLSI intends for epidemiologic surveillance testing.
- Consider leaving azithromycin, ceftriaxone, and cefixime in Tier 1.
- Consider moving ciprofloxacin and tetracycline move to lower tiers that need a more expert review.
- Disagreement that azithromycin should be in Tier 1.
- Consensus is to reevaluate *Neisseria gonorrhoeae* Table 1.

A motion to place gepotidacin in Table 1 Tier 1 for *Neisseria gonorrhoeae* was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

MINOCYCLINE DISK DIFFUSION BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Current breakpoints

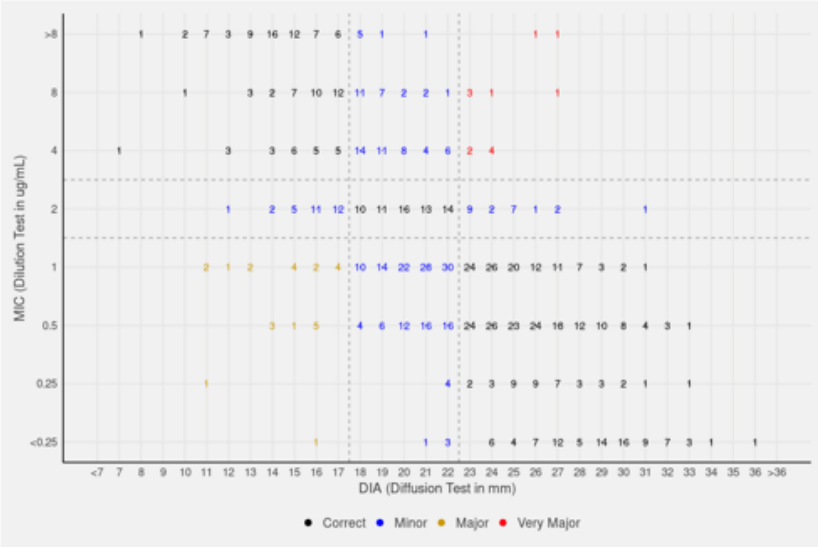
TETRACYCLINES								
Minocycline	30 µg	≥ 22	18–21	≤ 17	≤ 1	2	≥ 4	(13) If needed for treatment, confirmatory MIC testing is indicated for isolates with zones of 18–21 mm to avoid reporting false-intermediate results.

- Minocycline AST method study
  - Ayesha Khan, Betsy Hirsch, Micah Bhatti
  - N=100 global *Acinetobacter baumannii* isolates (JMI) isolated in 2024-2025
    - From diverse body sites
    - 60% carbapenem-resistant, 40% carbapenem-susceptible
  - Reference method
    - Broth microdilution performed in triplicate across three brands of cation-adjusted Mueller Hinton broth (BD, Remel, Hardy) with modal MICs analyzed
  - Test methods
    - Disk diffusion (BD BBL): minocycline, doxycycline, tetracycline
    - 3 brands of agar (BD BBL, Remel, Hardy)
  - Performance with current CLSI disk breakpoints, all agars

- Categorical agreement: 63.2% (569/900)
- Unacceptable VME and MI rates

MIC	No. of isolates	VME	ME	MI
≥ I + 2	135	7 (5.1%)	-	29 (21.5%)
I ± 1	414	8 (1.9%)	18 (4.3%)	170 (41%)
≤ I - 2	351	-	10 (2.8%)	39 (11%)

MIC range	M23 Acceptable Error Rates %		
	VME	ME	MiE
≥ I+2	<2	NA	<5
I±1	<10	<10	<40
≤ I-2	NA	<2	<5



- All data

Current CLSI Breakpoints (17,22mm)				
MIC	N	VME	ME	MI
≥ I + 2	135	7 (5.1%)	-	29 (21.5%)
I ± 1	414	8 (1.9%)	18 (4.3%)	170 (41%)
≤ I - 2	351	-	10 (2.8%)	39 (11%)

Manual best fit (18,22mm)				
MIC	N	VME	ME	MI
≥ I + 2	135	7 (5.1%)	-	13 (9.6%)
I ± 1	414	8 (1.9%)	28 (6.8%)	156 (37.7%)
≤ I - 2	351	-	14 (3.9%)	35 (10%)

dBETS best fit (15,21mm)				
MIC	N	VME	ME	MI
≥ I + 2	135	11 (8.1%)	-	61 (45%)
I ± 1	414	16 (3.9%)	9 (2.2%)	152 (36.7%)
≤ I - 2	351	-	5 (1.4%)	28 (8%)

- Exclusion of Hardy agar

Current CLSI Breakpoints (17,22mm)				
MIC	N	VME	ME	MI
≥ I + 2	45	2 (2.2%)	-	15 (16.7%)
I ± 1	138	5 (1.8%)	11 (4.0%)	132 (47.8%)
≤ I - 2	117	-	7 (3%)	34 (14.5%)

Manual best fit (16,20mm)				
MIC	N	VME	ME	MI
≥ I + 2	90	3 (3.3%)	-	23 (25.6%)
I ± 1	276	12 (4.3%)	8 (2.9%)	95 (34.4%)
≤ I - 2	234	-	7 (3%)	9 (3.8%)

dBETS best fit (15,20mm)				
MIC	N	VME	ME	MI
≥ I + 2	90	3 (3.3%)	-	34 (37.8%)
I ± 1	276	12 (4.3%)	6 (2.2%)	92 (33.3%)
≤ I - 2	234	-	4 (1.7%)	12 (5%)

- AHWG Discussion and Recommendation
  - Larger zone sizes with Hardy also seen with minocycline and *Stenotrophomonas* (also seen in QC)
  - Question of whether it is worth moving around correlates since none of the options are good and we already have a comment to confirm Intermediate results if needed?
  - Minocycline disk diffusion an important test in Latin America
  - Concern of laboratories using tetracycline or doxycycline disk diffusion results to predict minocycline not an issue anymore with removal of tetracycline and doxycycline disk breakpoints
- Breakpoints Working Group Discussion and Recommendation
  - Thought the juice not worth the squeeze to change breakpoints
  - Recommendation to keep breakpoints the same and keep the comment

SC DISCUSSION (MAIN POINTS)

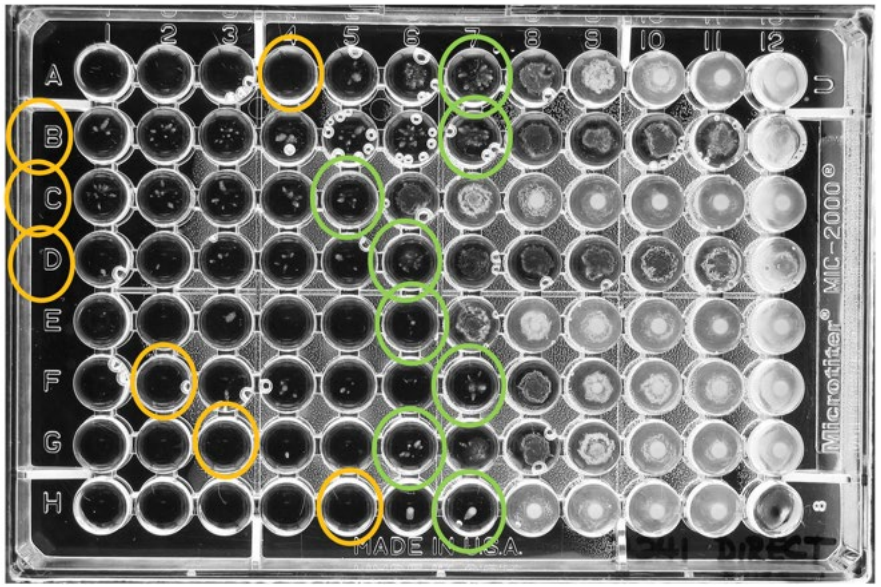
- Consensus to keep the minocycline breakpoints with the comment.

CEFTAZIDIME DISK DIFFUSION BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Current breakpoints

CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)							
Ceftazidime	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32

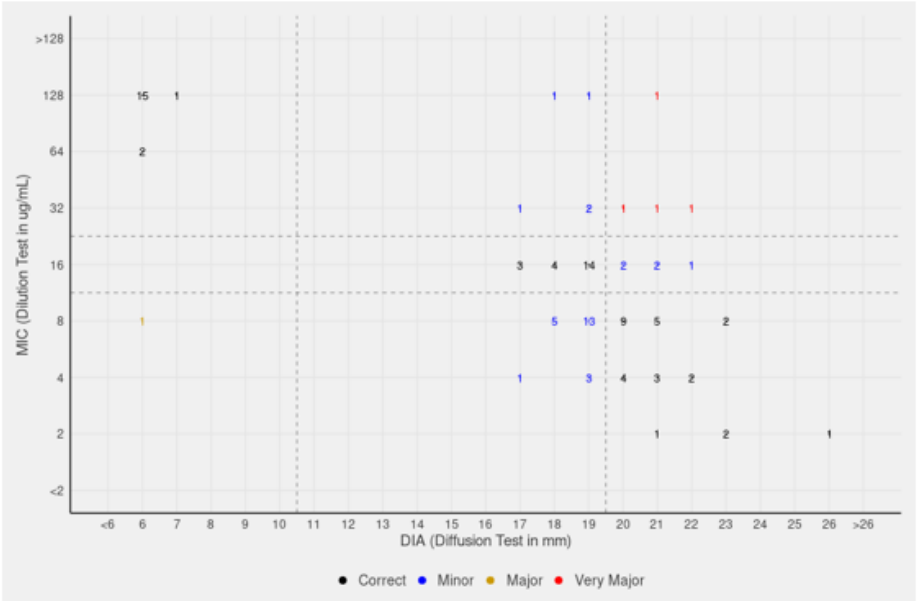
- Reading



Conservative read – no growth

Liberal read – ignored growth above an obvious endpoint

- DISK Study Data: Ceftazidime disk correlates



Current correlates
<=14 R, 15-17 I, >=18 S

	VME	ME	mE
> I + 1	14.3%	n/a	0.0%
I +/- 1	7.5%	1.5%	35.8%
< I - 1	n/a	0.0%	5.9%*

dBETS suggestion
<=10 R, 11-19 I, >=20 S

	VME	ME	mE
> I + 1	4.8%*	n/a	9.5%
I +/- 1	4.5%	1.5%	38.8%
< I - 1	n/a	0.0%	23.5%

- Breakpoints Working Group Discussion and Recommendation
  - Ayesha Khan leading a study that will provide more data next year
  - Motion to remove the ceftazidime breakpoints for *Acinteobacter* spp. with a comment from tobramycin in Table 3F-4: "The disk diffusion breakpoints have been temporarily removed pending additional review." WG Vote: 9-0-1-3.

SC DISCUSSION (MAIN POINTS)

- There are published papers that say ß-lactams and *Acinetobacter* have difficult zones to read, does that occur here? Response: CLSI will have more data next year to help address that.

A motion to remove the ceftazidime disk diffusion breakpoints for *Acinetobacter* spp. and add a comment stating, "The disk diffusion breakpoints have been temporarily removed pending additional review." was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

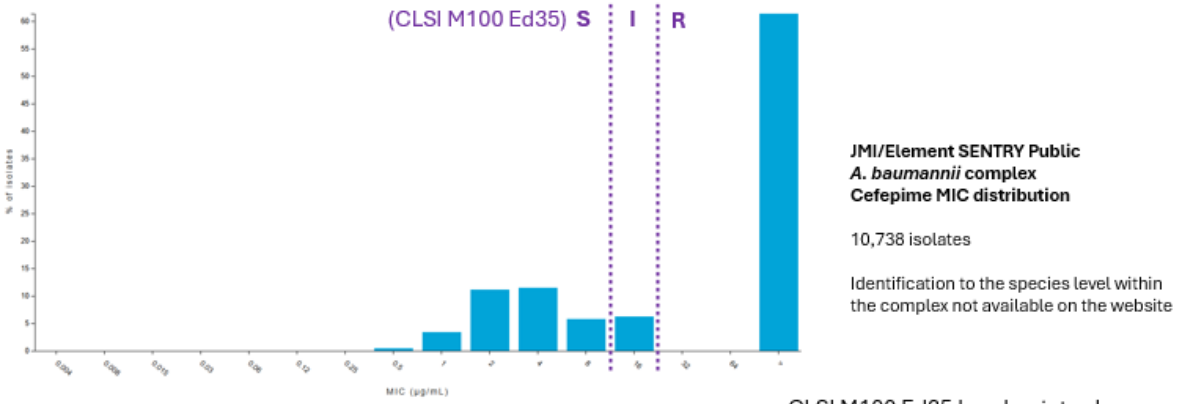
CEFEPIME BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Current breakpoints

CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)							
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

- No EUCAST breakpoints
- FDA does not recognize CLSI breakpoints
- Breakpoints/disks are the same as when Cefepime first introduced for most organisms in M100-S5

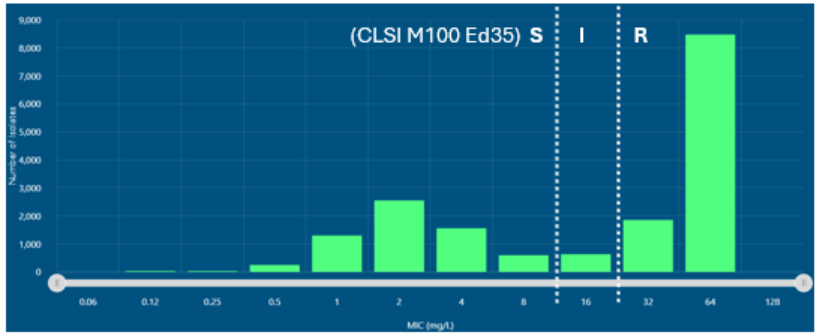
- Key mechanisms of resistance to cefepime in *Acinetobacter baumannii* complex
  - Outer membrane is intrinsically less permeable to cephalosporins (2- to 7-fold) compared to *P. aeruginosa*
    - Smaller number of outer membrane porins (OMPs)
    - OMPs are smaller than those in other gram-negative organisms
  - Chromosomal bla_{ADC} (class C; *Acinetobacter*-derived cephalosporinase)
    - Not inducible
    - Overexpression occurs in the presence of certain upstream insertion sequences
      - eg, IS*Aba1*-bla_{ADC-30} increases ceftazidime MIC 4-fold in an isogenic background
    - Substitutions within bla_{ADC} can also increase the hydrolysis of ceftazidime or broaden its hydrolytic spectrum (eg, to include cefepime)
  - Wide variety of other Ɓ-lactamases, including bla_{OXA} (which vary in their hydrolysis of cephalosporins) and, less commonly, other carbapenemases (including metallo-Ɓ-lactamases)
  - Efflux pump overexpression
- JMI SENTRY MIC distribution *A. baumannii* complex



CLSI M100 Ed35 breakpoints shown

Cefepime MIC, µg/mL	0.5	1	2	4	8	16	> 16
Isolates (cumulative %)	54 (0.5)	370 (3.9)	1197 (15.1)	1230 (26.6)	626 (32.4)	663 (38.6)	6598

- ATLAS MIC distribution *A. baumannii* complex



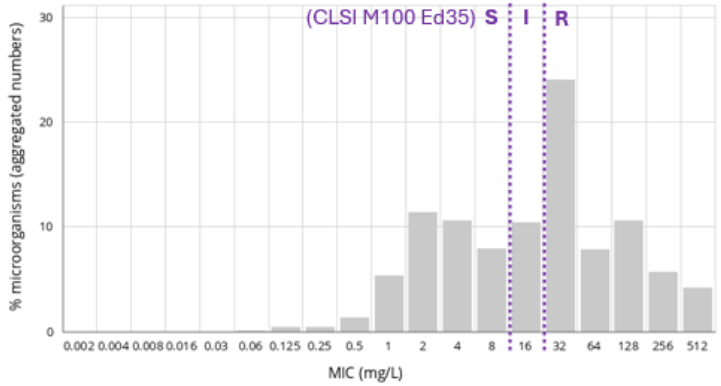
ATLAS (Pfizer)
***A. baumannii* complex**
Cefepime MIC distribution

17,285 isolates from all regions except the United States, 2019-2023

CLSI M100 Ed35 breakpoints shown

Cefepime MIC, µg/mL	0.12	0.25	0.5	1	2	4	8	16	32	>32
Isolates (cumulative %)	32 (0.2)	31 (0.4)	248 (1.8)	1299 (9.3)	2553 (24.1)	1560 (33.1)	596 (36.6)	632 (40.2)	1856 (51.0)	8478

- EUCAST MIC distribution *A. baumannii*



EUCAST
A. baumannii
Ceftazidime MIC distribution

6,142 isolates

"In MIC testing of *Acinetobacter* spp. there are great difficulties in the reading of endpoints for cephalosporins. The distributions, although abundant, are diverse, often truncated, and in general difficult to interpret. No ECOFF will be set on these."

"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

Cefepime 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)	0 (0)	5 (<0.1)	27 (0.5)	24 (0.9)	80 (2.2)	327 (7.5)	700 (18.9)	649 (29.5)	484 (37.4)	641 (47.8)	1476 (71.8)	480 (79.7)	647 (90.2)	347 (95.8)	255

- JMI + ATLAS + EUCAST: ECV = current susceptible breakpoint
  - "ECV" (97.5%) = 8 µg/mL
  - ECV was 8 also if exclude EUCAST and with weighted analysis
- *A. baumannii* is similar to *Pseudomonas aeruginosa* distribution
- Current CLSI cefepime breakpoints for gram-negative rods

	S	SDD	I	R
Enterobacterales MIC breakpoints (mg/L) Dose	≤ 2 1g q 8h or 2g q12h	4-8 2g q 8h over 3h	--	≥ 16
P. aeruginosa MIC breakpoints (mg/L) Dose	≤ 8 2g q 8h over 3h	--	16^	≥ 32
***Acinetobacter* spp.** MIC breakpoints (mg/L) Dose	≤ 8 --	--	16	≥ 32

  - Maximum FDA-approved dosage: 2 g q8h
  - IDSA guidelines for HAP/VAP and ventriculitis/meningitis: 2 g q8h
- PD targets in animal models for cefepime are similar across gram-negative rods

	Stasis	1-$\log_{10}$ kill	Citation
Enterobacterales (neutropenic mouse lung)	~25% *f*T > MIC	~35% *f*T > MIC	Muller AE et al. JAC 2022. PMID: 36253951.
P. aeruginosa (neutropenic thigh)	~30-40% *f*T > MIC	~45-50% *f*T > MIC	Ong CT et al. DMID 2007. PMID: 16930925
***Acinetobacter* spp.** (neutropenic mouse lung)	~31% *f*T > MIC	~39% *f*T > MIC	Bhagwat SS et al. AAC 2019. PMID: 30670419

- Cefepime non-clinical PK/PD targets for *A. baumannii*
  - Bhagwat SS et al. AAC 2019. PMID: 30670419
  - 5 carbapenemase-susceptible *A. baumannii* isolates included
  - Composite % fT>MIC for FEP:
    - Stasis = 30.9%
    - 1-log kill = 38.9%
- Clinical PK/PD targets for *P. aeruginosa* higher than non-clinical PK/PD targets from animals
  - Crandon JL… Nicolau DP. AAC 2010. PMID: 20038614
  - Retrospective cohort study (n = 56) of patients with a culture positive for *P. aeruginosa* from a sample from a non-urinary tract source, and active infection
  - CART analysis was used to partition fT > MIC at a value predictive of microbiological failure, a fT > MIC breakpoint of 60% identified
- Clinical exposures of cefepime for gram-negative bloodstream infections
  - Rhodes NJ…Scheetz MH. AAC 2015. PMID: 26666929

- Single center retrospective study of 180 adults with GNBSI, treated with cefepime (46.7% received combination therapy)
- 2 PK models used to impute exposure over first 24h
- CART identified fT>MIC threshold values for greater survival according to models 1 and 2 at >68% and >74%, respectively
- Each 1% increase in cefepime fT>MIC resulted in a 2% improvement in multivariate survival probability (P = 0.015)

- Monte Carlo simulations of cefepime
  - Frei CR et al. JAC 2008. PMID: 18252694
    - Probability of target attainment with 30-min infusions

Dosing regimen	*f* %*T* > MIC target	Infusion duration (h)	Maximum achievable MIC (mg/L)
1g q8h	50 60 70	0.5	4 2 2
2g q 12h	50 60 70	0.5	2 0.5 0.25
2g q8h	50 60 70	0.5	4 4 2

  - Crandon JL… Nicolau DP. AAC 2010
    - At current CLSI breakpoint of 8 g/ml, only a dose of 2 g every 8 h as either a 30-min or a 3-h infusion has a 82% likelihood of achieving at least 60% fT > MIC in patients with normal renal function
- Summary of PK/PD and MIC targets for ≥90% Probability of Target Attainment (PTA)
  - 2g Q8h is necessary to achieve >90% PTA against isolates with MICs (4-8 mg/L)
  - Extended infusion over 3-4h necessary to ensure adequate PTA at 8 mg/L
  - Cefepime-specific %fT>MIC thresholds differ between pre-clinical and clinical studies
  - Cefepime PTA is impacted primarily by %fT>MIC threshold, renal function, and dosing
- Clinical outcomes by MIC data mostly for other gram-negatives
  - Bhat SV...Paterson DL. AAC 2007
    - 204 episodes of bacteremia, from 197 patients, for which patients received cefepime (1g q 12h)
    - CART analysis used to define risk of 28-day mortality 8 mg/L
    - MV analysis showed that treating bacteremia with MIC ≥ 8 mg/L was independent predictor of mortality (p ≤ 0.001)
  - Rhodes NJ…Scheetz MH. DMID 2015.
    - Retrospective cohort study of patients (n = 91) with GNBSIs
    - Most patients received 2g q 8h
    - CART analysis identified an in-hospital mortality breakpoint at a cefepime MIC between 2 and 4 mg/L for patients with a modified APACHE II score ≤16.5 (4.2% versus 25%, respectively)
    - Multivariate logistic regression revealed increased odds of mortality at a cefepime MIC of 4 mg/L (adjusted odds ratio [aOR] 6.47; 95% confidence interval [CI] 1.25–33.4) and 64 mg/L (aOR 6.54, 95% CI 1.03-41.4)

- Cefepime breakpoints for *Acinetobacter* spp.: M23 criteria

	Data available?	Proposed breakpoint
ECV	☑	8 µg/mL
Nonclinical PK/PD cutoff	+/- (Mouse thigh infection models)	Targets differ from clinical outcomes data
Clinical exposure response (CER) cutoff	☑ Monte Carlo simulations	≥ 90% PTA using % *f*T > MIC ranging from 60-100% at MICs up to 8 (with 2g Q8h over 3h infusion)
Clinical cutoff	☑	Increased mortality at MIC ≥4/8

- AHWG Discussion and Recommendation
  - Proposal 1: Remove cefepime breakpoints. AHWG Vote: 0-5-0-0.
  - Proposal 2: Lower cefepime breakpoints 1 dilution to S ≤4, I 8, R ≥16. AHWG Vote: 0-5-0-0.
    - Rationale: ECV is around 8
  - Proposal 3: Keep current cefepime breakpoints (S ≤8, I 16, R ≥32). AHWG Vote: 5-0-0-0.
    - Based on dose of 2 g IV q 8 h over 3 h
    - Rationale: alignment with *P. aeruginosa* breakpoints, ECV is around 8, PK/PD modeling suggests 3h infusion is needed.
- Breakpoints Working Group Discussion and Recommendation
  - I^ (drugs that concentration in urine)?
    - Currently in Table 2 for Enterobacterales and *Pseudomonas aeruginosa* but not for *Acinetobacter* spp.
  - No Monte Carlo simulations for achieving pre-clinical PK/PD targets that were developed specifically for *Acinetobacter*
  - Pre-clinical PK/PD targets from animals lower than clinical targets for other gram-negatives
    - Reasonable to presume that clinical targets for *Acinetobacter* may also be higher than targets from mice
    - Would be the more conservative approach
  - Motion to keep the current cefepime breakpoints (S ≤8, I 16, R ≥32 µg/mL) for *Acinetobacter* spp. and add a dosing comment, "2 g over 3 hours every 8 hours". WG Vote: 9-0-1-3.
    - Matches dosage for S-DD for Enterobacterales and S for *Pseudomonas aeruginosa*)

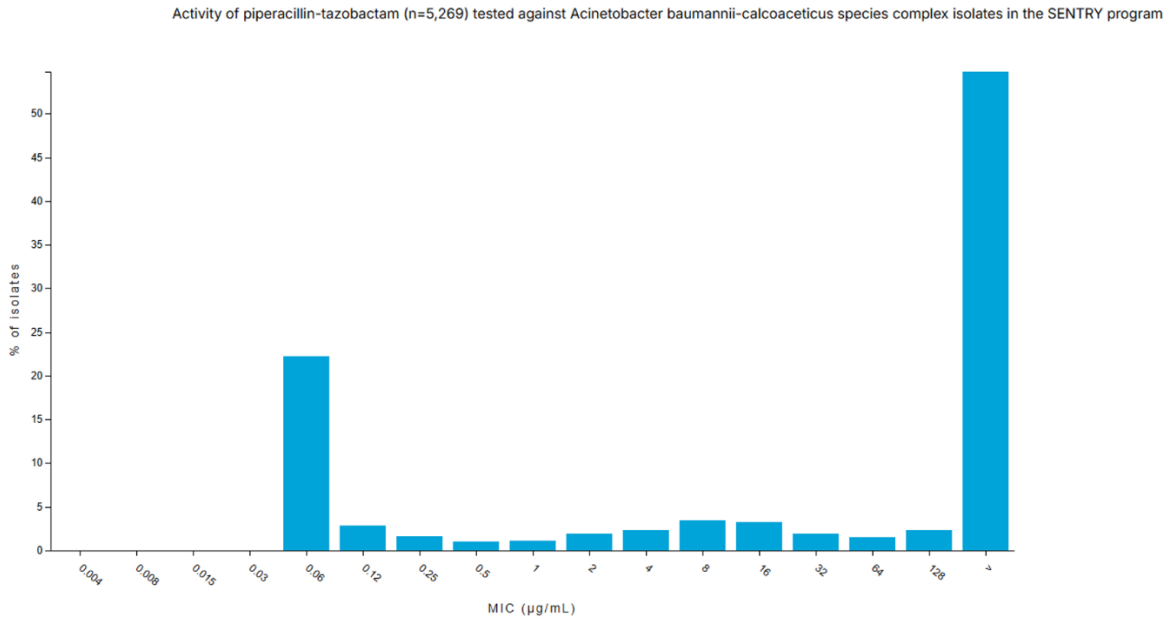
A motion to keep the current cefepime MIC breakpoints (S ≤ 8, I 16, R ≥ 32 µg/mL) for *Acinetobacter* spp. and add dosing comment for a dose of 2 g IV q 8 h over 3 h was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

PIPERACILLIN-TAZOBACTAM BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Current breakpoints

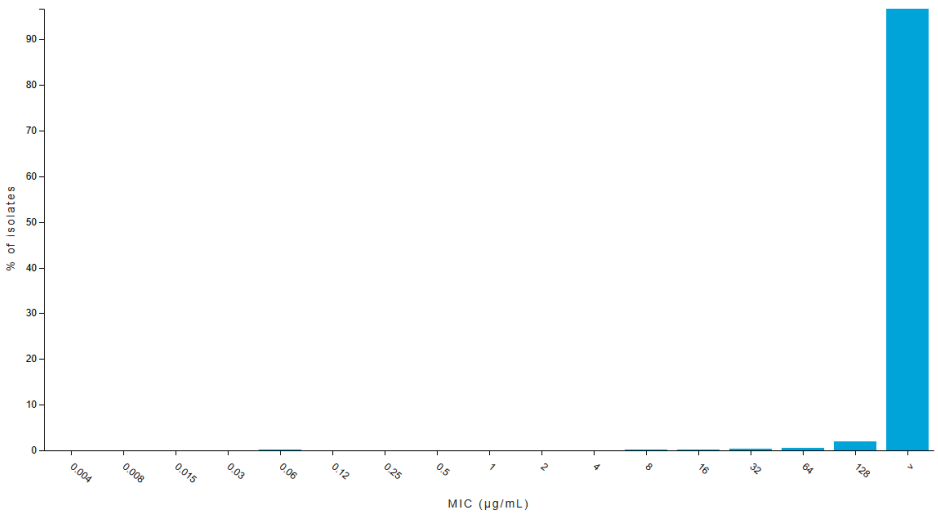
β-LACTAM COMBINATION AGENTS								
(4) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Ampicillin-sulbactam	10/10 µg	≥ 22	17-21	≤ 16	≤ 8/4	16/8	≥ 32/16	
Piperacillin-tazobactam	100/10 µg	≥ 21	18-20	≤ 17	≤ 16/4	32/4-64/4	≥ 128/4	
Sulbactam-durlobactam	10/10 µg	≥ 17	14-16	≤ 13	≤ 4/4	8/4	≥ 16/4	
Ticarcillin-clavulanate*	75/10 µg	≥ 20	15-19	≤ 14	≤ 16/2	32/2-64/2	≥ 128/2	

  - No EUCAST Breakpoints
  - FDA does recognize breakpoints and disk correlates
  - Breakpoints and disk correlates are the same as originally published for "other gram-negatives" (not *Pseudomonas aeruginosa*) in M100-S5
- Piperacillin-tazobactam (Pip-tazo) exposure in US data set: increased mortality
  - Appaneal et al, AAC 2022:66;3:e01975-21
    - 4,599 inpatients with *A. baumannii* infection included across multiple centers (2010–2019)
    - Retrospective cohort study of inpatients with *A. baumannii* cultures and treatment from 2010 to 2019
    - Fluoroquinolones were the most frequently used antibiotic (26.8% of patients)
    - Piperacillin-tazobactam ranked second in overall antibiotic use (24% of patients)
    - Carbapenems were used in 15.6%
    - Pip-tazo group with significant mortality (24.8%)
      - No pip-tazo-specific mortality rates directly compared to other agents
  - Alrahmany et al, Antibiotics 2021, 10, 630. https://doi.org/10.3390/antibiotics10060630
    - Only 17% of *A. baumannii* isolates demonstrated susceptibility to piperacillin-tazobactam
    - Despite this low susceptibility rate, 28% of patients received PIP/TAZ-based therapy (monotherapy or combination)
    - P/T use was associated with high odds of adverse events
    - Poor mortality outcomes were observed, particularly in infections caused by multidrug-resistant *A. baumannii* (MDRAB)
- JMI Sentry Data



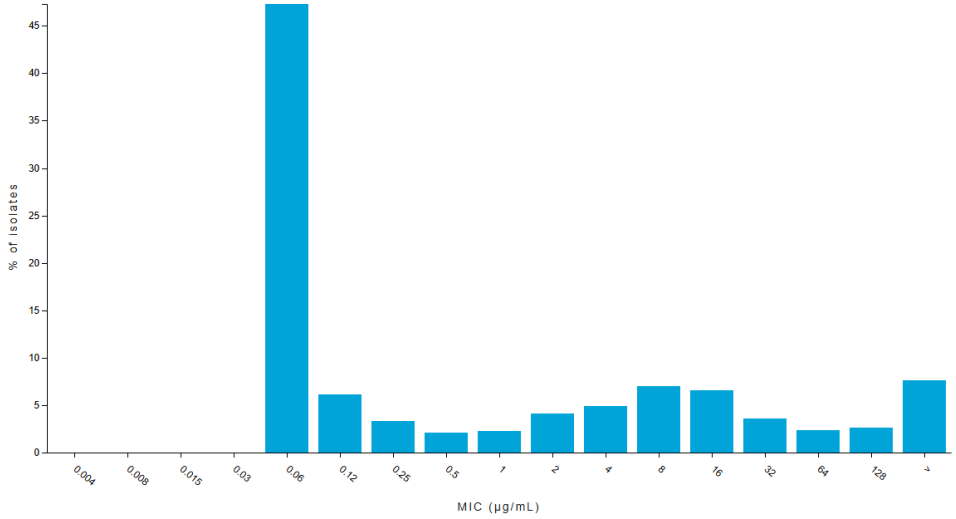
- Meropenem Resistant Isolates

Activity of piperacillin-tazobactam (n=2,784) tested against meropenem-R (CLSI 2026) Acinetobacter baumannii-calcoaceticus species complex isolates in the SENTRY program



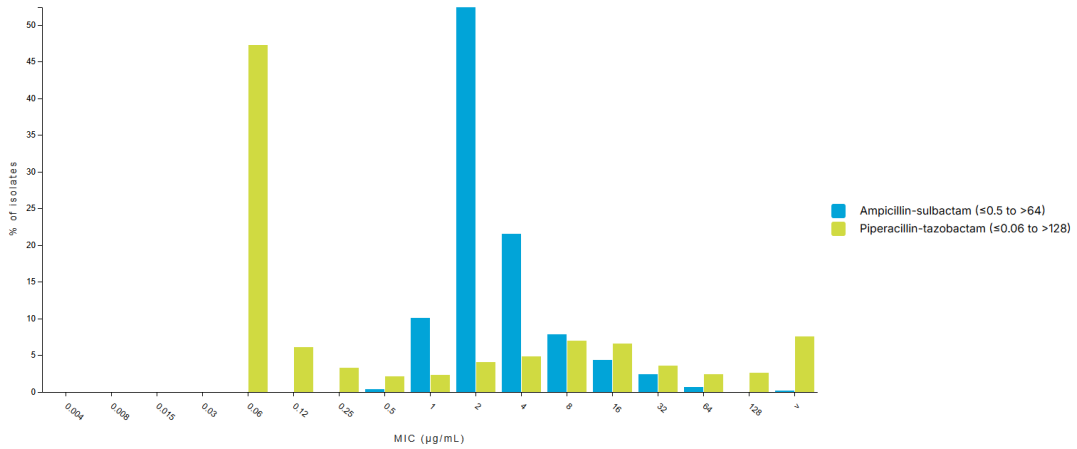
- Meropenem Susceptible Isolates

Activity of piperacillin-tazobactam (n=2,467) tested against meropenem-S (CLSI 2026) Acinetobacter baumannii-calcoaceticus species complex isolates in the SENTRY program

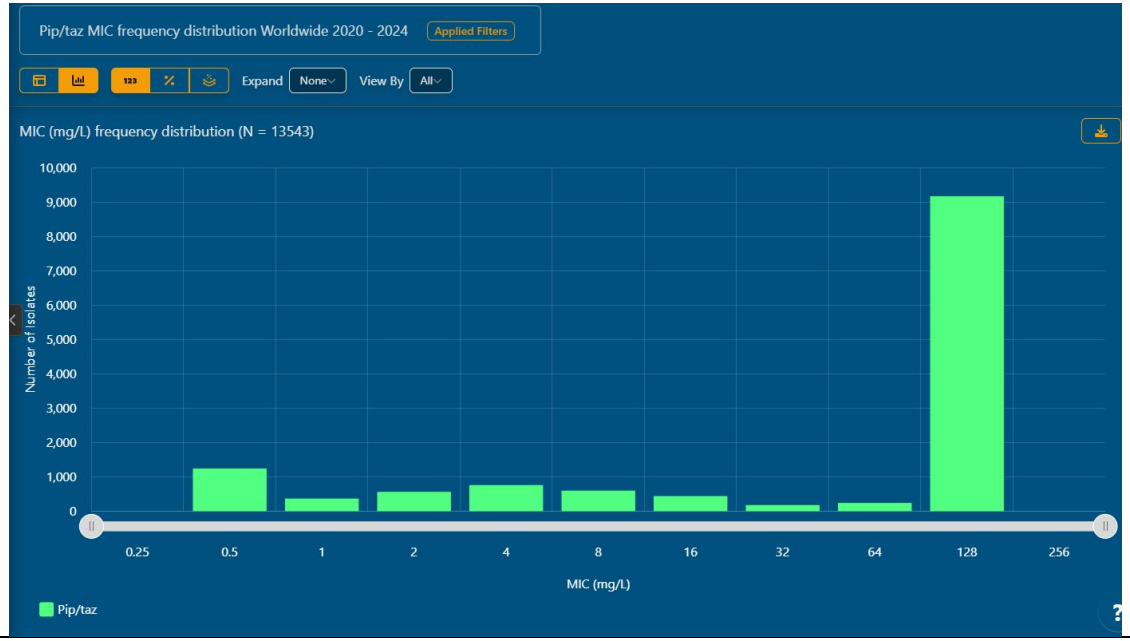


- Meropenem Susceptible Isolates with Ampicillin-sulbactam

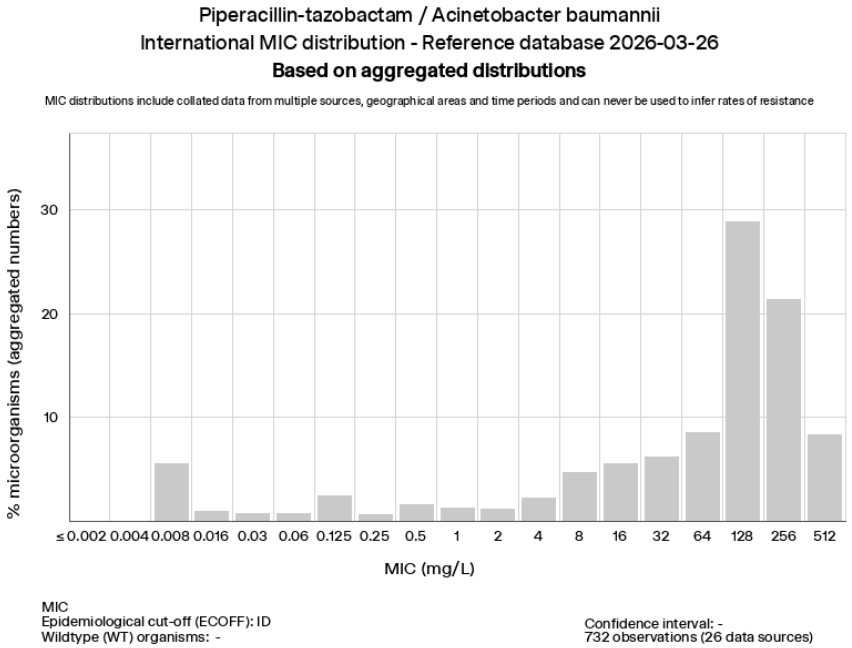
Activity of ampicillin-sulbactam (n=2,523) and piperacillin-tazobactam (n=2,467) tested against meropenem-S (CLSI 2026) Acinetobacter baumannii-calcoaceticus species complex isolates in the SENTRY program



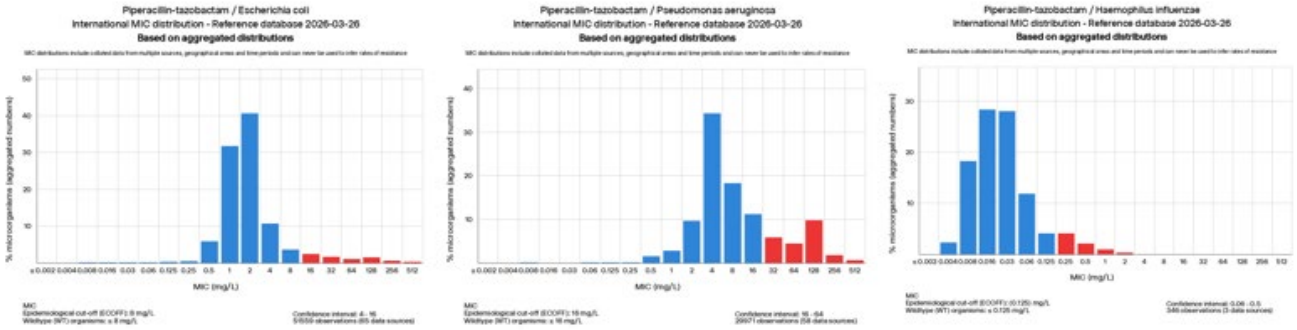
- ATLAS Data



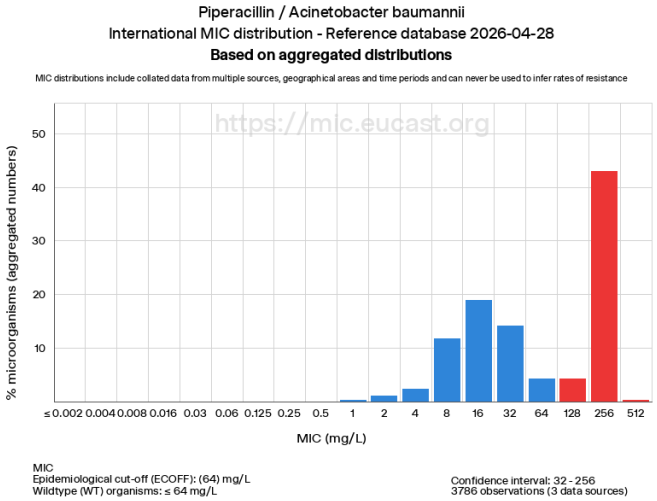
- EUCAST Data



  - “Normal” Pip-tazo Distributions



  - Piperacillin Distribution



- Tazobactam has activity against *Acinetobacter baumannii*
  - Tazobactam appears to be more potent than piperacillin
  - Higgins, Antimicrobial Agents and Chemotherapy, 2004
- Tazobactam has increased activity compared to piperacillin
  - Problem: testing uses a fixed concentration of tazobactam
  - Suh DMID 1995
- Breakpoints Working Group Discussion and Recommendation
  - Ad hoc working group asked for a path forward
  - Having a fixed tazobactam concentration is problematic if tazobactam has intrinsic activity
  - With low MICs, that includes a tazobactam concentration of 4 µg/mL (would be much lower if fixed ratio not fixed concentration)
  - Clinical need? Why not use ampicillin-sulbactam for *Acinetobacter*
  - Mixed infections where need anti-pseudomonal or enterococcal coverage?
  - Motion to remove piperacillin-tazobactam breakpoints for *Acinetobacter* spp. with comment in overview of changes in M100. WG Vote: 9-0-0-4.

SC DISCUSSION (MAIN POINTS)

- The test as it exists is really a tazobactam breakpoint, it is not splitting piperacillin susceptible and resistant.
- There is a problematic comment that states if an organism is susceptible to piperacillin that can be used to predict piperacillin-tazobactam. There is also a comment in Table 1.
- Need piperacillin MIC distribution on meropenem susceptible isolates.

A motion to remove the piperacillin-tazobactam MIC and disk diffusion breakpoints for *Acinetobacter* spp. with a comment in the Overview of Changes in M100 stating, "temporarily removed pending additional review" was made and seconded. Vote: 13 for, 3 against, 0 abstain, 0 absent (Pass)

Against Vote Reasoning:

- Cannot vote to get rid of without getting rid of piperacillin.
- Need to make clear that the drug is still active and the test does not work.

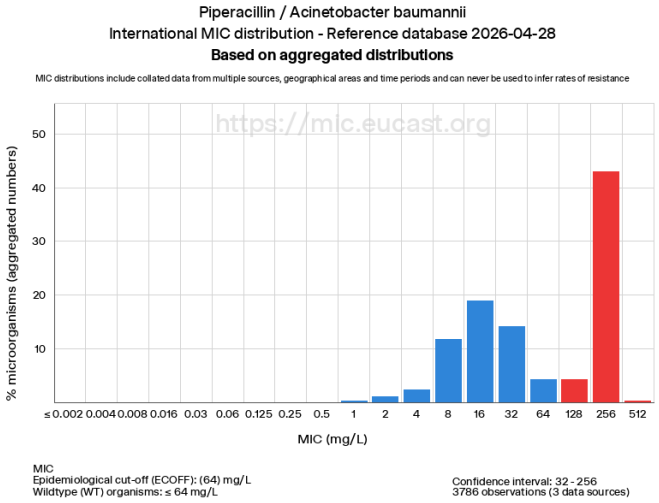
PIPERACILLIN BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Is there concern about leaving Piperacillin alone given the BL/BLI comment?
    - The Piperacillin EUCAST ECOFF is 64.

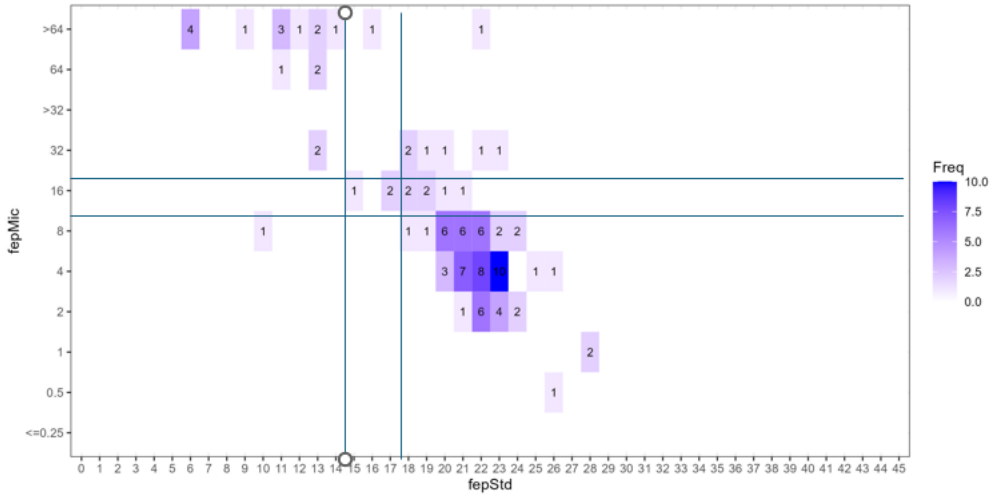
Table 2B-2. *Acinetobacter* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Piperacillin*	100 µg	≥ 21	18-20	≤ 17	≤ 16	32-64	≥ 128	
β-LACTAM COMBINATION AGENTS								
(4) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Ampicillin-sulbactam	10/10 µg	≥ 22	17-21	≤ 16	≤ 8/4	16/8	≥ 32/16	
Piperacillin-tazobactam	100/10 µg	≥ 21	18-20	≤ 17	≤ 16/4	32/4-64/4	≥ 128/4	
Sulbactam-durlobactam	10/10 µg	≥ 17	14-16	≤ 13	≤ 4/4	8/4	≥ 16/4	
Ticarcillin-clavulanate*	75/10 µg	≥ 20	15-19	≤ 14	≤ 16/2	32/2-64/2	≥ 128/2	
CEPHEMS (PARENTERAL) (including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								

- Piperacillin Distribution



- Piperacillin Data



14.3% VME in the I+/-1

SC DISCUSSION (MAIN POINTS)

- If piperacillin is not removed, users may think to test piperacillin to get a piperacillin-tazobactam result.

	• Suggestion to remove comment 4, which is the prediction comment.
	A motion to remove the piperacillin breakpoints MIC and disk diffusion breakpoints for *Acinetobacter* spp. with a comment in the Overview of Changes in M100 stating, "temporarily removed pending additional review" and remove comment 4 in Table 2B-2 was made and seconded. Vote: 15 for, 0 against, 0 abstain, 1 absent (Pass)
3.	**ADJOURNMENT** Dr. Mathers thanked the participants for their attention. The meeting was adjourned at 5:30 PM Central Time (US).

2026 JUNE AST MEETING SUMMARY MINUTES PLENARY 3: Tuesday, 2 June 2026 7:30 AM – 12:00 PM Central Time (US)	
#	**Description**
1.	**OPENING** Dr. Mathers opened the meeting at 7:30 AM Central Time (US).

2. **METHODS WORKING GROUP (T. DINGLE AND A. FERRELL)**

KLEBICINS ALTERNATIVE METHOD AD HOC WORKING GROUP REPORT

- Background from New Drug Alternative Methods AHWG
  - Is a modification to an accepted reference method necessary to determine the MIC of a new antimicrobial agent for non-fastidious bacteria?
  - Reference method defined by CLSI, EUCAST, ISO as Cation-Adjusted Mueller-Hinton Broth (CAMHB)
  - Modification – any modification of previously accepted reference method
  - Disk diffusion and Commercial Methods – designed and validated to produce results that are substantially equivalent to reference method
  - Establishing reference MIC method for new antimicrobial agent is crucial step in early development
    - Prediction of antimicrobial agent activity for different pathogens
    - Used in PK/PD analyses for strategies of dosing and mode of administration
  - In later stages of antimicrobial agent development, reference AST methods are used by diagnostic test manufacturers to establish and validate disk diffusion and other commercial tests. Also used by surveillance programs and reference laboratories to monitor changes in susceptibility.
  - Document Contents
    - Justification for change: Guidance for evaluation of methods:
      - Comparative data with reference AST method and alternative methods
      - MICs determined using reference method with modifications for strains tested in animal infection models.
      - Strains tested should include a spectrum of "resistance" phenotypes to ascertain whether the exposure-response relationship is affected by a specific "resistant" subgroup.
    - Is there lack of reproducibility of MIC tests with reference method and not with modification of reference method – QC strain testing, and clinical isolate testing
      - Trailing endpoints? Skipped wells? Medium interference?
      - Have modifications already approved by CLSI and/or EUCAST evaluated first? (ID-CAMHB-cefiderocol; additional Ca2+ - daptomycin; fresh medium – tigecycline; addition of polysorbate-80 – oritavancin)
    - Distribution of reference AST method MICs for relevant target species should be presented
    - *In vivo* PK/PD studies should show correlation between MIC and efficacy relative to *in vivo* exposure
  - These reasons do not justify a change from reference method:
    - No physiological justification
    - Lowering MIC values to demonstrate greater activity than competitor antimicrobial agents.
    - Modification to support growth of both fastidious and non-fastidious bacteria.
- AHWG Process
  - Presentation from GangaGen was distributed to all prior to 1st conference call with company (What we didn't do but next time would – AHWG only meet first to review process)
  - 1st conference call with GangaGen 3/25 – we made it through ¾ of slides. AHWG compiled questions and sent to them (included in background materials)
  - GangaGen provided answers to questions prior to 2nd conference call 4/10. Another question came up about QC

- AHWG met 4/23 and discussed – had they met the process. We followed up with what appeared to be out of spec QC but was a technical error on their initial internal testing of ceftazidime/avibactam in CAA

- What are casamino acids?
  - Water-soluble mixture of amino acids and small peptides derived from the acid hydrolysis of bovine casein. Widely used as nitrogen source.
- Klebicins: Protein antibiotics targeting *Klebsiella* spp.
  - Klebicins belong to a class of natural protein antibiotics called "Large bacteriocins" with molecular weight of 30-60 kDa
  - Highly genus specific, multi-domain protein structures that specifically kill *Klebsiella* spp.
  - Unique MoA: cell entry using cell surface receptors, translocation to substrate compartment and killing via cell wall damage or nucleic acid degradation
- GAN Kleb cocktail 001 (Klebicin CT)

Components of cocktail	Receptor	Mechanism of Translocation	Mechanism of cytotoxicity	Mw/PI
P628	IutA	Tol	RNase	59.2/9.21
P764	OmpC	Ton	Pore forming	39.8/8.96
P801	FhuA	Ton	Peptidoglycan synthesis inhibitor	29.8/8.9

- Salient features
  - Receptor mediated entry:
    - Active transport, overcomes the outer membrane permeability issues of Gram-negative bacteria
    - IutA and FhuA are reported to be involved in iron uptake
  - Novel molecules: No pre-existing resistance
  - Combination of Klebicins as a cocktail: diverse mode of action mitigate emergence of resistance
  - Narrow spectrum antibacterial: Specific only to *Klebsiella* species including *K. pneumoniae*, microbiome preserving
- Target Product Profile
  - Overall goal: Develop Klebicins as novel intravenous antibacterial agents to treat nosocomial pneumonia including HAP/VAP and CAP caused by *K. pneumoniae*

Variable	Minimal Requirement	Ideal Requirement
Primary Indication/ covering	HABP/VABP *K. pneumoniae*	HABP/VABP and hospitalized CABP *K. pneumoniae*
Delivery Mode	Intravenous infusion	Intravenous bolus
Treatment Duration	7 to 14 days	7 days
Regimen	2 to 3 times a day	1 to 2 times a day
Dosage Form	IV compatible solution in liquid form	
Dosing	In combination with SoC	Stand-alone
Efficacy	Non inferiority compared to SoC (monotherapy) and superiority on MDR *K. pneumoniae* (combination with SoC)	Superiority compared to SoC on all strains of *K. pneumoniae*
Combination with SoC antibiotics	No antagonism with broad spectrum antibiotics	Synergy with broad spectrum antibiotics against *K. pneumoniae*
Risk/Side Effect	Mild events or minor side effects with causal relation with reversible changes and do not lead to cessation of therapy.	No/ very limited adverse effects anticipated or observed

- Product development: A cocktail of 3 Klebicins
  - Three unique Klebicins P628, P764 and P801 with different mechanism of action that showed potent *in vitro* MICs and *in vivo* lung CFU reduction were designated as “Lead Klebicins”.
  - Lead Klebicins individually covered 60-89% of *K. pneumoniae* isolates. To achieve antibacterial activity against ≥95% *K. pneumoniae* isolates these three lead Klebicins were developed as a cocktail.
  - While the Frequency of resistance (FoR) of individual Klebicins were in the range of 10-6 to 10-7 the combination of the three Klebicins in the Klebicin cocktail reduced the FoR to ≤10-9 and hence has very low risk for resistance development.
  - The Minimum Effective Dose (MED) - the dose affording at least a one-log reduction in lung CFU) was determined for each individual Klebicin.
  - We initially tested the Klebicin cocktail composition with all the three Klebicins in equal concentrations (1:1:1 ratio), followed by other ratios derived based on the MED of individual Klebicins.
  - The most promising results were achieved with an optimized Klebicin ratio of 24:16:1 for P628, P764 and P801 respectively. The corresponding MED of Klebicins in combination are 60 mg/kg of P628, 40 mg/kg of P764 and 2.5 mg/kg of P801 (Total dose of 102.5mg/kg).
  - This optimized combination in 10mM Sodium Acetate Buffer, pH 5.0 with 8% Sucrose and 0.05% Tween 80 was designated as GAN Kleb cocktail 001.
  - GAN Kleb cocktail 001 was validated by both *in vitro* MICs and *in vivo* efficacy studies in the neutropenic lung infection model
  - IV administered GAN Kleb cocktail 001 was found to be well tolerated in mice and rats.
- Requirement for an iron deficient media for testing Klebicins

- Klebicins were tested in the standard CLSI - recommended medium CAMHB, but the MIC of GAN Kleb cocktail 001 were high and did not correlate well with the observed *in vivo* efficacy.
- As two of the three Klebicins in the Klebicin cocktail utilize iron-siderophore receptors for bacterial cell entry, we hypothesized that an iron-deficient medium is necessary to obtain accurate and relevant results. Hence the klebicins were tested in ID-CAMHB (CLSI approved for testing Cefiderocol) and other naturally iron-deficient media like Cas Amino Acids medium (CAA).
- Southwell JW et al. Enhancement of growth media for extreme iron limitation in Escherichia coli,Access Microbiol. 2024 Jun 3;6(6):000735.v4.
- Iron content in both ID-CAMHB and CAA are similar
- Concentrations of other cations vary considerably between CAA and ID-CAMHB

- Comparative MICs of Klebicins in different media
  - Klebicins were tested against a panel of drug sensitive, drug resistant and hypervirulent *K. pneumoniae*
  - Many QC strains will be tested in the next stage.
  - The Klebicins demonstrated poor activity in CAMHB despite showing good *in vivo* activity.
  - Despite lower iron levels in ID-CAMHB, Klebicin MICs remained high and similar to that seen in CAMHB.
  - MICs are lower with a consistent distribution in CAA than in other media.
  - Enhanced potency in CAA media was observed even for Klebicin P764, which utilizes the Omp C cell surface receptor.
- *In vitro* activity/*in vivo* efficacy correlation – wider panel of strains
  - Activity of Klebicin cocktail in CAMHB, ID-CAMHB and CAA were compared to draw a correlation with *in vivo* efficacy data.
  - *In vivo* efficacy:
    - 24 h Neutropenic Mouse Lung infection model
    - Challenge: Intranasal
    - Dose: Single dose IV administration at Klebicin Cocktail (102.5 mg/kg)
    - Efficacy parameter: Stasis or reduction in lung CFU.
    - Stasis: No increase in lung CFU's from the 2 hour baseline.
  - *In vitro – in vivo* correlation is considered when Klebicin cocktail demonstrates stasis or reduction in lung CFU and MIC of ≤ 32 µg/mL
  - Overall significant correlation of 96% was obtained with CAA while that with ID-CAMHB was 64% and with CAMHB was 52%.
- Investigating possible reasons for better activity of Klebicins in CAA
  - Effect of divalent cations in both CAA and ID-CAMHB
    - Adjusting the levels of calcium, magnesium, and zinc in both CAA and ID-CAMHB did not result in equivalent Klebicin activity between the two media
  - Components of CAMHB to CAA
    - Addition of starch to CAA did not affect the MIC
    - Supplementing CAA with Beef extract
      - Beef extract: The beef extract currently using is sourced from SRL Chem, India. Due to current import restrictions in India, it is difficult to source this item from alternative international brands.
      - An increase in the MIC of GAN Kleb cocktail 001 against *K. pneumoniae* isolates when tested in CAA supplemented with both chelated and unchelated Beef Extract was observed. The resulting MICs were similar to those obtained in ID-CAMHB. This suggests that Beef Extract may inhibit the activity of the GAN Kleb cocktail 001.

- Supplementing CAA with Beef extract (MP Biomedicals, USA): Similar to the previous source of beef extract, observed an increase in the MIC of GAN Kleb cocktail 001 against *K. pneumoniae* isolates when using the second brand of beef extract from MP Biomedicals.

- Conclusions
  - Calcium, Magnesium and Zinc Levels: Adjusting the levels of calcium, magnesium, and zinc in both CAA and ID-CAMHB did not result in equivalent Klebicin activity between the two media.
  - Beef Extract: An increase in the MIC of the Klebicin cocktail against K. pneumoniae isolates was observed when CAA was supplemented with both chelated and unchelated Beef Extract tested from two sources (SRL chemicals, India and MP Biomedicals USA). The resulting MICs were similar to those obtained in ID-CAMHB. This strongly suggests that Beef Extract may inhibit the activity of the Klebicin cocktail.
  - The data consistently demonstrate that the Klebicin activity observed in CAA was absent when MIC was performed in ID-CAMHB (or in CAA supplemented with Beef Extract). As Klebicins are proteins that can interact with various media components, the data clearly shows that ID-CAMHB and CAMHB are not suitable for MIC testing Klebicins.

- MIC in Cas Amino Acids medium across different CAA manufacturers
  - GAN Kleb cocktail 001 MIC is robust when tested in CAA medium from different manufacturers and MIC correlates with *in vivo* efficacy as well
- Testing of Klebicins and GAN Kleb cocktail 001 on global *K. pneumoniae* isolates
  - Two independent studies performed at IHMA, Europe
  - Total 648 recent (2021-2023) global *K. pneumoniae* isolates tested by MIC
  - Isolates: Drug-resistant, hypervirulent and MDR *K. pneumoniae* isolates from all body locations
  - Antibiotic sensitivity/resistance pattern already known for the tested isolates
  - Test items: Klebicins and GAN Kleb cocktail 001, microtiter plates were prepared and used fresh on the day of testing.
  - Testing media: Cas Amino Acids (CAA) media- [naturally iron-deficient media] for testing Klebicins and Cation adjusted Mueller Hinton broth for Ceftazidime/Avibactam and Meropenem
  - Inoculum : 5×10^5 CFU/mL
  - The following CLSI quality control (QC) strains were tested each day of testing:
    - *Escherichia coli* ATCC 25922
    - *Pseudomonas aeruginosa* ATCC 27853
    - *K. pneumoniae* ATCC 700603
  - MIC tests were performed by broth microdilution method in line with CLSI susceptibility testing standards, with an alternate medium (CAA medium)
- Summary of MIC studies with the Klebicin cocktail (Study 1 and 2)
  - Total 648 recent (2021-2023) global *K. pneumoniae* isolates tested by MIC
  - Two independent studies at IHMA
  - MDR isolates from multiple geographies, infection sites included
  - 32 µg/mL of the optimized Klebicin cocktail covers 95% of 648 global *K. pneumoniae* isolates tested
  - Summary of IHMA studies :
    - Testing was done in CAA medium
    - Klebicins were active on MDR isolates including colistin resistant ones and irrespective of the geography or source

- Summary
  - Klebicins are a novel class of narrow-spectrum antibiotics with a unique mechanism of action.
  - They are highly potent and specific only to *Klebsiella* species, including *K. pneumoniae*, particularly in iron-limited conditions.
  - Cas Amino Acids medium (CAA) is the most suitable medium for testing Klebicins due to its naturally low iron content, which mimics the iron-limited *in vivo* environment.
  - Comparative *in vitro* MIC results in CAMHB, ID-CAMHB, and CAA, along with the *in vitro-in vivo* correlation, confirm CAA is the best testing medium for Klebicins.
  - Reduced/lack of activity in ID-CAMHB
    - Beef extract present in ID-CAMHB has an inhibitory effect on Klebicin activity.
    - Adjusting the levels of calcium, magnesium, and zinc in both CAA and ID-CAMHB did not result in equivalent Klebicin activity between the two media
  - The MIC of the Klebicin cocktail is robust across different CAA manufacturers.
- AHWG Discussion and Recommendation
  - Sponsor Proposal: Cas Amino Acids (CAA) medium as the reference medium for testing the GAN Kleb cocktail 001 against *K. pneumoniae* using the CLSI Broth Microdilution Method.
  - AHWG questions covered testing of meropenem and ceftazidime/avibactam in CAA, final pH, possibility of disk zone diffusion, cocktail stability (>12 months, -20C), and bottle labels. R2 of answers included with background materials.
  - AHWG concurred that the sponsor proposal was acceptable and that criteria had been met. The test does not work in CAMHB or CAMHB-ID and alternate reference method using CAA is acceptable.
- Methods Working Group Discussion and Recommendation
  - Motion to approve the alternative broth microdilution method using CAA medium for testing of the GangaGen Klebicin cocktail against *Klebsiella pneumoniae*. WG Vote: 10-0-0-2.

SC DISCUSSION (MAIN POINTS)
- Is the CAA medium readily available? Yes.
- Another sponsor has come to CLSI in the past with a lysin compound and also requested CAS, so it is possible.
- Consider if CLSI wants to standardize a CAS method in the future.
- This method is for *Klebsiella* species.
- Will not get placed in M100. Intention of the vote is for GangaGen to move forward.

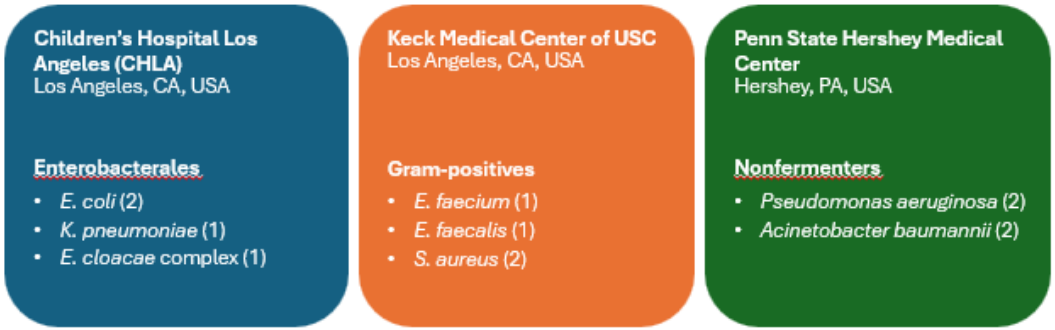
A motion to approve the alternative broth microdilution method using CAA medium for testing of the GangaGen Klebicin cocktail against *Klebsiella* spp. was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

EARLY GROWTH AST AD HOC WORKING GROUP REPORT

- Study Design: Phase I
  - Assess a small subset of isolates 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)

- Initial testing of Quality Control (QC) organisms will be included to ensure results are acceptable per CLSI standards.
- Motion: "To approve moving forward with the Phase II study as outlined in the summary of this presentation"
- This vote passes at the Methods WG 10-0-0-2 with a request to "consider including both S and R strains for colony counts to see if the lower counts impact the MIC/interp of the R strains."

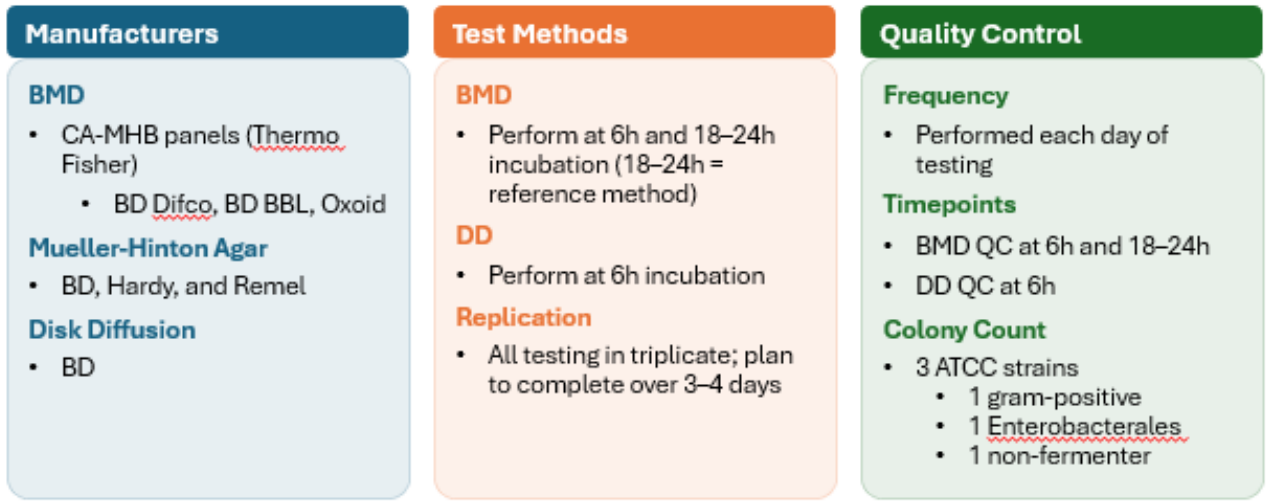
- Phase I Study Participants



- Phase I Antibiotics Tested

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

- Phase I Methods and Materials

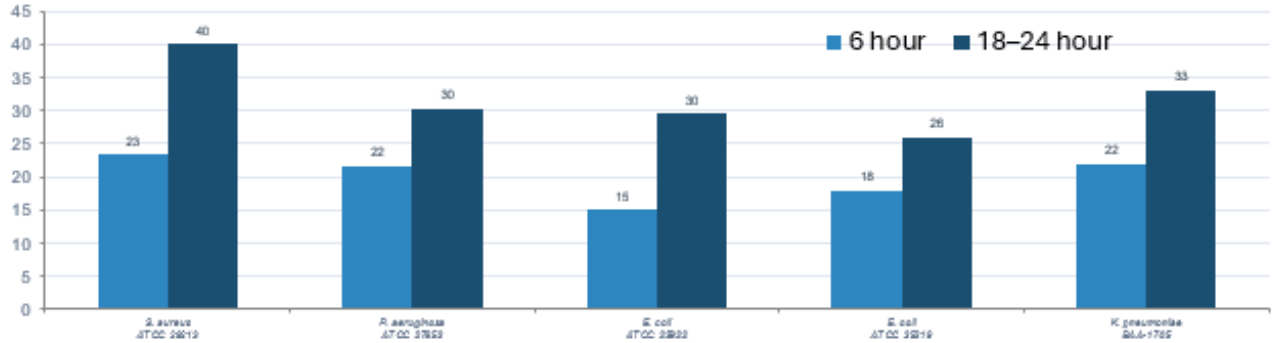


- Phase I QC Organisms

Gram Positives	Nonfermenters	Enterobacterales
S. aureus, ATCC 29213*,%	*P. aeruginosa*, ATCC 27853*	*K. pneumoniae*, ATCC BAA-1705
S. aureus, ATCC 25923#	*E. coli*, ATCC 25922^	*E. coli*, ATCC 25922*
E. faecalis, ATCC 29212		*E. coli*, ATCC 35218
E. faecalis, ATCC 51299		

*perform colony count
#For disk diffusion only for both *S. aureus* and enterococci
%For BMD only
^For ampicillin/sulbactam only

- Colony Count



- Comparison of QC isolates at 6 hours vs 18-24 hours growth
  - Majority of QC ranges were not fully covered within the BMD panel (dilutions did not cover the lower range)
    - All MICs were consistent between 6 hours and 18-24 hours except for one datapoint. Only one MiE (1, *E. coli* 25922, ampicillin on Oxoid BMD)
  - DD showed perfect concordance between 6 hours and 18-24 hours growth
    - All DD QC comparisons (6 hours vs 24 hours) achieved 100% correlation across every organism and drug tested at all sites

Site	QC Strain	DD 6h vs 24h, %CA	BMD 6h vs 24h, %CA	Colony Count 6h / 24h	QC Pass (Y/N)
USC	*S. aureus* 29213	—	100%	23.3 / 40	Y
USC	*S. aureus* 25923	100%	—	—	Y
USC	*E. faecalis* 29212	—	100%	—	Y
USC	*E. faecalis* 51299	100%	—	—	Y
PSU	*P. aeruginosa* 27853	100%	100%	21.7 / 30.3	Y
CHLA	*E. coli* 25922	100%	98.9%*	15 / 29.5	Y
CHLA	*E. coli* 35218	100%	100%	18 / 26	Y
CHLA	*K. pneumoniae* BAA-1705	100%	100%	22 / 33	Y

- Enterobacterales: Disk Diffusion

DISK DIFFUSION: 6h vs 24h BMD Reference

Antibiotic	N	S	I	R	CA% (All)	CA% (BD)	CA% (Hardy)	CA% (Remel)	MiE% (All)	ME% (All)	VME% (All)
ampicillin (10 µg)	12	0	0	12	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0 (0/36)	0	0
cefazolin (30 µg)	12	0	0	12	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0 (0/36)	0	0
cefepime (30 µg)	12	3	0	9	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0 (0/36)	0	0
ceftriaxone (30 µg)	12	0	0	12	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0 (0/36)	0	0
ciprofloxacin (5 µg)	12	3	0	9	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0 (0/36)	0	0
gentamicin (10 µg)	12	9	0	3	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0 (0/36)	0	0
meropenem (10 µg)	12	6	0	6	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0 (0/36)	0	0
nitrofurantoin (300 µg)*	9	3	3	3	88.9 (24/27)	100 (9/9)	66.7 (6/9)	66.7 (6/9)	22.2 (6/27)	0	0
TMP/SMX (25 µg)	12	3	0	9	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0 (0/36)	0	0

- Enterobacterales: Broth Microdilution

BROTH MICRODILUTION: 6h vs 24h BMD Reference

Antibiotic	N	S	I	R	CA% (All)	EA% (All)	CA% (BBL)	CA% (Difco)	CA% (Oxoid)	MiE% (All)	ME% (All)	VME% (All)
ampicillin (10 µg)	12	0	0	12	100 (36/36)	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0	0	0
cefazolin (30 µg)	12	0	0	12	100 (36/36)	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0	0	0
cefepime (30 µg)	12	3	0	9	100 (36/36)	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0	0	0
ceftriaxone (30 µg)	12	0	0	12	100 (36/36)	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0	0	0
ciprofloxacin (5 µg)	12	3	0	9	100 (36/36)	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0	0	0
gentamicin (10 µg)	12	9	0	3	94.4 (34/36)	94.4 (34/36)	100 (12/12)	100 (12/12)	83.3 (10/12)	5.6 (2/36)	0	0
meropenem (10 µg)	12	6	0	6	88.9 (32/36)	88.9 (32/36)	91.7 (11/12)	91.7 (11/12)	83.3 (10/12)	11.1 (4/36)	0	0
nitrofurantoin (300 µg)*	9	3	3	3	96.3 (26/27)	96.3 (26/27)	100 (9/9)	88.9 (8/9)	100 (9/9)	3.7 (1/27)	0	0
piperacillin tazobactam (100/10 µg)	12	0	0	12	100 (36/36)	94.4 (34/36)	100 (12/12)	100 (12/12)	100 (12/12)	0	0	0
TMP/SMX (25 µg)	12	3	0	9	100 (36/36)	100 (36/36)	100 (12/12)	100 (12/12)	100 (12/12)	0	0	0

- Nonfermenters: Disk Diffusion

DISK DIFFUSION: 6h vs 24h BMD Reference											
Antibiotic	N	S	I	R	CA% (All)	CA% (BD MH)	CA% (Hardy MH)	CA% (Remel MH)	MiE% (All)	ME% (All)	VME% (All)
ampicillin-sulbactam (10/10 µg)	6	0	0	6	83.3% (15/18)	83.3% (5/6)	66.7% (4/6)	100% (6/6)	16.7% (3/18)	0	0
cefepime (30 µg)	12	3	3	6	80.6% (29/36)	91.7% (11/12)	75% (9/12)	75% (9/12)	19.4% (7/36)	0	0
ceftazidime (20 µg)	12	0	3	9	94.4% (34/36)	100% (12/12)	83.3% (10/12)	91.7% (11/12)	5.6% (2/36)	0	0
ciprofloxacin (5 µg)	12	3	0	9	100% (36/36)	100% (12/12)	100% (12/12)	100% (12/12)	0	0	0
tobramycin (10 µg)	12	6	0	6	100% (36/36)	100% (12/12)	100% (12/12)	100% (12/12)	0	0	0
meropenem (10 µg)	12	3	0	9	100% (36/36)	100% (12/12)	100% (12/12)	100% (12/12)	0	0	0

- Nonfermenters: Broth Microdilution

BROTH MICRODILUTION: 6h vs 24h BMD Reference												
Antibiotic	N	S	I	R	CA% (All)	EA% (All)	CA% (BBL)	CA% (Difco)	CA% (Oxoid)	MiE% (All)	ME% (All)	VME% (All)
ampicillin-sulbactam (10/10 µg)	6	0	0	6	100% (18/18)	100% (18/18)	100% (6/6)	100% (6/6)	100% (6/6)	0	0	0
cefepime (30 µg)	12	3	3	6	91.7% (33/36)	100% (36/36)	100% (12/12)	91.7% (11/12)	83.3% (10/12)	8.3% (3/36)	0	0
ceftazidime (20 µg)	12	0	3	9	94.4% (34/36)	100% (36/36)	100% (12/12)	83.3% (10/12)	100% (12/12)	5.6% (2/36)	0	0
ciprofloxacin (5 µg)	12	3	0	9	100% (36/36)	100% (36/36)	100% (12/12)	100% (12/12)	100% (12/12)	0	0	0
tobramycin (10 µg)	12	6	0	6	97.2% (35/36)	100% (36/36)	91.7% (11/12)	100% (12/12)	100% (12/12)	2.8% (1/36)	0	0
meropenem (10 µg)	12	3	0	9	100% (36/36)	100% (36/36)	100% (12/12)	100% (12/12)	100% (12/12)	0	0	0
piperacillin tazobactam (100/10 µg)	12	0	0	12	91.7% (33/36)	100% (36/36)	100% (12/12)	0.916666667	83.3% (10/12)	8.3% (3/36)	0	0

- Gram positives: Disk Diffusion

S. aureus: 6h vs 24h BMD Reference

Antibiotic	N	S	I	R	CA% (All)	CA% (BD)	CA% (Hardy)	CA% (Remel)	MiE% (All)	ME% (All)	VME% (All)
cefoxitin (30 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0
clindamycin (2 µg)	6	3	0	3	83.3 (15/18)	50 (3/6)	100 (6/6)	100 (6/6)	16.7 (3/18)	0	0
doxycycline (30ug)*	6	3	0	3	50 (9/18)	50 (3/6)	50 (3/6)	50 (3/6)	50 (9/18)	0	0
erythromycin (15 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0
linezolid (30 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0
nitrofurantoin (300 µg)	6	6	0	0	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	N/A
TMP/SMX (25 µg)	6	6	0	0	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	N/A

*Doxycycline: 24h BMD yielded Remel Intermediate, Hardy and BD Resistant. Majority rules → Resistant reference

Enterococcus: 6h vs 24h BMD Reference

Antibiotic	N	S	I	R	CA% (All)	CA% (BD)	CA% (Hardy)	CA% (Remel)	MiE% (All)	ME% (All)	VME% (All)
ampicillin (10 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0
ciprofloxacin (5 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0
linezolid (30 µg	6	6	0	0	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	N/A
**nitrofurantoin (300 µg)	3	3	0	0	100 (9/9)	100 (3/3)	100 (3/3)	100 (3/3)	0	0	N/A
vancomycin (30 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0 (0/9)

**Nitrofurantoin: Omitted USC-EFAC-10; all 3 media types gave different 24h BMD interpretation

- Gram Positives: Broth Microdilution

S. aureus: 6h vs 24h BMD Reference

Antibiotic	N	S	I	R	CA% (All)	CA% (BD)	CA% (Hardy)	CA% (Remel)	MiE% (All)	ME% (All)	VME% (All)
cefoxitin (30 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0
clindamycin (2 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0
doxycycline (30ug)*	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0
erythromycin (15 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0
linezolid (30 µg)	6	6	0	0	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	N/A
nitrofurantoin (300 µg)	6	6	0	0	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	N/A
TMP/SMX (25 µg)	6	6	0	0	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	N/A
vancomycin (30 µg)	6	6	0	0	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	N/A

Enterococcus: 6h vs 24h BMD Reference

Antibiotic	N	S	I	R	CA% (All)	CA% (BD)	CA% (Hardy)	CA% (Remel)	MiE% (All)	ME% (All)	VME% (All)
ampicillin (10 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0
ciprofloxacin (5 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0
linezolid (30 µg)	6	6	0	0	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	N/A
***nitrofurantoin (300 µg)*	3	3	0	0	100 (9/9)	100 (3/3)	100 (3/3)	100 (3/3)	0	0	N/A
vancomycin (30 µg)	6	3	0	3	100 (18/18)	100 (6/6)	100 (6/6)	100 (6/6)	0	0	0

**Nitrofurantoin: Omitted USC-EFAC-10; all 3 media types gave different 24h BMD interpretation

- Disk Diffusion: Categorical Agreement Summary

6h DD read vs 24h BMD reference — CA% across all media

100% 90–99% <90% — n/a

Antibiotic	Enterobacterales	*S. aureus*	*Enterococcus*	*A. baumannii*	*P. aeruginosa*
ampicillin	100%	—	100%	—	—
ampicillin-sulbactam	—	—	—	33.3%	—
cefazolin	100%	—	—	—	—
cefepime	100%	—	—	100%	61.1%
cefoxitin	—	100%	—	—	—
ceftazidime	—	—	—	100%	83.3%
ceftriaxone	100%	—	—	—	—
ciprofloxacin	100%	—	100%	100%	100%
clindamycin	—	33.3%	—	—	—
doxycycline*	—	50%	—	—	—
erythromycin	—	100%	—	—	—
gentamicin	100%	—	—	—	—
linezolid	—	100%	100%	—	—
meropenem	100%	—	—	100%	100%
nitrofurantoin	83.3%	100%	100%	—	—
tobramycin	—	—	—	100%	100%
TMP/SMX	100%	100%	—	—	—
vancomycin	—	—	100%	—	—

*Doxycycline: 24h BMD reference interpretation ambiguous (media disagreed on S vs I vs R)

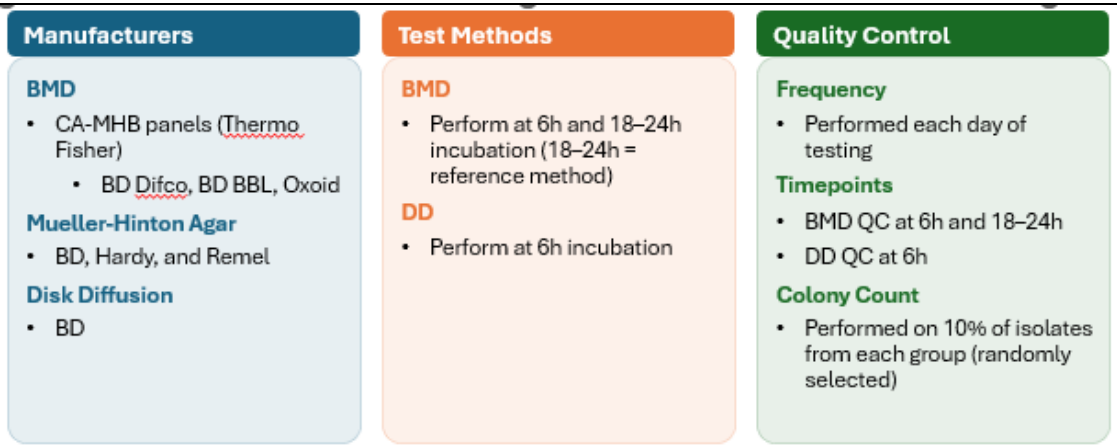
- Broth Microdilution: Categorical Agreement Summary

6h BMD read vs 24h BMD reference — CA% across all media

100% 90–99% <90% — n/a

Antibiotic	Enterobacterales	S. aureus	Enterococcus	A. baumannii	P. aeruginosa
ampicillin	100%	—	100%	—	—
ampicillin-sulbactam	—	—	—	100%	—
cefazolin	100%	—	—	—	—
cefepime	100%	—	—	100%	83.3%
cefoxitin	—	100%	—	—	—
ceftazidime	—	—	—	100%	83.8%
ceftriaxone	100%	—	—	—	—
ciprofloxacin	100%	—	100%	100%	100%
clindamycin	—	100%	—	—	—
doxycycline	—	100%	—	—	—
erythromycin	—	100%	—	—	—
gentamicin	94.4%	—	—	—	—
linezolid	—	100%	100%	—	—
meropenem	83.8%	—	—	100%	100%
nitrofurantoin	98.3%	100%	100%	—	—
pip-tazo	100%	—	—	100%	83.3%
tobramycin	—	—	—	94.4%	100%
TMP/SMX	100%	100%	—	—	—
vancomycin	—	100%	100%	—	—

- Phase II
  - Collection of 170 clinical isolates
    - 50 Enterobacterales
    - 60 Nonfermenters
      - 30 *Acinetobacter baumannii* complex
      - 30 *Pseudomonas aeruginosa*
    - 60 Gram positive
      - 30 *Staphylococcus* species
      - 30 *Enterococcus* species
  - Broad range of susceptible and resistant phenotypes
  - Obtained from geographically distinct sites (children and adults)
  - Isolated primarily from sterile sites
  - Testing at single site: CHLA
- Phase II Methods and Materials



- Phase II Supplies and Materials

Material/Supplies	Number of isolates tested	Manufacturer / Supplier	Catalogue #	Total Required (N)
BBL MHA II, 150mm	170 + QC	BD	221800 (24/pk)	240
Remel MHA, 150 mm	170 + QC	Remel/ThermoFisher	R04052 (40/pk)	240
Hardy MHA HDx, 150 mm	170 + QC	Hardy	H11 (10/pk)	240
Blood agar plate	170 + QC X 3 (1X DD and 2X BMD)	Any manufacturer		600
Inoculator Assembly	170 + QC For BMD X 2 (6 hr and 24 hr) X 2 (assuming 2 panels will be needed per isolate)	ThermoFisher	YP0050 (50/pack)	800
Demineralized water	170 + QC	ThermoFisher	YT3338, 29ml (20/pk)	240
BMD	60 Gram pos isolates + QC. Need panel with 3 different broths. 80 X 2 (6 and 18-24 hr) 110 gram neg isolates + QC. Gram neg panel with 3 different broths. 140 X 2 (6 and 18-24 hr)	ThermoFisher	Made by request	160 Gram pos panels 280 Gram neg panels Note: to accommodate all drugs and dilutions will likely need 2 panels per isolate
0.85% saline	170 + QC			240
Disks (BD)	30 + QC X 3 (media)			150 to 500 disks per drug

- Methods Working Group Discussion and Recommendation
  - BMD plate maps will be updated to include QC ranges

- Bias and trending analysis would be helpful when analyzing the Phase II results
- Motion to approve moving forward with the Phase II study as described. WG Vote: 10-0-0-2.
- Request to include both S and R strains for colony counts to see if the lower counts impact the MIC/interpretation of the R strains.

SC DISCUSSION (MAIN POINTS)

- Laboratories would want to pull the colony off the primary plates, which does not represent real life.
- Will this be considered a reference method? Can it be used for breakpoint setting studies? If this is going to be a reference method, then CLSI will need more data to compare.
- The idea is great and will greenlight laboratories to validate their own in-house susceptibility method, but unsure of the impact.
- Concern laboratories are not aware of the log phase of growth method.
- The study should use other timepoints beyond 6 hours, so laboratories can test a range.
- The study should try to mimic a mixed subculture.
- There will still be a purity plate, so mixed cultures would not be an issue.
- The intent was not to be a new reference method.
- Suggestion to use representative classes of drugs to cut down on the number of drugs and to test additional time points including longer time points like 30 hours.
- Was the QC near the mode or on the higher or lower end? That data was missing here. There is concern that this is not enough isolates, it is single center, and level of details for CLSI to accept this are not present.
- Does the log phase growth method have colony counts that show the same lower colony counts seen in this study?
- The log phase growth method might have isolates listed in CLSI M07 that this method does not work for, so make sure those organisms work with the early growth method.
- Laboratories will have to validate this method and there are some published studies that indicate this works. Laboratories can validate this method themselves without CLSI.
- There is a concern that the resources are not available that would be needed to successfully complete the data needed for CLSI to approve.
- The discussion points will be taken back to the AHWG to review.

APPENIDX A AD HOC WORKING GROUP REPORT

- Mission: To review Appendix A with bacterial, fungal, and veterinary subcommittees to align definitions for Category I, II, and III.
- Update
  - Fungal Group Update
    - Created an AHWG
    - 2 separate documents, mold and yeast supplement, just updated in March updates will be added to the next supplements
  - Update the definition of Categorical I
  - Update the table to move Drug/bug categories from I to II
  - Make sure if we update Category I should update notes in Table 2
  - Discuss deletion of Quinupristin-dalfopristin from table as it was discontinued in 2022
- Mock Up

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date with clinical and/or epidemiological significance	Uncommon in most institutions	May be common but generally considered of epidemiological concern
			Action Steps:		
			• Confirm ID and susceptibility.. • Report to infection prevention. • Check with public health department to determine appropriate reporting and isolate referral procedures. • Save isolate. NOTE: It may be appropriate to notify infection prevention of preliminary findings before confirmation of results.	• Confirm ID and susceptibility if uncommon in the institution.. • Check with infection prevention in the facility to determine whether special reporting procedures or additional actions are needed. • Check with public health department to determine appropriate reporting and isolate referral procedures.	• Confirm ID and susceptibility if uncommon in the institution.. • Check with infection prevention in the facility to determine whether special reporting procedures or additional action are needed.
Any Enterobacterales	β-Lactam combination agents	**Aztreonam-avibactam – I or R**	X		
		Ceftazidime-avibactam – R Imipenem-relebactam – I or R Meropenem-vaborbactam – I or R		X	
	Cephems	Cefiderocol – I or R	X	X	
	Carbapenems	Any carbapenem – I or R[b]		X	
	Aminoglycosides	Amikacin, gentamicin, and tobramycin – R			X
		Plazomicin – R (except *Proteus mirabilis*)	X	X	
	Lipopeptides	Colistin/polymyxin B – R[c]	X	X	

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date **with clinical and/or epidemiological significance**	Uncommon in most institutions	**May be common but generally considered of epidemiological concern**
Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, and P. mirabilis	Cephems	Cephalosporin III/IV – I/SDD or R			X
Salmonella and Shigella spp.[d]	Cephems	Cephalosporin III – I or R		X	
	Macrolides	Azithromycin – R		X	
	Fluoroquinolones	Any fluoroquinolone – I or R		X	
Pseudomonas aeruginosa	β-Lactam combination agents	Ceftazidime-avibactam – R Ceftolozane-tazobactam – I or R Imipenem-relebactam – I or R			X
	Cephems	Cefiderocol – I or R	X	X	
	Carbapenems	Any carbapenem[e] – I or R			X
	Aminoglycosides	Amikacin and tobramycin – R			X
	Lipopeptides	Colistin/polymyxin B – R	X	X	
Acinetobacter baumannii complex	β-Lactam combination agents	Sulbactam-durlobactam – I or R	X		
	Cephems	Cefiderocol – I or R	X	X	
	Carbapenems	Any carbapenem[e] – I or R			X
	Lipopeptides	Colistin/polymyxin B – R	X	X	
Stenotrophomonas maltophilia	Cephems	Cefiderocol – NS	X		
	Folate pathway antagonists	**Trimethoprim-sulfamethoxazole – I or R**			X

SC DISCUSSION (MAIN POINTS)

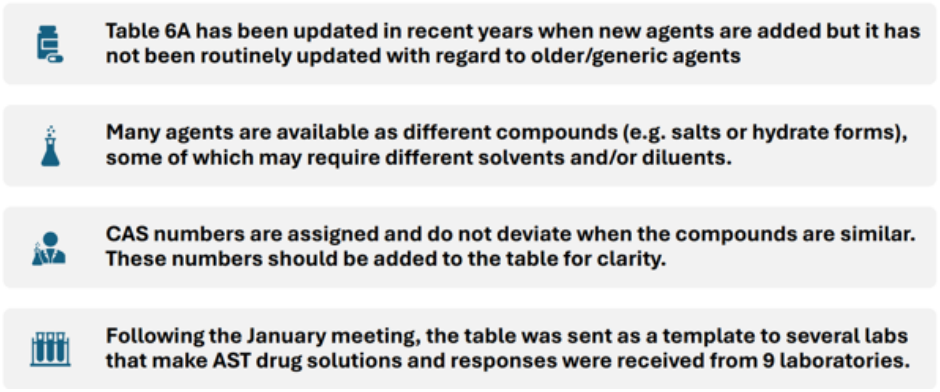
- Quinupristin-dalfopristin is no longer used, remove it from the document.
- Suggestion to also remove ticarcillin-clavulanate.

- Consider where teicoplanin will need to go.
- Agreement to remove quinupristin-dalfopristin.
- The categories are confusing for laboratories.
- Consensus was that Appendix A needs to be clarified more with the AHWG.

A motion to remove quinupristin-dalfopristin throughout M100 was made and seconded. Vote: 15 for, 0 against, 0 abstain, 1 absent (Pass)

SOLVENTS TABLE AD HOC WORKING GROUP REPORT

- Background



- A copy of the proposed Table 6A was included in the meeting background materials
- Future
  - Which compound is correct, is there a way to determine this.
  - Some concerns for standardization retroactively could require revalidation
  - Concerns about removing alternate versions that work because of the need for redundancy for back orders.
  - How to know if something did not work. Working with device manufacturers can help because they have an interest in knowing which versions work.
- Methods Working Group Discussion and Recommendation
  - Motion to approve the revised Table 6A for publication in the M100-Ed37, pending final formatting according to CLSI style. WG Vote: 10-0-0-2.

SC DISCUSSION (MAIN POINTS)

- Concern about the heating statement in new footnote. Confirmed to be “gentle heating”.
- Surotomycin is no longer made by Merck.
- Quinupristin-dalfopristin will be removed per the prior vote.

A motion to approve the revised Table 6A pending CLSI style formatting, change to "gentle heat" in new footnote, and remove surotomycin was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

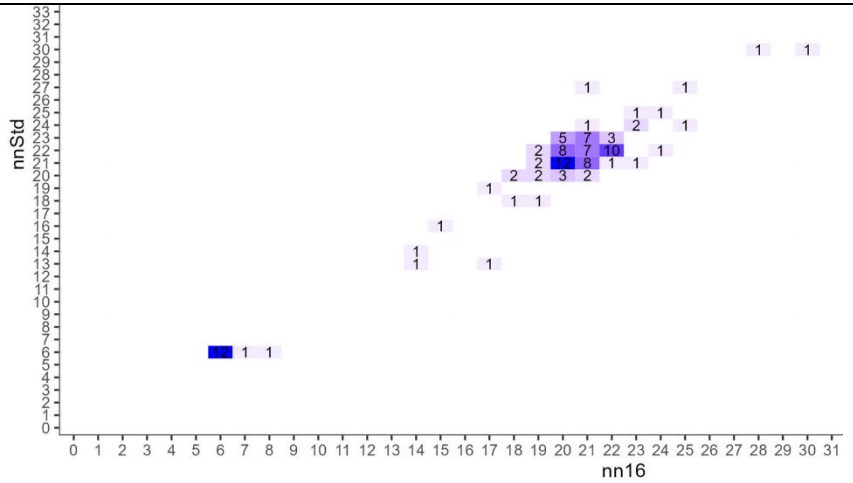
DIRECT BLOOD CULTURE SUSCEPTIBILITY AD HOC WORKING GROUP REPORT

- Tobramycin 16-18 hour vs Std Disk Diffusion *Acinetobacter*

	Std DD			
16-18 hr	S	I	R	Grand Total
S	89	1		90
I		3		3
R			14	14
Grand Total	89	4	14	107

CA	106/107	99.1%
VME	0/14	0
ME	0/89	0
mE	1/107	<1%

Standard zone cutoffs (mm)		
S	I	R
≥17	13-16	≤12

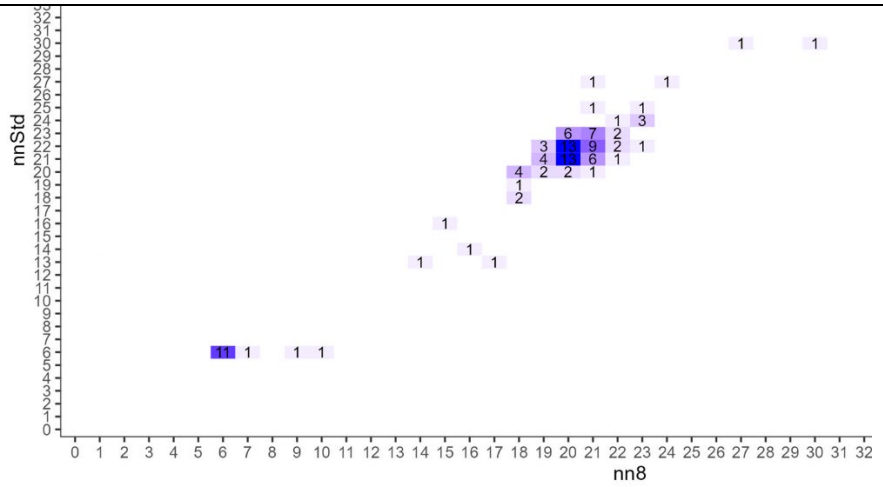


- Tobramycin 8-10 hour vs Std disk Diffusion Acinetobacter

	Std DD			
8-10 hr	S	I	R	Grand Total
S	89	1		90
I		3		3
R			14	14
Grand Total	89	4	14	107

CA	106/107	99.1%
VME	0/14	0
ME	0/89	0
mE	1/107	<1%

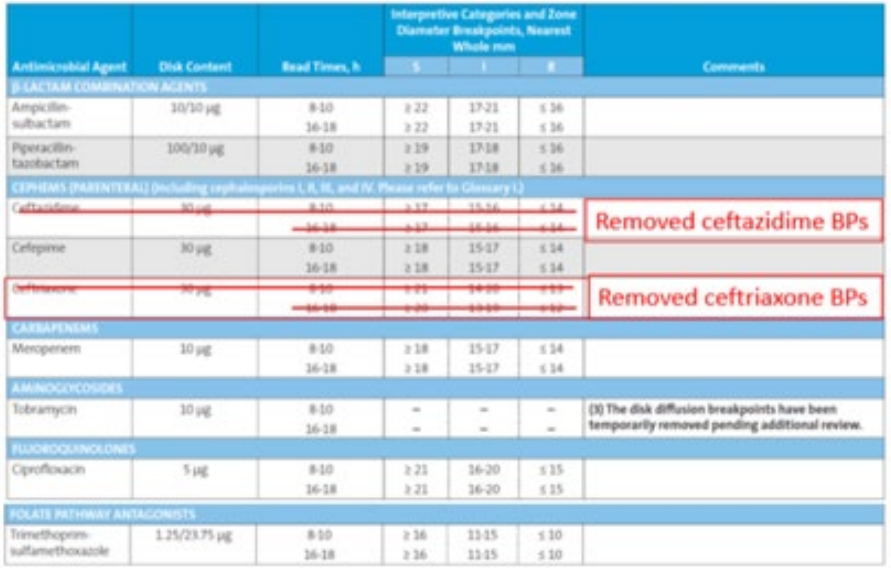
Standard zone cutoffs (mm)		
S	I	R
≥17	13-16	≤12



- Methods Working Group Discussion and Recommendation
  - Motion to accept the direct from blood culture disk diffusion tobramycin breakpoints (S ≥17, I 13-16, R ≤ 12 mm) for 16-18 hours and 8-10 hours. WG Vote: 8-0-0-4.

A motion to accept the direct from blood culture tobramycin disk diffusion breakpoints (S ≥ 17, I 13-16, R ≤ 12) for *Acinetobacter* spp. at 16-18 hours and 8-10 hours was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

- Zone Diameter Disk diffusion Breakpoints for *Acinetobacter* Direct from Blood Culture (Table 3F-4)



Antimicrobial Agent	Disk Content	Read Times, h	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm S	I	R	Comments
β-LACTAM COMBINATION AGENTS						
Ampicillin-sulbactam	10/10 µg	8-10	≥ 22	17-21	≤ 16	
		16-18	≥ 22	17-21	≤ 16	
Piperacillin-tazobactam	100/10 µg	8-10	≥ 19	17-18	≤ 16	
		16-18	≥ 19	17-18	≤ 16	
CEPHEMS (PARENTERAL) (including cephalosporins I, II, III, and IV. Please refer to Glossary I.)						
~~Ceftazidime~~	~~30 µg~~	~~8-10~~	~~≥ 17~~	~~15-16~~	~~≤ 14~~	Removed ceftazidime BPs
		~~16-18~~	~~≥ 17~~	~~15-16~~	~~≤ 14~~	
Cefepime	30 µg	8-10	≥ 18	15-17	≤ 14	
		16-18	≥ 18	15-17	≤ 14	
~~Ceftriaxone~~	~~30 µg~~	~~8-10~~	~~≥ 21~~	~~14-20~~	~~≤ 13~~	Removed ceftriaxone BPs
		~~16-18~~	~~≥ 20~~	~~13-19~~	~~≤ 12~~	
CARBAPENEMS						
Meropenem	10 µg	8-10	≥ 18	15-17	≤ 14	
		16-18	≥ 18	15-17	≤ 14	
AMINOGLYCOSIDES						
Tobramycin	10 µg	8-10	–	–	–	(3) The disk diffusion breakpoints have been temporarily removed pending additional review.
		16-18	–	–	–	
FLUOROQUINOLONES						
Ciprofloxacin	5 µg	8-10	≥ 21	16-20	≤ 15	
		16-18	≥ 21	16-20	≤ 15	
FOLATE PATHWAY ANTAGONISTS						
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	8-10	≥ 16	11-15	≤ 10	
		16-18	≥ 16	11-15	≤ 10	

Ad hoc WG will reassess direct DD breakpoints once standard DD breakpoints have been determined

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SC DISCUSSION (MAIN POINTS)

- Concern piperacillin-tazobactam disk diffusion method does not work.

A motion to remove ceftriaxone direct blood culture disk diffusion breakpoints with no comment and to remove ceftazidime and piperacillin-tazobactam direct blood culture disk diffusion breakpoints for *Acinetobacter* spp. with a comment in the Overview of Changes in M100 stating, "The disk diffusion breakpoints have been temporarily removed pending additional review." was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

- Customer Service Question
  - Is the MHA plate mentioned in the procedure a 150 mm or 100 mm plate?
  - Will results be the same if using 4 drops to inoculate 150 mm and 100 mm MHA plate sizes?
  - AHWG Discussion and Recommendation
    - Because the study design used only 150 mm plates, and all data are based off that, it is uncertain as to whether use of 100 mm plates would impact results
    - AHWG agreed that adding 150 mm plate into the methods was of value in Table 3F-1

Test	Direct Disk Diffusion
Test method	Disk diffusion using positive blood culture broth
Organism group	Enterobacterales, *Pseudomonas aeruginosa,* and *Acinetobacter* spp.
Medium	150-mm MHA plate
Antimicrobial concentration	Standard disk contents for the antimicrobial agents are detailed in Table 3F-2 (Enterobacterales), Table 3F-3 (*P. aeruginosa*), and Table 3F-4 (*Acinetobacter* spp.).
Inoculum	Positive blood culture broth with gram-negative bacilli, used within 8 hours of flagging positive by the blood culture system
Test procedure	1. Invert blood culture bottle 5–10 times to thoroughly mix. 2. Sterilize the top of the bottle with an alcohol wipe (allow to dry) and insert 20-gauge venting needle into the blood culture bottle. 3. Dispense 4 drops of blood culture broth onto a150-mm MHA plate. As a purity check, use an inoculated blood agar plate streaked for isolation. 4. Spread blood culture broth across the entire surface of the MHA plate using a sterile cotton swab. 5. Repeat this procedure by streaking twice more, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. 6. Leave the lid ajar for 3–5 minutes (ideally) but no more than 15 minutes. 7. Dispense antimicrobial disks onto the surface of the inoculated MHA plate.

SC DISCUSSION (MAIN POINTS)

- Consensus agreed with the proposed revision.

BETA-HEMOLYTIC STREPTOCOCCI TRIMETHOPRIM-SULFAMETHOXAZOLE (TMP-SMX) CORRELATES AND MEDIA

- Background on TMP-SMX testing of beta-hemolytic streptococci
  - CLSI M100 adopted BMD using standard media for beta-strep (MHB w/LHB) with MIC breakpoints for TMP-SMX beta-hemolytic strep testing at the June 2025 meeting.
  - Disk diffusion breakpoints were not evaluated due to thymidine concerns in the sheep blood agar and agar-based testing was discouraged in the M100 text.
  - Previously, high thymidine concentrations in sheep blood-containing media caused falsely elevated MICs among beta-hemolytic streptococci
    - "Since 2006, when the thymidine content of MHA became strictly regulated by the Clinical and Laboratory Standards Institute (CLSI) to maintain a low level of thymidine and hence avoid inhibition (M6-A2 protocol) (9), it has no longer been necessary to add lysed horse blood to the medium for AST."
    - *M6-A2: "Each manufacturer should test the potential candidate lots of Mueller-Hinton agar to determine that the medium contains acceptable thymidine content…. Reject any candidate lot that does not meet these criteria from further consideration. This can be done by preparing ten plates of each candidate lot and then testing five replicates each of *E. faecalis* ATCC® 33186 against trimethoprim-sulfamethoxazole…. Acceptable performance is: For *E. faecalis* ATCC 33186 (or ATCC 29212) and trimethoprim-sulfamethoxazole, the inhibition zone must be ≥ 20 mm, with clarity comparable to the reference medium."
- What is the difference between sheep and horse blood?
  - Lysed horse blood contains thymidine phosphorylase, which neutralizes thymidine

        - Thymidine phosphorylase converts thymidine to thymine and hence overcomes the inhibition of folate metabolism that occurs in the presence of thymidine
        - Can no longer serve as an exogenous source of thymidine
        - No other mammalian blood carries thymidine phosphorylase
    - Thymidine content regulated in MHA (>0.03 mg/L)
        - *Enterococcus faecalis* 29212/33186 used to QC thymidine content in media
- CLSI current recommendations
    - MH-F is acceptable for TMP-SMX testing of *S. pneumoniae*
    - MH-F is acceptable for testing of *Haemophilus influenzae*, except with TMP-SMX, which requires HTM

Table 2H-1. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. β-Hemolytic Group

Testing Conditions	
Medium:	Disk diffusion: MHA with 5% sheep blood Broth dilution: CAMHB with LHB (2.5% to 5% v/v); the CAMHB should be supplemented to 50 µg/mL calcium for daptomycin (see CLSI M07[1] for instructions for preparation of LHB). Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.
Inoculum:	Colony suspension, equivalent to a 0.5 McFarland standard, using colonies from an overnight (18- to 20-hour) sheep blood agar plate
Incubation:	35°C ± 2°C Disk diffusion: 5% CO_2; 20-24 hours Dilution methods: ambient air; 20-24 hours (CO_2 if necessary, for growth with agar dilution)

FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	–	–	–	–	**≤ 0.5**	**1**	**≥ 2**	**(14) Agar-based methods using MHA with 5% sheep blood (eg, disk diffusion, gradient diffusion, agar dilution) should not be performed due to increased thymidine content leading to false resistance.** **(15) Testing and reporting should be limited to isolates recovered from skin and skin structure infections; it is not indicated for isolates from other sources (eg, pharyngeal).**

- EUCAST current recommendations

Streptococcus groups A, B, C and G

Expert Rules and Expected Phenotypes | Guidance documents

EUCAST Clinical Breakpoint Tables v. 16.0, valid from 2026-01-01

For abbreviations and explanations of breakpoints, see the Notes sheet

MIC determination (broth microdilution according to ISO standard 20776-1)
Medium: Cation-adjusted Mueller-Hinton broth + 5% lysed horse blood and 20 mg/L β-NAD (MH-F broth)
Inoculum: $5x10^5$ CFU/mL
Incubation: Sealed panels, air, 35±1°C, 18±2h (for glycopeptides 24h)
Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth. See "EUCAST Reading Guide for broth microdilution" for further information.
Quality control: *Streptococcus pneumoniae* ATCC 49619. For agents not covered by this strain, see EUCAST QC Tables.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1°C, 18±2h
Reading: Unless otherwise stated, read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light. See "EUCAST Reading Guide for disk diffusion" for further information.
Quality control: *Streptococcus pneumoniae* ATCC 49619. For agents not covered by this strain, see EUCAST QC Tables.

This group of bacteria includes many species, which can be grouped as follows:
Group A: *S. pyogenes*
Group B: *S. agalactiae*
Group C: *S. dysgalactiae* (plus the more rarely isolated *S. equi*)
Group G: *S. dysgalactiae* and *S. canis*
S. dysgalactiae includes the subspecies *equisimilis* and *dysgalactiae*. *S. equi* includes the subspecies *equi* and *zooepidemicus*.

Miscellaneous agents	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)		
	S ≤	R >	ATU		S ≥	R <	ATU
Trimethoprim (uncomplicated UTI only), *S. agalactiae* (group B streptococci)	Note[3]	Note[3]			Note[B]	Note[B]	
Trimethoprim-sulfamethoxazole[4]	0.5	0.5		**1.25-23.75**	16	16	

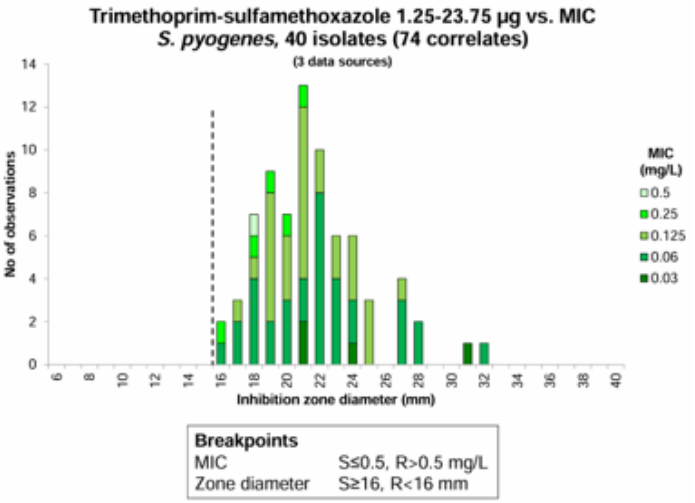
3/B. The activity of trimethoprim is uncertain against *S. agalactiae* and it is not possible to predict clinical outcome. The ECOFF to categorise isolates as wild type or non-wild type is 2 mg/L.
4. Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.

- Antimicrobial Susceptibility Testing Methods for BHS
  - disk content the same for CLSI and EUCAST Breakpoints: 1.25/23.75 ug

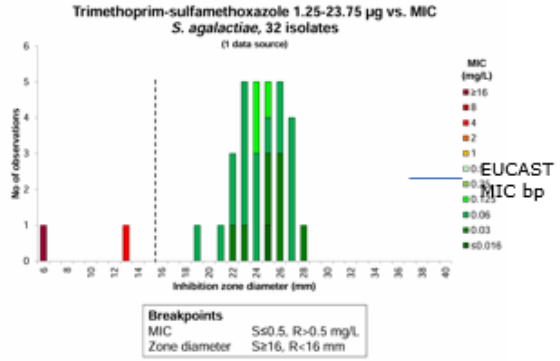
	Disk Diffusion	Broth Microdilution	Agar Dilution
CLSI	MHA with **5% sheep blood** 35°C ± 2°C 5% CO_2; 20–24 hours	**CAMHB with lysed horse blood** (2.5% to 5% v/v) 35°C ± 2°C ambient air; 20–24 hours	**MHA with sheep blood** (5% v/v) 35°C ± 2°C ambient air; 20–24 hours (CO_2 if necessary, for growth with agar dilution)
EUCAST	Mueller-Hinton agar + **5% defibrinated horse blood** and 20 mg/L β-NAD (**MH-F)** Sealed panels, air, 35±1ºC, 18±2h (for glycopeptides 24h)	**Cation-adjusted Mueller-Hinton broth + 5% lysed horse blood** and 20 mg/L β-NAD (**MH-F broth**) Sealed panels, air, 35±1ºC, 18±2h	Guidance not available

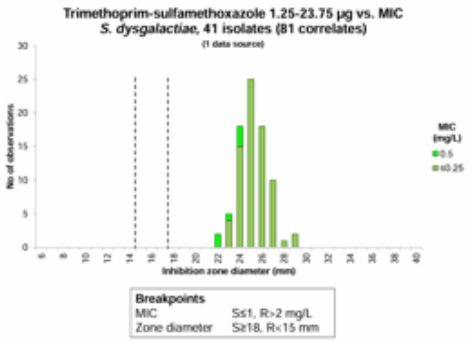
- EUCAST data

- EUCAST MH-F broth MIC vs MH-F disk diffusion

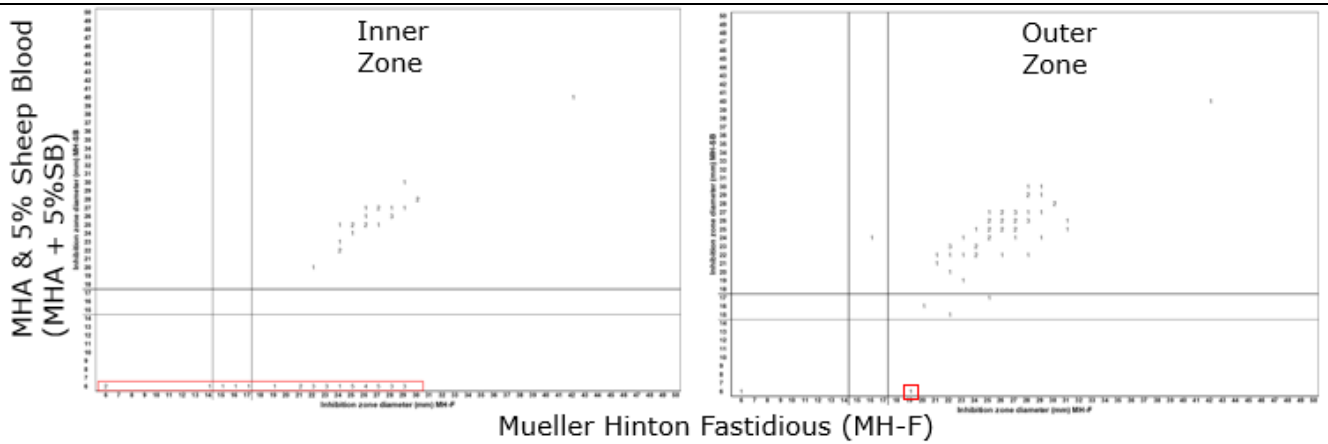


*9 MICs from E-test

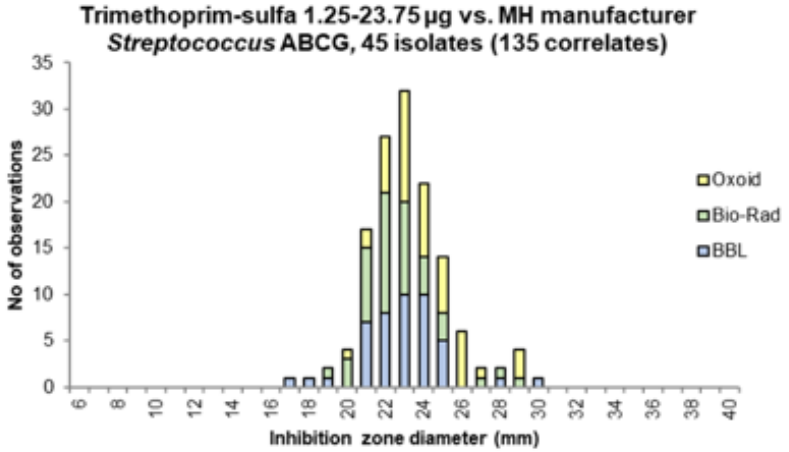




- EUCAST Data Comparing MHA + 5% SB vs MH-F
  - Zones easier to read on MH-F compared to MHA + 5% SB
  - Outer zones on MHA + 5% SB correlate better than inner zones



- Zone diameter distributions using MHA + 5% SB from three manufacturers (BBL, Bio-Rad and Oxoid) to produce zone diameter distributions
  - Hazy growth was observed within the zone for several isolates when tested on BBL and Bio-Rad MH-S, but zones are reproducible when the outer zone diameter was read.
  - Hazy growth within zones ignored and outer zone read



- Proposed study for beta-hemolytic streptococci TMP-SMX disk diffusion testing
  - 2-3 testing laboratories:
    - 2 agar manufacturers per media type
    - 2 disk brands
  - 3-method comparison,

        - MH-F x2 manufacturers
        - MHA-SB x2 manufacturers
        - reference BMD x1 (MHB-LHB, frozen)
    - Record inner and outer zones
    - 100 isolates, beta-hemolytic streptococci only (clinical, from any site):
        - 30 *S. pyogenes* (Group A)
        - 30 *S. agalactiae* (Group B)
        - 30 *S. dysgalactiae* (Groups C, G)
        - 10 less common beta *Streptococcus* species (eg, *S. canis*)
    - QC to include *Enterococcus faecalis* 29212/33186 for thymidine concentration and standard QC for TMP-SMX
    - Call for manufacturer donations and resistant beta-streptococci isolates; below are suggestions, open to others
        - Frozen BMD panels (Thermo Fisher)
        - MH-F (Hardy and Liofilchem)
        - MHA + 5% sheep blood (BBL and Remel)
        - TMP-SMX disks (BBL and Oxoid)
- Methods Working Group Discussion and Recommendation
    - CDC *Streptococcus* group might be a good place to acquire resistant isolates from
    - Approximate numbers of each item is requested by manufacturers
    - Hardy is willing to provide media and disks
    - Standard MHA without SB will not be tested – literature review indicates this media won't work and there is difficulty with reading
    - Motion to accept the TMP-SMX BHS proposed study as outlined. WG Vote: 8-0-0-4.

SC DISCUSSION (MAIN POINTS)

- Hardy and Liofilchem are going to make media for this study. The MH-F agar is not currently available in the United States, but having the breakpoints will create a market.
- The goal is to get the TMP-SMX disk diffusion up and running rather than test all drug combinations for the streptococci on different media.

A motion to approve the trimethoprim sulfamethoxazole disk diffusion study for beta-hemolytic streptococci as outlined was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

PSEUDOMONAS AERUGINOSA CARBAPENEMASE TESTING AD HOC WORKING GROUP REPORT

- Objectives
    - To review the current literature assessing and comparing clinical relevance, PK/PD parameters and testing performance between carbapenem resistant- carbapenemase-producing and non-producing *P. aeruginosa*
    - To explore several drug combinations to promote detection of carbapenemases in *P. aeruginosa*, contrasting global datasets.
    - To provide suggested criteria to apply carbapenemase testing in carbapenem-resistant *P. aeruginosa*, balancing sensitivity and specificity with the corresponding text to be discussed and considered for inclusion in the M100-37th edition 2027.
- 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.
- The CLSI M100 includes several 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*.
- Unexpected AST phenotypes are also appearing in some geographic regions, and this could lead to treatment failures. (eg, KPC producing *P. aeruginosa* displaying ceftolozane/tazobactam susceptibility).

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

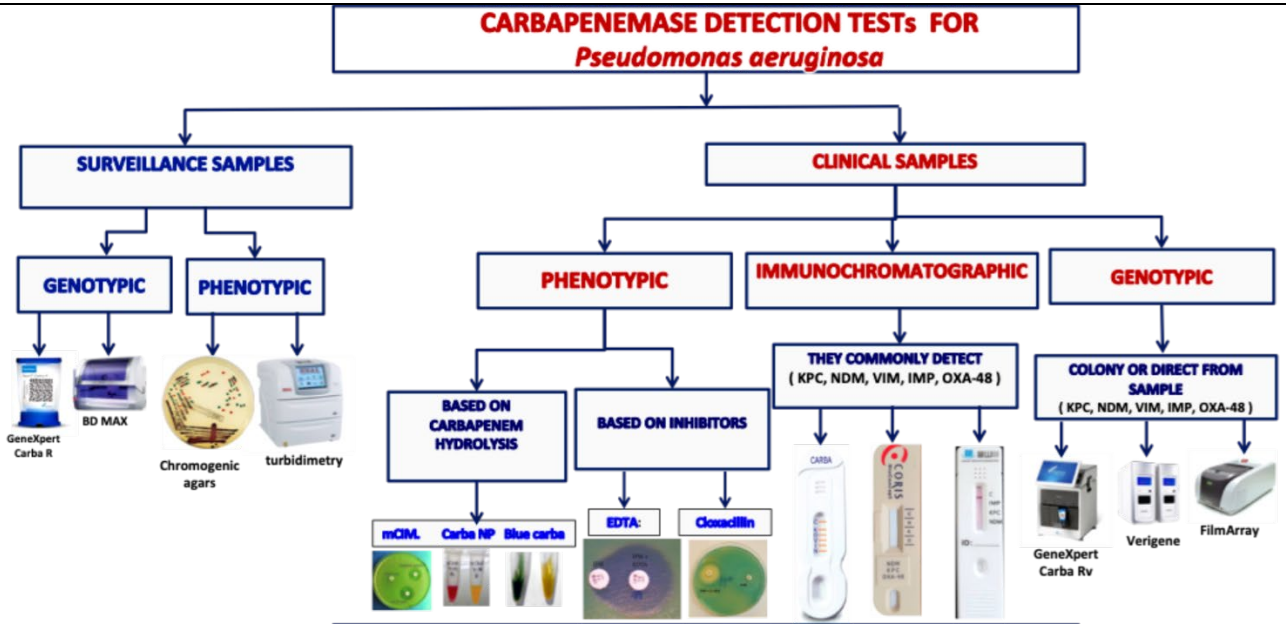
Introduction to Tables 3B and 3C. Tests for Carbapenemases in Enterobacterales and *Pseudomonas aeruginosa*

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 1 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 1 or more agents in cephalosporin subclass III (eg, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone). However, some isolates that produce carbapenemases, such as OXA-48, SME, or IMI, often test susceptible to these cephalosporins.

	Tests Used for Carbapenemase Detection			
	Carba NP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Organisms	Enterobacterales and *P. aeruginosa* that are not susceptible to 1 or more carbapenems	Enterobacterales and *P. aeruginosa* that are not susceptible to 1 or more carbapenems	Enterobacterales that are positive by mCIM	Enterobacterales and *P. aeruginosa* that are not susceptible to 1 or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by Carba NP or mCIM
Strengths	Rapid	No special reagents or media necessary	No special reagents or media necessary	Determines type of carbapenemase in addition to absence or presence of the enzyme

- Test availability and carbapenemase detection performances
  - Simner PJ, Pitout JDD, Dingle TC. Laboratory detection of carbapenemases among Gram-negative organisms. Clin Microbiol Rev. 2024 Dec 10;37(4):e0005422



- Literature review- Phenotypic methods to detect carbapenemases in *P. aeruginosa*

Method	Source	Isolates and enzymes included	Enzymes missed	Sensitivity	Specificity
mCIM (standard CLSI method)	Simner et al 2017 PMID 29118172	**30** (7 *bla*VIM, 3 *bla*IMP, 2 *bla*KPC, 2 *bla*NDM, and 1 *bla*SPM)	1 *bla*VIM 2 *bla*NDM	98% (86.7-100%)	94.7% (93.3-96.7%)
	Gallego M et al 2022 Hechos Microbiol. 2022;13(2).	**20** (4 *bla*VIM, 6 *bla*KPC, 2 *bla*KPC+*bla*VIM)	0	96% (86.3-99.5%)	96.3% (87.3-99.5%)
sCIM Colonies smeared onto imipenem disks	Jing et al 2018 PMID: 30425686	**158** (1 *bla*VIM-4, 13 *bla*VIM-1, 2 *bla*VIM-2, 4 *bla*VIM-4)	1 *bla*VIM-4	83.2%	100%
rCIM Colonies smeared onto imipenem disks 6-8h of incubation	Jing et al 2018 PMID: 30425686	**236** (2 blaKPC-2, 1 blaIMP-4, 20 blaVIM-1, 3 blaVIM-2, 12 blaVIM-4	1 *bla*VIM-4	97.4%	100%
CIMTrisII Standard mCIM vs Tris-HCl buffer and different carbapenem disks	Uechi K et al 2019 PMID: 30526741	**95** (1 *bla*DIM, 1 *bla*DIM+blaIMP, 1 *bla*DIM+*bla*VIM, 24 *bla*GES, 21 *bla*VIM, 1 *bla*NDM, 10*bla*VIM	Missed by standard mCIM *bla*GES-5 (21/23), *bla*GES-6 (1/1), *bla*VIM-2 (2/6)	100% CIMtris II **72.3%** mCIM	100% CIMtrisII 100% mCIM

Method	Source	Isolates and enzymes included	Enzymes missed	Sensitivity	Specificity
EDTA modified mCIM	Gill C et al 2020 PMID: 32690021	**96** (27 *bla*VIM, 22 *bla*IMP, 13 blaNDM, 14 *bla*SPM, *12* *bla*GES, 8 *bla*KPC.	**mCIM** *bla*GES-5 (6/12), **eCIM** *bla*IMP (22/22), *bla*SPM (14/14)	93% (86 to 97%)	85% (66 to 96%)
BD Phoenix™ CPO	Whitley V et al 2020 PMID: 32132195	**215.** (not disclosed carbapenemase types)	False positive 5.1% False negatives 1.4%	PPA 95.9%	NPA 92.3%
	Murata M et al 2023 PMID: 37162344	**33** (14 *bla*IMP)	False positive (5/19) They all were CRPA non-carbapenemase producers	PPA 100%	NPA 73. 7%
	Berneking L et al 2021 PMID: 33245470	**108** (42 *bla*VIM, 2 *bla*IMP, 1 blaOXA48, 2 *bla*GES,)	False positive 51/60 False negatives 2/48	98.3%	17.8%

Method	Source	Isolates and enzymes included	Enzymes missed	Sensitivity	Specificity
Carba NP	Dortet L et al 2012 PMID: 23070158	**21** (3 $_{bla}$KPC-2, 3 $_{bla}$VIM-2, 2 $_{bla}$VIM-2, 2 $_{bla}$VIM-4, 2 $_{bla}$IMP-1, 1 $_{bla}$IMP-2, 2 blaNDM-1, 1 $_{bla}$SPM, 3 $_{bla}$AIM-1, 4 $_{bla}$GIM-1	0	100%	100%
	Simner et al 2017 PMID 29118172	**30** (7 $_{bla}$VIM, 3 $_{bla}$IMP, 2 $_{bla}$KPC, 2 $_{bla}$NDM, and 1 $_{bla}$SPM)	1 $_{bla}$VIM-4	97.8 (88.2–99.9)	97.8 (88.2–99.9)
	Boulash Z et al 2020 PMID: 31899068 (review)	**Original CLSI carba nP** **214.** (96 $_{bla}$VIM, 38 $_{bla}$IMP, 14 $_{bla}$KPC, 10 $_{bla}$GES, 7 $_{bla}$NDM, 6 $_{bla}$SPM, 8 $_{bla}$GIM, 3 $_{bla}$AIM, 1 $_{bla}$FIM	3 $_{bla}$GES-5 2 $_{bla}$GES-18	95% (90–97%)	99% (97–100%)
		Rapidec® Carba NP **334.** (62 $_{bla}$VIM, 30 $_{bla}$IMP, 14 $_{bla}$KPC, 6 $_{bla}$GES, 8 $_{bla}$NDM, 5 $_{bla}$SPM, 2 $_{bla}$GIM, 1 $_{bla}$AIM, 1 $_{bla}$FIM	3 $_{bla}$GES-2, 2 $_{bla}$GES-5	96% (91–99%)	92% (88–95%)
NitroSpeed-Carba NP	Mustafá S et al 2021 PMID: 33321426	**202** (52 $_{bla}$VIM, 16 $_{bla}$IMP, 4 $_{bla}$KPC, 3 $_{bla}$GES, 8 $_{bla}$NDM, 4 $_{bla}$SPM, 2 $_{bla}$GIM, 2 $_{bla}$AIM, 1 $_{bla}$DIM, 1blaOXA-181	1 $_{bla}$GES-2	99%	100%
Blue Carba	Pires J et al 2013 PMID: 24108615	**14** (12 $_{bla}$VIM-2, 1 $_{bla}$SPM)	0	100%	100%

Method	Source	Isolates and enzymes included	Enzymes missed	Sensitivity	Specificity
Cloxacillin	Fournier D et al 2013 PMID: 23966511	**118** (3 $_{bla}$KPC, 32 $_{bla}$VIM, 16 $_{bla}$IMP, 1 $_{bla}$NDM-1, 1 $_{bla}$SPM, 5 $_{bla}$GES, 1 $_{bla}$GES$_{bla}$VIM, 1 $_{bla}$IMP$_{bla}$VIM	0	100%	100%
Boronic Acid Dipicolinic Acid Cloxacillin	Pasteran F et al 2011 PMID: 21689207	**149** (36 $_{bla}$KPC, 21 $_{bla}$VIM, 9 $_{bla}$IMP, 8 $_{bla}$SPM,	1 $_{bla}$VIM-11 17 non-MBL were positive with dipicolinic acid.	KPC 97% MBLs : 97% Cloxacillin: 97%	KPC 97% MBLs: **81%** Cloxacillin: 97%
Dipicolinic acid Cloxacillin EDTA	Heinrichs A 2015 PMID: 25896858	**188** (1 $_{bla}$KPC, 51 $_{bla}$VIM, 10 $_{bla}$IMP, 1 $_{bla}$SPM, 1 $_{bla}$GIM, 2 $_{bla}$OXA198,	Cloxacillin $_{bla}$VIM-2 (2/51)	EDTA 95% DPA 100%	EDTA 100% DPA 33%

- Literature review- Immunochromatographic assays to detect carbapenemases in *P. aeruginosa*

Method	Source	Isolates and enzymes included	Enzymes missed	Sensitivity	Specificity
NG CARBA 5	Jenkins S et al 2020 PMID: 32376668	**69** (2 *bla*KPC, 9 *bla*VIM, 5 *bla*IMP,)	0	PPA 100%	NPA 100%
	Pasteran F et al , 2024 34th ECCMID Abstract number: 01270	**12** (3 *bla*KPC, 4 *bla*VIM, 5 *bla*IMP,)	*bla*IMP-16 (1/5)	95%	100%
	Potron A, et al 2019 PMID: 31285233	96 (3 *bla*KPC, 24 *bla*VIM, 17 *bla*IMP, 4 *bla*GES, 2 *bla*NDM, 1 *bla*OXA198, 2 *bla*GIM, 2 *bla*AIM, 1 *bla*SPM, 1 *bla*DIM)	*bla*IMP (9/24) (IMP-15, IMP-13, IMP-18, IMP-63) *bla*GES (0/4) 2 *bla*GIM, 2 *bla*AIM, 1 *bla*SPM, 1 *bla*DIM)	blaKPC and blaVIM 100% *bla*IMP 63%	blaKPC and blaVIM 100% *bla*IMP 100%

- Literature review- molecular assays to detect carbapenemases in *P. aeruginosa*

Method	Source	Isolates and enzymes included	Enzymes missed	Sensitivity	Specificity
GeneXpert Carba R	Traczewski M et al 2018 PMID: 29848561	**80** (1 *bla*KPC, 31 *bla*VIM, 16 *bla*IMP,)	*1 bla*IMP (false +)	100%	98%

- Summary of carbapenemase testing for *P. aeruginosa*
  - blaGES enzymes can go undetected with mCIM and carba NP.
  - Performance of EDTA based assays is suboptimal for IMPs. (although good for VIMs).
  - Cloxacillin is an interesting screening test. Negative results in imipenem resistant *P. aeruginosa* suggest the production of carbapenemases. Need more data and unfortunately, cloxacillin is not available in certain locations.
  - Immunochromatography and PCR (eg. GeneXpert carba R) are good options to detect blaKPC, blaVIM, blaNDM, blaIMP, blaOXA-48. However, they are missing some frequent enzymes in specific locations like the (blaGES). The challenge is that not all blaGES are carbapenemases.
  - It is important to mention that blaGIM, blaAIM, blaFIM, blaDIM and blaSPM, are not included in commercial PCR methods or immunochromatography assays. But they are unusual and located in specific geographic regions.
- Literature based criteria for carbapenemase testing for *P. aeruginosa*

Citation	Population	Criteria	Sensitivity	Specificity
Gill CM, *et al*. *Open Forum Infect Dis* 2021;9:ofab617.	807 CR-PA, 33% CP + Global collection	IPM or MEM-R plus FEP and CAZ-NS or C/T-NS	92% (88-95%)	54% (50-59%)
Vallabhaneni S, *et al*. J Clin Microbiol. 2021 May 19;59(6):e02874-20.	6,192 PA isolates 3% CP + United States	IPM or MEM-R and C/T-NS	100%	86%
Ocal M, *et al*. *Diagn Microbiol Infect Dis* 2024;110:116495.	256 CR-PA, 13% CP+ Turkey	IPM or MEM-R plus FEP/CAZ-NS or C/T-NS	88% (73-97%)	36% (29-42%)
Mancini S, et al. *Microbiol Spectr* 2025;13:e0319624.	136 CR-PA Challenge set 71 CP+ isolates	IPM+MEM+C/T-NS Disk diffusion IPM+MEM+TOB-NS Disk diffusion	93 (84-98%) 93 (84-98%)	66 (53-77%) 74% (62-84%)
Ellem JA, *et al*. *JAC Antimicrob Resist* 2026;8:dlaf249.	100 PA isolate Challenge Set 57 CP+	MEM + CAZ + TOB MIC ≥ 8mg/L IPM or MEM R CAZ and/or FEP NS and C/T NS	100% (84-100%) 81% (60-93%)	97% (87-99%) 81% (69-90%)

- Testing criteria re-assessed

Source	Population	Criteria	Number of isolates meeting criteria	Sensitivity	Specificity
JMI	2,358 imi +mero NS PsA, ~10% CP+ Europe > Asia Pacific>USA >>> Lat Am	IPM + MEM +C/T- NS	431	99%	81%
		MEM + C/T-NS	476	99%	85%
		MEM + CAZ-NS	1300	100%	36%
GEMARA Database	~3200 Psa, 2% CP Spain	IPM + MEM +C/T- NS	103	91% (82-97%)	99% (98-99%)
		MEM + C/T-NS	113	91% (82-97%)	98% (98-99%)
		MEM + CAZ-NS	360	88% (78-94%)	90% (89-91%)
CDC EIP	~1200 Psa, ~400 with documented CP USA	MEM + C/T-NS	465/~1200	96%	85%
		MEM + CAZ-NS	2775/~4700	97%	54%
CDC GAIHN-AR	902 PsA, 315 CR-PA India >50% CP	IPM +MEM + C/T- NS Low number tested so far	31/36	100%	67%
		IPM or MEM + C/T- NS Low number tested so far		100%	67%

- 12,051 isolates from IHMA molecularly categorized
  - 97% IPM-NS; 81% MEM-NS

Region	N of isolates (%CP+)	Criteria	Sensitivity	Specificity
All	12,501 (19%)	IPM+MEM+C/T-NS MEM + C/T-NS MEM + CAZ-NS	96% (95-97%) 97% (96-97%) 96% (95-97%)	88% (87-89%) 87% (87-88%) 63% (62-64%)
Europe	4,000 (18%)	MEM + C/T-NS MEM + CAZ-NS	97% (94-98%) 96% (94-97%)	84% (83-86%) 60% (58-61%)
Middle East	826 (11%)	MEM + C/T-NS MEM + CAZ-NS	98% (93-99.7%) 97% (91-99%)	87% (85-90%) 55% (51-59%)
Africa	555 (40%)	MEM + C/T-NS MEM + CAZ-NS	99% (96-99.7%) 95% (91-97%)	90% (86-93%) 73% (68-77%)
South Pacific	534 (7%)	MEM + C/T-NS MEM + CAZ-NS	100% (91-100%) 100% (91-100%)	94% (91-96%) 83% (79-86%)
North America	1,637 (1%)	MEM + C/T-NS MEM + CAZ-NS	90% (67-99%) 95% (74-99.9%)	89% (87-90%) 62% (60-65%)
Asia	2,056 (23%)	MEM + C/T-NS MEM + CAZ-NS	97% (95-98%) 98% (96-99%)	88% (87-90%) 63% (61-66%)
Latin America	2443 (28%)	MEM + C/T-NS MEM + CAZ-NS	95% (92-97%) 94% (92-96%)	89% (88-91%) 64% (61-66%)

- Criteria false negatives

Data Set	Criteria	False negative, N	False Negatives, enzymes
IHMA, Global	MEM + C/T-NS	75	**VIM, n = 27; GES, n = 11**;IMP, n = 8; KPC, n =7;BKC, n = 5, KPC + VIM, n = 5; NDM, n=4; OXA-181-like, n = 3; No known CP, n =4
	MEM + CAZ- NS	95	**VIM, n = 49; GES, n = 19**;IMP, n = 7; KPC, n =2;BKC, n = 5, KPC + VIM, n = 4; NDM, n=3; OXA-181-like, n = 1;IMP + VIM, n =1; No known CP, n =4
GEMARA, Spain	MEM + C/T-NS	6	GES, n = 5; VIM, n = 1
	MEM + CAZ- NS	13	GES, n = 7; VIM, n = 6
CDC EPI	MEM + C/T-NS	16	KPC, n =8; VIM, n = 3; NDM, n = 2; IMP, n = 2; OXA-48-like, n = 1*
	MEM + CAZ- NS	38	KPC, n =8; **VIM, n = 20**; NDM, n = 4; IMP, n = 2; OXA-48-like, n = 4*
JMI Sentry	MEM + C/T-NS	2	IMP-13, n = 1; OXA-232, n = 1

- Carbapenemase testing criteria for *P. aeruginosa*

- Similar to the literature, a ceftolozane/tazobactam not susceptible criteria performs the best across a variety of settings including in different CP-prevalence settings
  - Simplified criteria of MEM+C/T-NS may be practical for implementation
  - Can consider MEM + CAZ-NS though more false positives leading to more testing
- Where available, tobramycin-based criteria may be appropriate in some settings though local validation is warranted
  - Amikacin cannot be used interchangeably

- AHWG Discussion and Recommendation
  - Proposal for criteria to guide carbapenemase testing
  - Suggest option 1, as it provides the best balance between sensitivity and specificity. These criteria will have the appropriate text in table 2. Most commercial AST panels for *P. aeruginosa* used worldwide contain both meropenem and ceftolozane/tazobactam. Some commercial AST panels do not contain imipenem.

Proposal	Criteria	Sensitivity Range Across Datasets	Specificity Range Across Datasets	Comments
1	Meropenem + ceftolozane/tazobactam-NS	91-99%	85-98%	Ceftolozane/tazobactam not tier 1 drug for PsA
2	Meropenem + Imipenem + Ceftolozane/tazobactam-NS	91-99%	81-99%	Some labs do not routinely test imipenem
3	Meropenem + ceftazidime-NS	88-100%	36-90%	Poor specificity, high number of isolates tested

- Methods Working Group Discussion and Recommendation
  - Preferred offering both 1 and 3 as criteria for testing, to accommodate laboratories that do not test ceftolozane/tazobactam.
  - WG preferred option 1 text below. Mirrors Table 2A-1 Enterobacterales intro to carbapenems.
  - Those in favor of option 2 text below because prefer shorter, more concise language.
- Option 1 Table 2B-1

Carbapenem resistance in *P. aeruginosa* can arise through the interplay of multiple mechanisms. These include, but are not limited to, porin mutations (eg, OprD loss or alteration), increased production of Pseudomonas-derived cephalosporinase (PDC) enzymes (also known as pseudomonal AmpC enzymes), upregulation of efflux pumps (eg, MexAB-OprM), and acquisition of carbapenemase enzymes. The prevalence and distribution of carbapenemases among P. aeruginosa vary substantially by geographic region, healthcare setting, and patient population.

Carbapenemase-producing *P. aeruginosa* is more commonly associated with metallo-ß-lactamases (MBLs) such as VIM, IMP, NDM, and SPM although other serine carbapenemases such as KPC and GES variants with carbapenemase activity, may also be encountered.

	Because the presence of a carbapenemase has important implications for antimicrobial selection, infection prevention measures, and epidemiologic surveillance, targeted carbapenemase testing may be considered for selected carbapenem-resistant *P. aeruginosa* isolates. These results do not replace antimicrobial susceptibility testing. Depending on local epidemiology and the antimicrobial agents available for antimicrobial susceptibility testing, isolates that are meropenem not susceptible (ie, intermediate or resistant) and not susceptible to ceftolozane-tazobactam (Sensitivity 91-99%; Specificity 85-98%) or, alternatively, meropenem not susceptible and not susceptible to ceftazidime (Sensitivity 88-100%; Specificity 36-90%) may be selected for carbapenemase testing using phenotypic and/or molecular assays. These assays should identify and ideally differentiate the presence of specific carbapenemases (eg, VIM, IMP, NDM, etc.). The reported sensitivity and specificity estimates were derived from a dataset of 19,270 molecularly characterized *P. aeruginosa* isolates from five International surveillance programs and may vary depending on local epidemiology and the prevalence of specific carbapenemase mechanisms. The high sensitivity of both screening approaches supports their use for identifying potential carbapenemase-producing isolates, whereas differences in specificity will influence the number of isolates requiring confirmatory carbapenemase testing. Decisions related to carbapenemase testing and reporting are best made by each laboratory in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. • Option 2 Table 2B-1 Because the presence of a carbapenemase has important implications for antimicrobial selection, infection prevention measures, and epidemiologic surveillance, targeted carbapenemase testing may be considered for selected carbapenem-resistant *P. aeruginosa* isolates. These results do not replace antimicrobial susceptibility testing. Depending on local epidemiology and the antimicrobial agents available for antimicrobial susceptibility testing, isolates that are meropenem not susceptible (ie, intermediate or resistant) and not susceptible to ceftolozane-tazobactam (Sensitivity 91-99%; Specificity 85-98%) or, alternatively, meropenem not susceptible and not susceptible to ceftazidime (Sensitivity 88-100%; Specificity 36-90%) may be selected for carbapenemase testing using phenotypic and/or molecular assays. These assays should identify and ideally differentiate the presence of specific carbapenemases (eg, VIM, IMP, NDM, etc.). The reported sensitivity and specificity estimates were derived from a dataset of 19,270 molecularly characterized *P. aeruginosa* isolates from five International surveillance programs and may vary depending on local epidemiology and the prevalence of specific carbapenemase mechanisms. Decisions related to carbapenemase testing and reporting are best made by each laboratory in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. • Proposed text to Tables 3B and 3C

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 and may inform treatment decisions in carbapenem-resistant *P. aeruginosa* infections.

Carbapenemase-producing isolates ~~of Enterobacterales~~ usually test intermediate or resistant to 1 or more carbapenems using the current breakpoints as listed in Table 2A-1 for Enterobacterales or Table 2B-1 for *P. aeruginosa*. ~~(**NOTE:** Testing not susceptible to ertapenem is often the most sensitive indicator of carbapenemase production for Enterobacterales. 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 isolates usually test resistant to 1 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.~~

See Table 2A-1 Comment 26 for Enterobacterales or Table 2B-1 Comment X for *P. aeruginosa* for additional guidance on when to consider performing carbapenemase testing among isolates.

	Tests Used for Carbapenemase Detection			
	Carba NP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Organisms	Enterobacterales and *P. aeruginosa* ~~that are not susceptible to 1 or more carbapenems.~~	Enterobacterales and *P. aeruginosa* ~~that are not susceptible to 1 or more carbapenems~~	Enterobacterales that are positive by mCIM	Enterobacterales and *P. aeruginosa* ~~that are not susceptible to 1 or more carbapenems~~ isolates to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by Carba NP or mCIM
Strengths	Rapid	No special reagents or media necessary	No special reagents or media necessary	Determines type of carbapenemase in addition to absence or presence of the enzyme

	Tests Used for Carbapenemase Detection			
	Carba NP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Limitations	Special reagents are needed, some of which necessitate in-house preparation (and have a short shelf life). Invalid results occur with some isolates. Certain carbapenemase types (eg, OXA-type, chromosomally encoded) are not consistently detected. Does not determine the type of carbapenemase In geographic regions where GES carbapenemases are frequent, false negative Carba NP test may occur and alternative methods should be considered including PCR or whole genome sequencing where available.	Requires overnight incubation Does not determine the type of carbapenemase In geographic regions where GES carbapenemases are frequent, false negative mCIM test may occur and alternative methods should be considered including PCR or whole genome sequencing where available.	Requires overnight incubation False-negative results are likely to occur for isolates coproducing a serine carbapenemase and a metallo-β-lactamase. Does not determine the type of serine carbapenemase or metallo-β-lactamase	Special reagents and equipment are needed. Specific to targeted genes; false-negative result if specific carbapenemase gene present is not targeted.

Abbreviations: Carba NP, carbapenemase Nordmann-Poirel; eCIM, EDTA-modified carbapenem inactivation method; EDTA, ethylenediaminetetraacetic acid; mCIM, modified carbapenem inactivation method.

- Proposed text to Table 2A-1

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
CARBAPENEMS										
(26) Following evaluation of PK/PD properties, limited clinical data, and MIC distributions that include recently described carbapenemase-producing strains, revised breakpoints for carbapenems were first published in June 2010 (CLSI M100-S20-U) and are listed below. Because of limited treatment options for infections caused by organisms with carbapenem MICs or zone diameters in the intermediate range, clinicians may wish to design carbapenem dosage regimens that use maximum recommended doses and possibly prolonged IV infusion regimens, as has been reported in the literature.[5-8] Consultation with an infectious diseases specialist is recommended for isolates for which the carbapenem MICs or zone diameter results from disk diffusion testing are in the intermediate or resistant ranges. Carbapenemase-producing Enterobacterales usually test resistant to 1 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 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). Decisions related to carbapenemase testing and reporting are best made by each laboratory in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. These results do not replace antimicrobial susceptibility testing, but are important for treatment decisions, and to inform infection control and prevention interventions and/or epidemiologic investigations. Testing not susceptible to ertapenem is often the most sensitive indicator of carbapenemase production. Depending on local epidemiology and available resources, carbapenemase testing for *E. cloacae* complex and *K. 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. See Appendix G, Table G3 regarding suggestions for reporting when mechanism of resistance-based testing (molecular and phenotypic methods) is discordant with phenotypic AST. The following information is provided as background on carbapenemases in Enterobacterales that are largely responsible for MICs and zone diameters in the intermediate and resistant ranges, and thus the rationale for setting revised carbapenem breakpoints: • The clinical effectiveness of carbapenem treatment of infections produced by isolates for which the carbapenem MIC or disk diffusion test results are within the intermediate range is uncertain due to lack of controlled clinical studies. Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (eg, MICs in the intermediate or resistant range) than meropenem or doripenem MICs. These isolates may have elevated imipenem MICs by mechanisms other than production of carbapenemases.									

SC DISCUSSION (MAIN POINTS)

- This will help laboratories not send unnecessary isolates.
- Is it worth adding ceftazidime-avibactam to the criteria? This drug is helpful if there are metallo-ß-lactamases. But if there are no metallo-ß-lactamases then the other isolates (eg., KPC and GES) will be missed
- What about resistance to meropenem or imipenem plus ceftolozane-tazobactam? Can use either meropenem or imipenem, it does not matter. Imipenem performs well for VIMs, but the AHWG preferred meropenem.

- Consider meropenem plus ceftolozane-tazobactam and add a note that would use imipenem instead of meropenem.
- If laboratories do not have ceftolozane-tazobactam then ceftazidime can help.
- Some ESBLs can be meropenem susceptible and ceftolozane-tazobactam not susceptible.
- Clarify what the sensitivity and specificity will be. Clarify this is for carbapenemase detection.
- The comment is too long. Should remove sensitivity and specificity because those numbers will change as the epidemiology of the organisms changes.
- Suggestion to add an explanation as to why carbapenemase testing is important.
- Enterobacterales and *P. aeruginosa* should both have the same length of information. Both are currently too long.
- This will save the laboratory and public health extra time by reducing unnecessary work.

A motion to approve option 1 text for Table 2B-1 regarding *Pseudomonas aeruginosa* isolates that should be considered for carbapenemase testing with the meropenem + ceftolozane-tazobactam-NS testing as the preferred testing and either imipenem + ceftolozane-tazobactam-NS or meropenem + ceftazidime-NS as the less preferred testing to accommodate laboratories that do not test ceftolozane-tazobactam pending wordsmithing was made and seconded. Vote: 15 for, 0 against, 1 abstain, 0 absent (Pass)

INTRINSIC RESISTANCE DEFINITION AD HOC WORKING GROUP REPORT

- Goals
  - Revisit the CLSI definition of intrinsic resistance for possible adjustments
    - Attempt to standardize the definition amongst SC on Antifungal Tests, AST SC, and VAST
  - Explore a concept of “reduced susceptibility” when IR not achieved but antimicrobial should not be used
- AHWG Discussion and Recommendations
  - Reporting Guidance for Expected Resistance (ER) (Including IR and Expected Clinical Failure [ECF])
    - Discussed reporting as R, IR, R*, ECF, or other
      - Decided to provide options to laboratories as the determinations will be a) based on LIS and testing capabilities, and b) decided with input by clinicians and other end users
      - Discovered that R* is a possible reporting option in some systems, but decided against recommending this as a reporting option
      - AHWG recommended reporting as R or not at all (for ER)
    - Discussed reporting with or without MIC/zone diameters
      - AHWG recommended reporting without MIC or zone diameters
    - Discussed need to distinguish between IR and ECF for clinical providers
      - AHWG decided to recommend against differentiating between IR and ECF for clinical providers
      - Laboratories may consider use of report comments for clarification
      - Laboratory educational events will be provided by CLSI (eg, webinar)
  - Placement of IR/ECF Guidance in Documents
    - Discussed whether to list IR and ECF separately or in the same table (with color coding to accentuate differences)
      - AHWG recommended listing IR and ECF in same table
      - Voting for this item will take place through the respective subcommittees (ie, AST SC M100 IR AHWG)

- Various SC groups have mocked up tables for their own documents and shared with IR Definition AHWG
- Edited Wording in Introductory Text for ER Table(s)
  - "Systemic infection"
    - Different connotations of this phrase
      - Could mean bloodstream infection (as opposed to respiratory or wound infections), OR
      - It could mean systemic administration (as opposed to topical or inhaled antimicrobial routes of administration)
    - AHWG included this phrase since the definition states that we exclude topical or inhaled drug administration
  - "Should test resistant or with a high modal MIC" (first sentence of third paragraph)
    - Try to avoid use of phrase "test resistant" when there are no breakpoints
    - We retained the term but stated "or with a high modal MIC" for clarification
- Timeline and Standardization of ER Definition Across Documents
  - Timeline to include ER text in documents
    - AST SC will plan for M100Ed38 (2028)
    - AFST will plan for 2028
    - VAST not determined yet
  - ER in other documents
    - M45 publication date (early to mid 2027)
      - Includes an Intrinsic Resistance Appendix which has older definition of IR and does not include ECF
      - No strict guidelines on reporting of IR, but text indicates in multiple places to "notify the clinical team of intrinsic resistance to XX"
      - The concept of reduced susceptibility/ECF is introduced but not called out as ECF
      - Future update (no anticipated year at this point) should include the updated ER definition
    - M39 wording states to report IR as R in cumulative antibiogram
- Future steps
  - Finalize wording for introductory text in tables of ER
  - Each subcommittee group (AFST, AST SC M100 IR AHWG, VAST) comprising this AHWG will finalize their own antimicrobial agent/organism combinations which did not meet the original definition of IR and will update their own document tables accordingly
  - Alignment anticipated between subcommittee groups:
    - Reporting
  - Alignment remains a goal:
    - Recommendation from IR Definition AHWG on reporting of IR/ECF in cumulative antibiograms (TBD)
    - IR designations between certain organism/antimicrobial combinations which straddle the AST SC M100 IR AHWG and the VAST IR AHWG (TBD)
    - Provide input to M45 group on inclusion of ER in that document

- Proposed Appendix X. Expected Resistance Introductory Text for Tables in CLSI Documents (edits highlighted in yellow)

The concept of "expected resistance" includes both intrinsic resistance (IR), which is a property inherent to an organism, and Expected Clinical Failure (ECF) ie, treatment failure or insufficient antimicrobial exposure in a host species. Antimicrobial agent-organism combinations qualify for expected

resistance, if they display either IR or ECF. IR is defined as inherent or innate (not acquired) antimicrobial resistance, evidenced by high MICs or reduced zone diameter values for specific antimicrobial agent-organism combinations for all or nearly all isolates (≥90%) of a microbial species or organism group (eg, species complex). The MIC distribution for an antimicrobial agent-organism combination exhibiting IR generally displays a high modal MIC. ECF includes antimicrobial agent-organism combinations for which available PK/PD data show insufficient antimicrobial exposure at clinically achievable concentrations for systemic infections, or when available microbiologic or clinical data demonstrate lack of efficacy against a species *in vivo*. Antimicrobial susceptibility testing (AST) is unnecessary for organisms considered to have expected resistance to an antimicrobial agent. However, if testing is performed, the AST result should be suppressed or reported as resistant. MIC and zone diameter values should not be reported, as some isolates may exhibit low MICs or large zone diameters despite lack of *in vivo* utility of the antimicrobial agent. See Appendix X.

These tables can be helpful in at least 4 ways: 1) they provide a way to evaluate the accuracy of AST methods; 2) they aid in the recognition of common phenotypes; 3) they can assist with verification of cumulative antimicrobial susceptibility test data, and 4) ECF may warn about some antimicrobial agent-organism combinations associated with poor clinical efficacy for systemic infections (ie, this does not apply for topical agents or inhaled antimicrobial agents).

In the IR tables, an "IR" occurring with an antimicrobial agent–organism combination means that strains should test resistant or with a high modal MIC. Ten percent (or fewer) isolates may appear susceptible due to method variation, mutation, or low levels of resistance expression.

Laboratories may include intrinsic resistance designations in their institutional antibiograms for antimicrobial stewardship purposes (see CLSI M39).

Each laboratory should decide which agents to test and report in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. Consideration may be given to adding comments regarding IR or ECF of agents not tested.

- Methods Working Group Discussion and Recommendation
    - Should body site reporting be included in ECF (eg, daptomycin from respiratory sites)
    - The acronym ECF is similar to the acronym ECOFF – could this cause confusion in the tables?
    - Add wording about ECF to the antibiogram sentence.
    - Motion to approve the addition of "systemic infection" in the definition and the proposed introduction text with the considerations to add sentence regarding ECF and institution antibiograms with the M39 statement and to change to "this may not apply to topical agents or inhaled antimicrobial agents." WG Vote: 8-0-0-4.

SC DISCUSSION (MAIN POINTS)

- Suggestion to change "systemic infection" to "systemic administration".
- Suggestion to move "(ie, this does not apply for topical agents or inhaled antimicrobial agents)" to the first paragraph of the definition.
- Suggestion to add sentence regarding ECF and institution antibiograms with the M39 statement.
- Suggestion to change to "this may not apply to topical agents or inhaled antimicrobial agents."
- The expected resistance definition and introduction text will not be added to the next edition of M100. The Antifungal Subcommittee and Veterinary AST Subcommittee will need to vote on these changes.
- Next steps are for the Intrinsic Resistance AHWG to review the tables and make the changes to the new definition.

A motion to approve the expected resistance definition and introduction text with the addition of "systemic administration" instead of "systemic infection", consideration to move "(ie, this does not apply for topical agents or inhaled antimicrobial agents)" to the definition, add a sentence regarding ECF and institution antibiograms with the M39 statement, and to change to "this may not apply for topical agents or inhaled antimicrobial agents." was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

Modified Expected Resistance text based on vote:

The concept of "expected resistance" includes both intrinsic resistance (IR), which is a property inherent to an organism, and Expected Clinical Failure (ECF) i.e., treatment failure or insufficient antimicrobial exposure in a host species. Antimicrobial agent-organism combinations qualify for expected resistance, if they display either IR or ECF. IR is defined as inherent or innate (not acquired) antimicrobial resistance, evidenced by high MICs or reduced zone diameter values for specific antimicrobial agent-organism combinations for all or nearly all isolates (≥90%) of a microbial species or organism group (eg, species complex). The MIC distribution for an antimicrobial agent-organism combination exhibiting IR generally displays a high modal MIC. ECF includes antimicrobial agent-organism combinations for which available PK/PD data show insufficient antimicrobial exposure at clinically achievable concentrations for systemic administration (ie, this may not apply for topical agents or inhaled antimicrobial agents), or when available microbiologic or clinical data demonstrate lack of efficacy against a species *in vivo*. Antimicrobial susceptibility testing (AST) is unnecessary for organisms considered to have expected resistance to an antimicrobial agent. However, if testing is performed, the AST result should be suppressed or reported as resistant. MIC and zone diameter values should not be reported, as some isolates may exhibit low MICs or large zone diameters despite lack of *in vivo* utility of the antimicrobial agent. See Appendix X.

These tables can be helpful in at least 4 ways: 1) they provide a way to evaluate the accuracy of AST methods; 2) they aid in the recognition of common phenotypes; 3) they can assist with verification of cumulative antimicrobial susceptibility test data, and 4) ECF may warn about some antimicrobial agent-organism combinations associated with poor clinical efficacy for systemic administration.

In the IR tables, an "IR" occurring with an antimicrobial agent–organism combination means that strains should test resistant or with a high modal MIC. Ten percent (or fewer) isolates may appear susceptible due to method variation, mutation, or low levels of resistance expression.

Laboratories may include intrinsic resistance or expected clinical failure designations in their institutional antibiograms for antimicrobial stewardship purposes (see CLSI M39).

Each laboratory should decide which agents to test and report in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. Consideration may be given to adding comments regarding IR or ECF of agents not tested.

INTRINSIC RESISTANCE AD HOC WORKING GROUP REPORT

- "All in one" tables vs. separate tables for IR/ECF
    - All-in-one vs. separate tables: One of the reasons for the proposed "all-in-one" ER table is so the different Appendixes look the same.
    - A separate table for Enterobacterales would only have one line – *Salmonella*/*Shigella*. A separate table for Non-Enterobacterales would have just a few breakpoint change ECFs.
    - The end result should be the same: the user either suppresses or reports as Resistant all of them.
- Abbreviations in tables: IR/ECF vs R/R*, R/ECF

- R/R*: exist today. R for Intrinsic Resistance; R* for *Salmonella/Shigella* and *Enterococcus* warnings which is expected clinical failure. Could just add R* for non-Enterobacterales breakpoint changes.
- R/ECF: Keep R as is; change R* to ECF
- Consensus (not unanimous) was that new definition of Expected Resistance is IR or ECF; should not those be the abbreviations in the tables?
- End result is same: user should suppress or report as resistant

- M100 Appendix B4
  - Current

B4. *Enterococcus* spp.

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

Abbreviation: R, resistant.

Footnote

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

M100-Ed36

  - Proposed

Expected Resistance									
Organism/Drug	**Cephalosporins**	**Vancomycin**	**Teicoplanin**	**Aminoglycosides**	**Clindamycin**	**Quinupristin-dalfopristin**	**Trimethoprim**	**Trimethoprim-Sulfamethoxazole**	**Fusidic Acid**
Enterococcus faecalis	ECF			ECF	ECF	IR	IR	ECF	IR
Enterococcus faecium	ECF			ECF	ECF		IR	ECF	IR
E. gallinarum, E. casseliflavus	ECF	IR		ECF	ECF	IR	IR	ECF	IR

Expected Clinical Failure: For Enterococcus spp., cephalospoorins, aminoglycosides (except for high-level testing), clindamycin, and trimethoprim-sulfamethoxazole may appear active in vitro but are not effective clinically and should be suppressed or reported as R

need to make same warning word change in Table 2

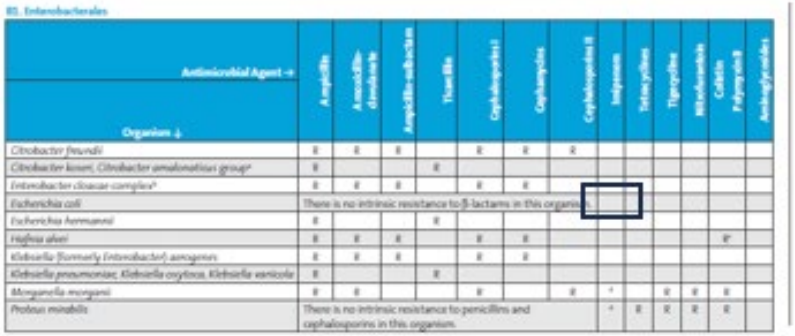
- Another option – showing 2 tables, with Different drugs

Expected Resistance			
Intrinsic Resistance			
Organism/Drug	**Vancomycin**	**Trimethoprim**	**Fusidic Acid**
Enterococcus faecalis		IR	IR
Enterococcus faecium		IR	IR
E. gallinarum, E. casseliflavus	IR	IR	IR

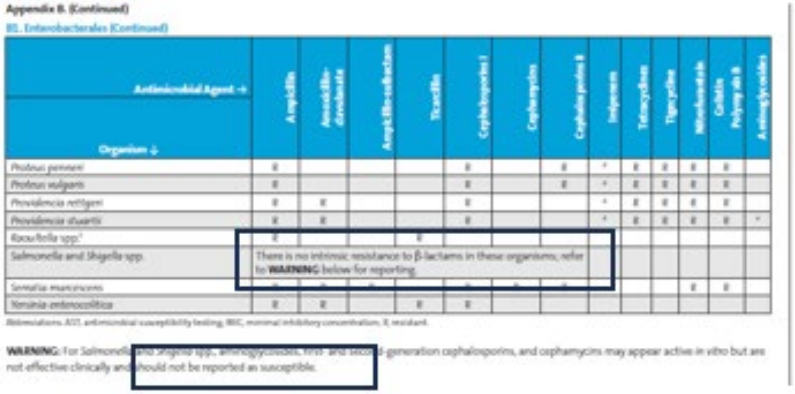
Expected Clinical Failure				
Organism/Drug	**Cephalosporins**	**Aminoglycosides**	**Clindamycin**	**Trimethoprim- Sulfamethoxazole**
Enterococcus faecalis	ECF	ECF	ECF	ECF
Enterococcus faecium	ECF	ECF	ECF	ECF
E. gallinarum, E. casseliflavus	ECF	ECF	ECF	ECF

Expected Clinical Failure: For Enterococcus spp., cephalospoorins, aminoglycosides (except for high-level testing), clindamycin, and trimethoprim-sulfamethoxazole may appear active in vitro but are not effective clinically and should not be reported as susceptible

- M100 Appendix B1
  - Current



Imipenem should be included with b-lactams



Wording changes

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- Proposed

Organism/Drug	Ampicillin	Amoxicillin-clavulanate	Ampicillin-Sulbactam	Ticarcillin	Cephalosporin I	Cephamycins	Cephalosporin II	Imipenem	Tetracycline	Tigecycline	Nitrofurantoin	Polymyxin/ Colistin	Aminoglycosides
Citrobacter freundii	IR	IR	IR		IR	IR	IR						
Citrobacter koseri, C. amalonaticus group[a] (C. amal gp includes C. amalonaticus, C. farmeri, C. sedlakii)	IR			IR									
Enterobacter cloacae complex[b] (E. cloacae gp. includes E. asburiale, E. cloacae, C. hormaechei)	IR	IR	IR		IR	IR							
Escherichia coli	There is no intrinsic resistance to β-lactams in this organism.												
Escherichia hermannii	IR			IR									
Hafnia alvei	IR	IR	IR		IR	IR						IR[c]	
Klebsiella (formerly Enterobacter) aerogenes	IR	IR	IR		IR	IR							
Klebsiella pneumoniae, K. oxytoca, K. variicola	IR			IR									
Morganella morganii	IR	IR			IR		IR	[d]		IR	IR	IR	
Proteus mirabilis	There is no intrinsic resistance to penicillins and cephalosporins in this organism.							[d]	IR	IR	IR	IR	
Proteus penneri	IR				IR		IR	[d]	IR	IR	IR	IR	
Proteus vulgaris	IR				IR		IR	[d]	IR	IR	IR	IR	
Providencia rettgeri	IR	IR			IR			[d]	IR	IR	IR	IR	
Providencia stuartii	IR	IR			IR			[d]	IR	IR	IR	IR	e (IR to Gm, To but not Ak)
Raoultella spp. ([f] R. ornithonolytica, R. terrigena, R. planticola)	IR			IR									
Salmonella and Shigella spp.	There is no intrinsic resistance to penicillins in these organisms.				ECF[x]	ECF[x]	ECF[x]						ECF[x]
Serratia marcescens	IR	IR	IR		IR	IR	IR				IR	IR	
Yersinia enterocolitica	IR	IR		IR	IR								
Yersinia pseudotuberculosis (in VETSED07 not M100)												IR	

X - Expected Clinical Failure: For Salmonella and Shigella spp., aminoglycosides, first- and second-generation cephalosporins, and cephamycins may appear active in vitro but are not effective clinically and should be **suppressed or reported as resistant.**

bz note: change wording in Table 2 also

Organism/Drug	Ampicillin, Amoxicillin	Cefotaxime	Ceftriaxone	Ceftazidime	Cefepime	Ertapenem	Polymyxin B/ Colistin	Aminoglycosides	Tetracyclines /Tigecycline	Trimethoprim	Trimet/Sulfa	Chloramphenicol	Fosfomycin
Acinetobacter baumannii, A. calcoaceticus complex	IR		xxxx			IR			ECF (Doxycycline and Tetracycline only) (footnote x)	IR		IR	IR
Burkhlderia cepacia complex [a] (Long BCEP footnote)	IR	footnote a	footnote a		footnote a	IR	IR	footnote a		footnote a			IR
Pseudomonas aeruginosa	IR	IR	IR			IR		ECF (Gentamicin only) (footnote y)	IR	IR	IR	IR	
Stenotrophomonas maltophilia	IR	IR	IR	ECF (footnote z)		IR		IR	footnote b (IR to Te, but not Doxy, Mino, or TGC)	IR			IR

Footnote x. Expected Clinical Failure - Acinetobacter baumannii, A. calcoaceticus complex with doxycycline and tetracycline, but not with minocycline or tigecycline. Therapuetic breakpoints for doxycycline and tetracycline with A. baumannii, A. calcoaceticus complex were removed in M100-Ed35.

Footnote y. Expected Clinical Failure - P. aeruginosa with gentamicin, but not with amikacin, tobramycin or netilmycin. Therapeutic breakpoints for systemic use of gentamicin with P. aeruginosa were removed in M100-Ed33.

Footnote z. Expected Clinical Failure. Therapeutic breakpoints for ceftazidime with S. maltophilia were removed in M100-Ed34.

xxx - Acinetobacter with Cax is not published yet?

- Footnotes
  - Footnote x. Expected Clinical Failure - *Acinetobacter baumannii*, *A. calcoaceticus* complex with doxycycline and tetracycline, but not with minocycline or tigecycline. Therapuetic breakpoints for doxycycline and tetracycline with *A. baumannii*, *A. calcoaceticus* complex were removed in M100-Ed35.
  - Footnote y. Expected Clinical Failure - *P. aeruginosa* with gentamicin, but not with amikacin, tobramycin or netilmycin. Therapeutic breakpoints for systemic use of gentamicin with *P. aeruginosa* were removed in M100-Ed33.
  - Footnote z. Expected Clinical Failure. Therapeutic breakpoints for ceftazidime with *S. maltophilia* were removed in M100-Ed34.
  - Should we add "systemic" to all footnotes?
  - Can add to all that results should be suppressed or reported as Resistant.
- Methods Working Group Discussion and Recommendation
  - Why not call everything R and then use color to differentiate IR and ECF?
  - Initially in favor of the 2 tables, but now appreciates the single table approach
  - Is it necessary to call out where there is no intrinsic resistance?

SC DISCUSSION (MAIN POINTS)

- What is the process to come up with ECF notation? Is the AHWG only looking at drugs currently in the IR table? Or will the AHWG look at additional bug/drug combinations? AHWG will start where there is strong data.
- For breakpoints that were removed earlier, it needs to be clear if they were removed because the test does not work or if they are an ECF.
- Consensus likes the direction the AHWG is moving in for the Appendix tables.

- *Y. pseudotuberculosis* IR to Colistin
  - Organisms in Vet01S7 Appendix B but not in M100
    - Vet01ED7 has *Y. pseudotuberculosis* IR to colistin, and *Achromobacter* (IR to numerous drugs) in Appendix B2

- *Achromobacter* Intrinsic Resistance is now in M45.
- *Y. pseudotuberculosis* IR to colistin

- Publications
  - Hulankova, R. (2022). Higher Resistance of Yersinia enterocolitica in Comparison to *Yersinia pseudotuberculosis* to Antibiotics and Cinnamon , Oregano and Thyme Essential Oils. Pathogens, 11, 1456. DOI 10.3390/pathogens11121456
    - 46 isolates from wild boars, with MIC50/90s of 16/16 for colistin, tested on Vitek 2
  - Reinhardt, M., Hammerl, J. A., Kunz, K., Barac, A., Nöckler, K., & Hertwig, S. (2018). *Yersinia pseudotuberculosis* Prevalence and Diversity in Wild. Applied and Environmental Microbiology, 84(18), e00675-18.
    - 9/10 isolates had colistin MICs >16 using Trek BMD panels
  - Rebeil, R., Ernst, R. K., Gowen, B. B., Miller, S. I., & Hinnebusch, B. J. (2004). Variation in lipid A structure in the pathogenic yersiniae. 52, 1363–1373. https://doi.org/10.1111/j.1365-2958.2004.04059.x
    - LPS acylation patterns show a 90x increase in polymyxin B MICs at 37C vs 21C.
    - Multiple other studies show modification of LPS responsible for polymyxin resistance (primarily aminoarabinose) were higher at 37C vs. 21C. (*Y. pestis* and *Y. enterocolitica* have more modifications at 21C than 37C).
- Thank you to Lori Selden, CLSI who looked at the Vet Subcommittee minutes.
- Vet – Appendix B first published in 2018, based on Appendix B in M100 and EUCAST Intrinsic Resistance Table 1
- No record of discussion at Vet Subcommittee about *Y. pseudotuberculosis* and colistin, included based on EUCAST Tabl3 1, V3.1 (2016 Sep 26)
- Methods Working Group Discussion and Recommendation
  - Motion to add intrinsic resistance to *Yersinia pseudotuberculosis* for colistin in Appendix A. WG Vote: 8-0-0-4.

A motion to add intrinsic resistance to *Yersinia pseudotuberculosis* for colistin in Appendix B was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

- *Citrobacter freundii* – complex or not?
  - Rationale
    - Conventional phenotypic assays have a species-level correct identification rate of approximately 46.8% for *Citrobacter* spp., with a significant rate of misidentification between species.
    - MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry), although much better than phenotypic methods, still has limitations at the species level for the *Citrobacter freundii* complex, especially when reference spectra in the database are incomplete or when atypical strains are present.
    - Members of the complex have intrinsic resistance to aminopenicillins, amoxicillin-clavulanate, ampicillin-sulbactam, and first and 2nd generation cephalosporins. *C. freundii* complex can have inducible AmpC expression, additional β-lactam resistance.
  - AHWG Discussion and Recommendation
    - Proposal to change *Citrobacter freundii* to *Citrobacter freundii* complex in Appendix B with a footnote stating, "*C. freundii* complex includes *C. freundii, C. braakii, C. gillenii, C. murliniae, C. sedlakii, C. youngae, C. werkmanii, C. cronae,* and *C. portucalensis*".
  - Methods Working Group Discussion and Recommendation
    - How often are complexes reviewed across documents with regards to taxonomy updates?

- This is important for IR expert rules in some commercial systems
- Motion to change *Citrobacter freundii* to *Citrobacter freundii* complex in Appendix B with a footnote stating, "*C. freundii* complex includes *C. freundii*, *C. braakii*, *C. gillenii*, *C. murliniae*, *C. sedlakii*, *C. youngae*, *C. werkmanii*, *C. cronae*, and C. *portucalensis*". WG Vote: 7-1-0-4. Did not pass.
- No vote would have liked to review the references to make sure that all of the species within the complex are IR.

A motion to change *Citrobacter freundii* to *Citrobacter freundii* complex in Appendix B with the footnote stating, "*C. freundii* complex includes *C. freundii*, *C. braakii*, *C. gillenii*, *C. murliniae*, *C. sedlakii*, *C. youngae*, *C. werkmanii*, *C. cronae*, and *C. portucalensis*" was made and seconded. Vote: 16 for, 0 against, 0 abstain, 0 absent (Pass)

NOTE: During the Text and Tables Working Group review it was noted that *C. sedlakii* was already listed in the footnote for *C. amalonaticus* group. In discussions with the IR AHWG chairholder and Methods Working Group chairholders, it was decided to remove *C. sedlakii* from this new listing until further taxonomic recommendations can be brought forth next year. Additionally, EUCAST is also reassessing *C. sedlakii* as *C. freundii* complex versus *C. amalonaticus* group (current classification).

3. **JOINT WORKING GROUP (J. HINDLER)**

WORKING GROUP DOCUMENTS

- M23S/SOP 11 (November 2024 2nd ed): Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria
  - Approved addition of disk source for Phase 1 and 2
  - Discussing increasing numbers of isolates for Phase 1 and 2
- M23S2/SOP 12 (November 2024 2nd ed): Process to Submit Disk Content (Potency) Data for Joint CLSI-EUCAST Working Group Review and Approval
- M23S3/SOP 13 (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)
  - Approved addition of paragraph re: pre-QC of CAMHB
  - Discussing performance of disks from different manufacturers
- M23S4/SOP 14 (under review; forthcoming 2026): Guidance for Antimicrobial Agent Developers Considering a Modification of The Standard Reference Method for Susceptibility Testing

DISK CONTENT SELECTION

WG Assigned Study #	Agent	Sponsor	Notes
JWG-2023-1	BWC0977	Bugworks (JMI)	Phase 1 completed. On hold.
JWG-2023-2	Piperacillin-tazobactam (reassessment)	JMI, EDL, Vanderbilt	Phase 2 ongoing with 15/5, 15/10, 20/5 and 20/10 µg disks
JWG-2024-4	FG-960	Blacksmith Medicines (Forge Therapeutics) (JMI)	Phase 2 with 30, 50, 75 µg disks in progress (data presented to client)
JWG-2025-2	Cefiderocol (reassessment)	JMI, EDL	Phase 1 discussed in Methods WG. Ongoing analysis per organism /species, inner/outer zones, Acinetobacter)
JWG-2025-4	Cefiderocol-xeurborbactam	Qpex Biopharma (JMI)	Phase 2 in progress (5/2, 7/5, 10/5, 12/6 µg disks)

MHA AGAR EVALUATIONS

WG Assigned Study #	Agent	Sponsor	Notes
JWG-2026-1	Meropenem-ANT3310	Antabio	In progress

SUMMARY OF FEEDBACK FROM DISK MANUFACTURERS ON DISK DEVELOPMENT PROCESSES – 4 MANUFACTURERS

Question	Disk Manufacturer Response				Conclusion
	A	B	C	D	
During disk development, do you evaluate multiple agar mediums from multiple suppliers with your disk to assess variation between media suppliers per disk?	No	Yes	Yes	No	Disk development typically occurs with only 1-2 media sources.
When approached to develop a disk by a sponsor, do you know the QC zone diameter range to target from the beginning?	Sometimes	Yes	Sometimes	Yes	QC zone diameter is sometimes known when development begins, but depends on how far along pharma is. If development begins very early, the zone might not be known.
If so, do you develop the disk to target the mode of the QC range during disk development?	Sometimes	Yes	No	No	All manufacturers aim to be in range; with half targeting the mode when known and is preferred/ideal.
Does the sponsor provide you with any QC range distribution data from handmade disks for reference prior to your own development?	Sometimes	Sometimes	Rarely	Rarely	QC range distribution data is rarely or occasionally available.
Would it be helpful if some kind of high-level disk development guidance/guideline was created by the WG to ensure that disks generally follow a similar development process, to reduce the potential disk variation amongst disks produced by multiple manufacturers?	Yes	Yes	Yes	Yes	It is welcomed to develop high level general guidance.

COMPARISON OF DISKS FROM DIFFERENT MANUFACTURERS

- Pre-tier 2 QC studies
- Designed to detect differences between MHA media manufacturers
    - The procedure is intended to confirm that MHA sources will perform reliably when a full QC study is performed, thereby avoiding media-related problems when QC ranges are established.
    - Performed after a disk content has been selected
- Data Analysis
    - For each QC strain-antimicrobial agent combination (test and control agent) measurement, calculate per MHA source tested:
        - Mean
        - Median
        - Standard deviation

- Range of zone diameters (minimum to maximum zone diameter)
  - Optimally, select MHA sources that demonstrate mean or median zone diameter values within ± 1 mm for the test agent.
    - If mean or median zone diameters are not within ± 1 mm for three media, perform three additional tests. Perform calculations using results from all six tests.
  - If criteria in step 2 are not met, select MHA sources that demonstrate mean or median zone diameter values within ± 2 mm for the test agent.
- Can we set the same criteria for disk variability?
  - Optimally, disks from different manufacturers should demonstrate mean or median zone diameter values within ± 1 mm on each test medium
    - If mean or median zone diameters are not within ± 1 mm for three media, perform three additional tests.
    - Perform calculations using results from all six tests.
  - If criteria in step 1 are not met for disks from two manufacturers, discuss with Joint WG. Then possibly work together with the disk manufacturers to align disk potency.

DATA ANALYSIS OF PHASE 1 AND 2 STUDIES

- Isolate Numbers for Combination Agents
- Disk Content Selection
  - Phase 1 – few isolates/many disks
    - 4 isolates/species
    - ≈10 different disk contents
    - 1 disk lot/content, 1 MHA source
  - Phase 2 – many isolates/few disks
    - 30 isolates/species or 60/group of organisms
    - 2-4 disk contents (2 lot each), 2 MHA sources
- Phase 1 - Single Agent and Small Number of Each Species

Table 3C. Zone Diameter Sizes (in mm) and Zone Differences (last row) When Data From Tables 3A and 3B Are Consolidated

Isolate ID	Species	MIC, µg/mL	WT/NWT (WT ≤ 0.5 µg/mL)	Disk Content (Potency), µg[a]									
				0.1	0.2	0.4	1	5	10	20	30	40	50
1	*E. coli*	0.06	WT	12	16	18	21	26	27	29	31	32	32
2	*E. coli*	0.25	WT	7	12	16	19	26	27	29	29	30	30
3	*E. coli*	8	NWT	6	6	6	9	16	19	21	23	24	23
4	*E. coli*	16	NWT	6	6	6	6	9	12	16	18	20	19
Difference between largest and smallest zone				**6**	**10**	**12**	**15**	**17**	**15**	**13**	**13**	**12**	**13**

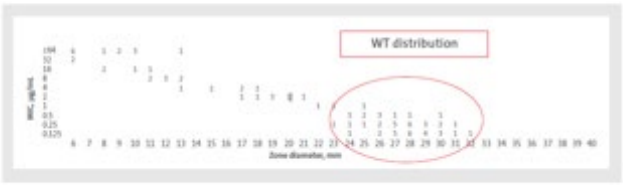
Isolate ID	Species	MIC, µg/mL	WT/NWT (WT ≤ 0.5 µg/mL)	Disk Content (Potency), µg[a]									
				0.1	0.2	0.4	1	5	10	20	30	40	50
5	*K. pneumoniae*	0.25	WT	10	13	16	18	24	26	28	28	28	29
6	*K. pneumoniae*	0.5	WT	11	15	17	21	26	28	30	31	30	31
7	*K. pneumoniae*	32	NWT	6	6	6	6	8	10	13	15	15	16
8	*K. pneumoniae*	32	NWT	6	6	6	6	6	8	11	14	15	15
Difference between largest and smallest zone				**5**	**9**	**11**	**15**	**20**	**20**	**19**	**17**	**15**	**16**

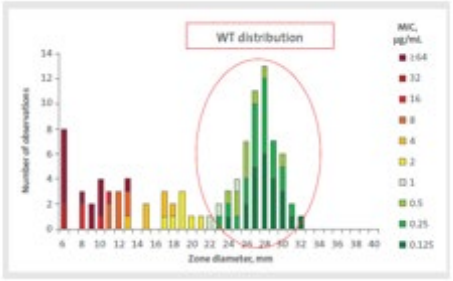
Isolate ID	Species	MIC, µg/mL	WT/NWT (WT ≤ 8 µg/mL)	Disk Content (Potency), µg[a]									
				0.1	0.2	0.4	1	5	10	20	30	40	50
9	*P. aeruginosa*	2	WT	6	6	8	13	21	24	27	27	28	29
10	*P. aeruginosa*	4	WT	6	6	6	6	15	20	23	25	25	28
11	*P. aeruginosa*	> 32	NWT	6	6	6	12	18	21	25	26	25	28
12	*P. aeruginosa*	> 32	NWT	6	6	6	6	15	18	20	21	22	25
Difference between largest and smallest zone				**0**	**0**	**2**	**7**	**6**	**6**	**7**	**6**	**6**	**4**

Abbreviations: ID, identification; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type.
[a] Disk contents (potencies) with both optimal zone sizes and the largest increase in zone sizes between two consecutive disk contents (potencies) for WT isolates are highlighted in yellow. The greatest difference (mm) between the largest and smallest zone within the optimal area is highlighted in green.

- Phase 2 - Single Agent

- Inhibition zone diameters are correlated to the corresponding MIC values and presented in two different graphical formats:
  - Species-specific scattergrams (see Figure 1A) and inhibition zone diameter histograms with corresponding MIC values as colored bars (see Figure 1B)
    - **NOTE:** Figures 1A and 1B represent the same dataset.





- Phase 1 and 2 Combination Agents
  - To select the optimal disk content (potency) for combination agents, a greater number of isolates than suggested for a single agent should be tested (see Table 4). **NOTE:** For Enterobacterales, a variety of target species should be included, and differences in MIC values between species should be considered when the laboratory is determining the numbers of each species to evaluate.

Table 4. Selection of Isolates for Phase 1 and Phase 2 Testing of Combination Agents

Phenotype	Phase 1	Phase 2
WT to the parent compound	≥ 2	≥ 10
WT to the combination (NWT to parent compound)	≥ 2	≥ 30
NWT to the combination	≥ 2	≥ 30

Abbreviations: NWT, non-wild-type; WT, wild-type.

  - It is advisable to include in the report information available on relevant resistance mechanisms in the test isolates and the effect of the inhibitor on these resistance mechanisms.
  - Results should be summarized in species-specific or organism-specific scattergrams (see Figure 1A) and inhibition zone diameter histograms with corresponding MIC values as colored bars (see Figure 1B).

Increase number of isolates recommended for combination agents

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SC DISCUSSION (MAIN POINTS)

	• For increasing the number of isolates for Phase 1 and Phase 2 studies, will that number be flexible? Yes, the number will be flexible as this is a guideline. **ACTION ITEMS** • M23S – modify recommendations to increase numbers of isolates for Phase 1 & 2 disk content selection studies, particularly for combination agents • M23S3 - define criteria for disk source performance (±1 mm) (Pre-Tier 2 QC) • Develop a protocol for sharing disk content selection QC data with disk manufacturers • Complete M23S4

4. **OUTREACH WORKING GROUP (A. SCHUETZ)**

WORKING GROUP GOALS

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

PRODUCTS OF WORKING GROUP

- January 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

- Quality Control
    - News Update article November 2025
    - Pending
        - Update ASM-CAP-CLSI MIC IQCP (based on revisions to CAP checklist MIC.21910 12/9/2025 version) and disk diffusion IQCP
        - Provide practical approaches to ensuring reliable AST results
        - Add more scenarios as examples
            - Old drugs on panel in use for 4 years
            - Newer disk introduced 3 months ago
- *Burkholderia cepacia* complex
    - News Update article November 2025
    - JCM manuscript submitted

CONTENT PRIORITIES 2026

- Disk diffusion validation resources and aztreonam-avibactam educational content in upcoming Summer 2026 News Update
- Commence proposal submissions to Association for Diagnostics and Laboratory Medicine (ADLM) conference session
- Update M100 Online Course
- Refresh Breakpoint Implementation Toolkit (BIT)
- Continue QC updates
- Alignment of duties with other education working groups (Antifungal SC and VAST SC)

AST SUBCOMMITTEE MEETING EDUCATION WORKSHOPS

- Maintain January workshop
    - Include topics applicable to Antifungal SC and VAST SC
- Discontinued June workshop
- Possible topics for upcoming January 2027 Education Workshop
    - Global perspective of CLSI (outside of North America)
    - Legislative impacts on AST
    - Artificial intelligence
    - Clinical trials and bringing a drug to market
    - Back to the basics: review of M23 content; PK/PD
    - Roles and services of reference laboratories

NEWS UPDATE

- AST News Updates are located under “Resources” tab of CLSI homepage
- Summer 2026
    - Feature: AST and molds
    - Case: NDM and Aztreonam-avibactam
    - Practical tips: Aztreonam-avibactam methods and validation of updated disk diffusion breakpoints
    - Hot topic: Increase in NDM
    - More News!: What’s in CLSI AST’s pipeline?
- Fall 2026
    - Feature: Rapid phenotypic AST
    - Case: Sulbactam-durlobactam
    - Practical tips: Sulbactam-durlobactam testing guidance
    - Hot topic: Pending
    - More News!: BIT 2026 updates featured

ATTENDEE ORIENTATION

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

WEBINARS/PRESENTATIONS

- CLSI Annual Update (24th)
  - The Latest in Antimicrobial Susceptibility Testing?
  - February 25, 2026
  - Speakers: April Bobenchik and Virginia Pierce
  - Moderator: Janet Hindler
  - Stats:
    - Live Attendance: 633
    - Live Q&A: 113
- CLSI/SIDP/ACCP Annual Webinar
  - From Update to Update: Implementing the CLSI M100 Standards to Advance Antimicrobial Stewardship and Clinical Practice
  - April 30, 2026
  - Speakers: Kevin Alby and Kate DeSear
  - Moderator: Lindsay Donohue
  - Stats:
    - Live Attendance: 520
    - Participants from 22 counties
- CLSI/CAP Annual Webinar
  - Fungi Fight Back
  - October 29, 2026
  - Speakers: Anisha Misra and Sean Zhang
  - Topics potentially to include:
    - *C. auris* and guidance on when to test
    - Trichophyton mentagrophytes new genotypes and AST patterns (although no terbinafine breakpoints currently, this is part of a new working group and a goal)
    - Mold AST (when to send for testing and usual AST patterns)
    - Intrinsic resistance for yeasts and molds (recent updates) and updated body site reporting restrictions
    - ECV use, highlighting data from CAP survey about uptake of ECVs

NON-CLSI CONFERENCES AND EVENTS

- ASM Microbe 2026
  - Ur-ine or Ur-out: Body Site Considerations When Reporting and Interpreting Antimicrobial Susceptibility Test Results
  - June 6, 2026
  - Speaker: James Lewis
- ADLM 2026
  - Confronting Antimicrobial Susceptibility Testing Challenges: Tools from CLSI
    - July 29, 2026
    - Speakers: Alexandra Bryson, Elizabeth Garrett, and Elizabeth Palavecino
    - Moderator: Alexandra Bryson

- Fungal Taxonomy Updates, *Candida auris*, and Antifungal Resistance Testing in Clinical Laboratories
  - July 29, 2026
  - Speaker: Amir Seyedmousavi

CLSI M100 EDUCATIONAL PROGRAM

- Update anticipated soon
- No fee
- Great for laboratory directors, training technologists and other trainees in laboratory

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.
- Publication on CLSI vs EUCAST methods for antifungal susceptibility testing
- Others

BREAKPOINT IMPLEMENTATION TOOLKIT (BIT)

- Updated and *New BPs previously not included in 2023 BIT

Enterobacterales	Pseudomonas aeruginosa	Acinetobacter spp.
Ceftolozane-tazo DD 2022		Amikacin 2026
Cefiderocol DD 2022		Amp-sulbactam DD 2025
		Gentamicin 2026
		Minocycline 2025
		Tobramycin 2026
		Cefiderocol DD 2022
***Aztreonam-avibactam 2026**		***Sulbactam-durlobactam 2024**

* New

Stenotrophomonas maltophilia	Neisseria gonorrhoeae
Cefiderocol (DD) 2026 DD MIC 2022	Cefixime 2026
Minocycline 2024	Ceftriaxone 2026

Staphylococcus	Beta Streptococcus	Viridans
Linezolid (DD) 2024	***Tedizolid (DD) 2024**	***Tedizolid (DD) 2024**
***Tedizolid (DD)** 2024	***Trimethoprim-sulfa 2026**	

- Actions for 2026 BIT
  - Expand goal to address verification
  - Align with upcoming CLSI M52 and M68 publications
  - Auto calculations for verification/validation
  - Address verification/validation disk diffusion
  - Continue to regularly keep up with 2023 BIT Part B
  - Education on AR Bank and isolates available elsewhere (include explanation of range, mode, etc.)
  - Fall 2026 CLSI video featuring sulbactam-durlobactam and aztreonam-avibactam verification

PODCAST/SOCIAL MEDIA OPPORTUNITIES/OTHER

- Let's Talk Micro" with Luis Plaza
- Session 226: CLSI M100-2026 Updates
- April Bobenchik, PhD
- Seeking volunteers to present

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
5.	**ADJOURNMENT** Dr. Mathers thanked the participants for their attention. The meeting was adjourned at 12:00 PM Central Time (US).

PLENARY ATTENDEES

Plenary 1	Plenary 2	Plenary 3
Abdul Azim Ahmed	Acevedo Sarah	Abdul Azim Ahmed
Acevedo Sarah	Adams Jennifer K.	Adams Jennifer K.
Adams Jennifer K.	Alby Kevin	Alby Kevin
Alby Kevin	Andermann Tessa	Andermann Tessa
Andermann Tessa	Asempa Tomefa	Asempa Tomefa
Arone Lisa	Austerman Ashley	Austerman Ashley
Asempa Tomefa	Barnett Katie	Bobenchik April M.
Austerman Ashley	Bhatti Micah M.	Bowden Robert
Barnett Katie	Bobenchik April M.	Boyle Bridget
Bhatti Micah M.	Bowden Robert	Brown Jekia
Bobenchik April M.	Boyle Bridget	Bryan, MD, PhD Andrew
Bowden Robert	Brown Carrine	Bryson Alexandra Lynn
Boyle Bridget	Brown Jekia	Bulman Zackery P.
Brown Carrine	Bryan, MD, PhD Andrew	Campbell Davina
Brown Jekia	Bryson Alexandra Lynn	Campeau Shelley
Bryan, MD, PhD Andrew	Bulman Zackery P.	Capraro Gerald A.
Bryson Alexandra Lynn	Campbell Davina	Carpenter Darcie E.
Bulman Zackery P.	Campeau Shelley	Carvalhaes M.D., Ph.D., D ABMM Cecilia
Campbell Davina	Capraro Gerald A.	Castanheira Mariana
Campeau Shelley	Carpenter Darcie E.	Chandrasekaran Sukantha
Capraro Gerald A.	Carvalhaes M.D., Ph.D., D ABMM Cecilia	CHEN YAMIN
Carpenter Darcie E.	Castanheira Mariana	Copsey-Mawer Sarah
Carvalhaes M.D., Ph.D., D ABMM Cecilia	Chandrasekaran Sukantha	Cullen Sharon K.
Castanheira Mariana	CHEN YAMIN	DeJonge Boudewijn
Chandrasekaran Sukantha	Copsey-Mawer Sarah	Dial Courtney
CHEN YAMIN	Cullen Sharon K.	Dingle Tanis
Copsey-Mawer Sarah	DeJonge Boudewijn	Donohue Lindsay
Cullen Sharon K.	Dial Courtney	Dressel Dana C.
DeJonge Boudewijn	Dingle Tanis	Dumm Rebekah
Dial Courtney	Donohue Lindsay	Duncan Elaine
Dingle Tanis	Dressel Dana C.	Esparza German
Donohue Lindsay	Dumm Rebekah	Estrada Sandy
Dressel Dana C.	Duncan Elaine	Fedorenko Marianna
Dumm Rebekah	Esparza German	Feng Hwa-ping
Duncan Elaine	Estrada Sandy	fernandez erica
Esparza German	Fedorenko Marianna	Ferrell Andrea L.
Estrada Sandy	Feng Hwa-ping	Fisher Mark A.
Fedorenko Marianna	fernandez erica	Fratoni Andrew
Feng Hwa-ping	Ferrell Andrea L.	Galas Marcelo F.
fernandez erica	Fisher Mark A.	Gancarz Barb

Ferrell Andrea L.
Fisher Mark A.
Fratoni Andrew
Galas Marcelo F.
Gancarz Barb
Garrett Elizabeth
Gatermann Sören
Gold Howard
Gomez Emily J.
Grande Roche Kerian
Gray Alice
Gray Kamisha
Griffin Natasha
Gupta Vikas
Haddock Christopher
Haring Jennifer
Hastey Christine
Hendrix Megan
Hindler Janet A.
Hirsch Elizabeth
Hoffard Rita
Howell Nicholas
Hsiung Andre
Huband Michael D.
Hufnagel David
Humphries Romney M
Igarashi Yuki
Jean Sophonie
Jimenez Pearson Antonieta
Johnson Brian
Khan Ayesha
Killian Scott B.
Kim Peter
Kirn Thomas
Klavins Anna
Kumaraswamy Monika
Lam Christine M.
Larson Kajal
LaVoie Stephen
Law Ashley
Lewis James S.
Liesman Rachael
Livesay Hannah
Fratoni Andrew
Galas Marcelo F.
Gancarz Barb
Garrett Elizabeth
Gatermann Sören
Gold Howard
Gomez Emily J.
Grande Roche Kerian
Gray Alice
Gray Kamisha
Griffin Natasha
Gupta Vikas
Haddock Christopher
Haring Jennifer
Hastey Christine
Hendrix Megan
Hindler Janet A.
Hirsch Elizabeth
Hoffard Rita
Howell Nicholas
Hsiung Andre
Huband Michael D.
Hufnagel David
Humphries Romney M
Igarashi Yuki
Jean Sophonie
Jimenez Pearson Antonieta
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Mindel Susan
Mitchell Stephanie L.
Monogue Maggie
Moran Angelica
Morrissey Ian
Moussa Samir
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Pham Andy
Pham Cau Dinh
Pierce Virginia M.
Pillar Chris
Poettker Anna
Ramos Karl Anthony
Rana Khurram
Reinagle Meagan
Riccobene Todd
Rice Felicia
Robinson Stephanie F.
Sabour Sarah
Satlin Michael
Scangarella-Oman Nicole
Scheetz Marc H.
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