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 Standard (US) time.	**Plenary 1:** Monday, 24 June 2024, 7:30 AM – 12:00 PM **Plenary 2:** Monday, 24 June 2024, 1:00 – 5:30 PM **Plenary 3:** Tuesday, 25 June 2024, 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 (35th).		
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):	
James S. Lewis, PharmD, FIDSA **AST** Subcommittee Chairholder	**Oregon Health and Science University**
Amy J. Mathers, MD, D(ABMM) AST Subcommittee Vice-Chairholder	**University of Virginia Medical Center**
Alexandra L. Bryson, PhD, D(ABMM) AST Subcommittee Secretary	**Virginia Commonwealth University Health**
Members Present:	
Sharon K. Cullen, BS, RAC	Beckman Coulter, Inc. Microbiology Business
Tanis Dingle, PhD, D(ABMM), FCCM	Alberta Precision Laboratories
German Esparza, MSc	Proasecal SAS Columbia
Romney M. Humphries, PhD, D(ABMM), FIDSA, FAAM	Vanderbilt University Medical Center
Thomas J. Kirn, MD, PhD	Rutgers Robert Wood Johnson Medical School
Joseph D. Lutgring, MD	Centers for Disease Control and Prevention
Navaneeth Narayanan, PharmD, MPH	Rutgers University, Ernest Mario School of Pharmacy
Elizabeth Palavecino, MD	Wake Forest Baptist Medical Center
Virginia M. Pierce, MD, FIDSA	University of Michigan Medical School
Audrey N. Schuetz, MD, MPH, D(ABMM)	Mayo Clinic (Rochester, MN)
Susan Sharp, PhD, D(ABMM), F(AAM)	Copan Diagnostics, Inc.
Patricia J. Simner, PhD, D(ABMM)	Johns Hopkins University School of Medicine, Department of Pathology
Pranita D. Tamma, MD, MHS	John Hopkins University School of Medicine, Department of Pediatrics
Melvin P. Weinstein, MD	Robert Wood Johnson University Hospital
Advisors Present:	
Kevin Alby, PhD, D(ABMM)	University of North Carolina Hospital
Amelia S. Bhatnagar, MPH	Centers for Disease Control and Prevention
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
Mariana Castanheira, PhD	Element/JMI Laboratories
Lindsay Donohue, PharmD, BCIDP	University of Virginia Medical Center
Andrea L. Ferrell, MLS(ASCP)	BD
Marcelo Galas, BSc	Pan American Health Organization
Natasha Griffin, PhD	FDA Center for Devices and Radiological Health
Janet A. Hindler, MCLS, MT(ASCP), F(AAM)	Los Angeles County Department of Public Health
Dmitri Iarikov, MD, PhD	FDA Center for Drug Evaluation and Research

Antonieta Jimenez Pearson, MQC, PhD	INCIENSA
Joe Kuti, PharmD, FIDP, FCCP	Hartford Hospital
Stephanie L. Mitchell, PhD, D(ABMM)	Cepheid, Inc.
Greg Moeck, PhD	Venatorx Pharmaceuticals, Inc.
Kiyofumi Ohkusu, PhD	Tokyo Medical University
Mike Satlin, MD	Weill Cornell Medicine
Reviewers and Guests (Non-SC-roster attendees): see Plenary Attendee List below	
Staff:	
Jennifer Adams, MT(ASCP), MSHA	CLSI
Emily Gomez, MS, MLS(ASCP)MB	CLSI
Barb Jones, PhD	CLSI
Christine Lam, MT(ASCP)	CLSI
Lori Selden, MS, MT(ASCP)	CLSI

Plenary Agendas

PLENARY AGENDA: SESSION 1 – IN PERSON Monday, 24 June 2024 7:30 AM – 12:00 PM Central Standard (US) Time			
Time	**Item**	**Presenter**	**Page**
7:30 AM – 7:40 AM (10 min)	Opening Remarks	J. Lewis	8
7:40 AM – 7:50 AM (10 min)	CLSI Welcome and Update	B. Jones	8
7:50 AM – 8:00 AM (10 min)	CLSI Awards	B. Jones	8
8:00 AM – 8:10 AM (10 min)	CLSI M100 35th Edition Publication and Reminders	E. Gomez	9
8:10 AM – 8:20 AM (10 min)	Veterinary AST Subcommittee Update	R. Bowden	10
8:20 AM – 9:50 AM (1 hr 30 min)	Quality Control WG	S. Cullen C. Pillar	13
9:50 AM – 10:10 AM (20 min)	Break		
10:10 AM – 10:40 AM (30 min)	Joint CLSI-EUCAST WG	J. Hindler	25
10:40 AM – 12:00 PM (1 hr 30 min)	Breakpoints WG: Part 1	N. Narayanan M. Satlin	30

PLENARY AGENDA: SESSION 2 – IN PERSON Monday, 24 June 2024 1:00 PM – 5:30 PM Central Standard (US) Time			
Time	**Item**	**Presenter**	**Page**
1:00 PM – 3:30 PM (2 hr 30 min)	Breakpoints WG: Part 2	N. Narayanan M. Satlin	41
3:30 PM – 3:50 PM (20 min)	Break		
3:50 PM – 5:30 PM (1 hr 30 min)	CLSI, Breakpoints, and FDA	R. Humphries	89

PLENARY AGENDA: SESSION 3 - IN PERSON Tuesday, 25 June 2024 7:30 AM - 12:00 PM Central Standard (US) Time			
Time	**Item**	**Presenter**	**Page**
7:30 AM – 9:00 AM (1 hr 30 min)	Methods Application and Interpretation WG	K. Johnson	96
9:00 AM – 9:30 AM (30 min)	Text and Tables WG	S. Campeau	116
9:30 AM – 9:50 AM (20 min)	Break		
9:50 AM – 10:20 AM (30 min)	Methods Development and Standardization WG	T. Dingle	137
10:20 AM – 10:50 AM (30 min)	Outreach WG	A. Schuetz	146
10:50 AM – 12:00 PM (1 hr)	Additional Items as Needed		152
12:00 PM	Closing Remarks	J. Lewis	152

Summary of Voting Decisions and Action Items

Summary of Passing Votes			
#	**Motion Made and Seconded**	**Results**[a]	**Page**[b]
1.	To accept the rifasutenizol QC ranges for *Helicobacter pylori* ATCC 43504 (0.001-0.008 µg/mL).	14-0-0-0	14
2.	To accept the zosurabalpin QC ranges for *Acinetobacter baumannii* NCTC 13304 (0.016 – 0.12 µg/mL) with the following footnotes: 1) "Zosurabalpin MIC testing was conducted in cation-adjusted Mueller-Hinton broth supplemented with 20% heat-inactivated horse serum" and 2) the standard footnote that "QC range was established with broth microdilution. Agar dilution equivalency has not been established."	14-0-0-0	16
3.	To accept the minocycline 30 µg disk diffusion QC ranges for *E. coli* ATCC 25922 (20 – 26 mm).	14-0-0-0	19
4.	To endorse the general direction the QC Working Group is going with the streamlined QC and to accept the following revisions to the Tables 2 QC Box: 1) Remove the reference to specific strains, 2) Refer to QC tables for ranges, 3) Refer to new tables for QC strain and frequency recommendations, 4) Refine statement about commercial tests to avoid redundant/conflicting comments.	13-0-1-0	22
5.	To accept the investigational breakpoints for cefepime/zidebactam (≤64/64 µg/mL) for Enterobacterales, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* to be published in a separate document in the CLSI free resources.	14-0-0-0	39
6.	To accept the susceptible cefepime dosage for *P. aeruginosa* as 2g IV q8h over 3 hrs.	14-0-0-0	47
7.	To form an ad hoc working group on trimethoprim-sulfamethoxazole breakpoints for ß-hemolytic streptococci.	14-0-0-0	50
8.	To accept the Table 2A-1 comment, "Isolates resistant to any carbapenem tested (eg, ertapenem, imipenem, meropenem) except *Proteus* spp., *Providencia* spp., or *Morganella* spp. only resistant to imipenem, should undergo carbapenemase testing using a phenotypic and/or molecular assay to identify and ideally differentiate the presence of particular carbapenemases (eg, KPC, NDM, OXA-48, VIM, IMP). The decision of testing and reporting is best made by each laboratory in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. These results are important for treatment decisions and inform infection control and prevention interventions and/or epidemiologic investigations, but do not replace antimicrobial susceptibly testing for new agents. Depending on local epidemiology and resources, laboratories may consider omitting carbapenemase testing for *Enterobacter cloacae* complex and *Klebsiella aerogenes* isolates that are only resistant to ertapenem, because carbapenemases are currently uncommon in such isolates. See Appendix G, Table G3 regarding suggestion for reporting when new mechanism of resistance-based testing (molecular and phenotypic methods) is discordant with phenotypic AST."	12-2-0-0	56
9.	To accept the Tables 3B and 3C comment, "Institutional treatment guidelines, infection prevention procedures, or epidemiological investigations may necessitate identification of carbapenemase-producing Enterobacterales or *P. aeruginosa*. Tests that detect the type of carbapenemase are recommended to inform treatment decisions for carbapenem-resistant Enterobacterales isolates."	14-0-0-0	57
10.	To not change the current ampicillin-sulbactam MIC breakpoints (S≤8/4, I 16/8, R ≥32/16 µg/mL) for *Acinetobacter* spp. and to accept adding based on a dosage of 3g AMP-SUL (2g AMP and 1g SUL) q6h as an extended infusion of ≥3 hours.	14-0-0-0	71

Summary of Passing Votes			
11.	To accept the ampicillin-sulbactam disk breakpoints (S≥22, I 17-21, R≤16 mm) for *Acinetobacter* spp. and to remove the direct disk diffusion breakpoints until reviewed.	14-0-0-0	74
12.	To accept the minocycline MIC breakpoints (S≤1, I 2, R≥4 µg/mL) for *Acinetobacter* spp. based on a dosage of 200mg q12h.	13-0-0-1	84
13.	To accept the minocycline disk breakpoints (S≥22, I 18-21, R≤17 mm) for *Acinetobacter* spp. with a comment to test the MIC with an intermediate result.	13-0-1-0	88
14.	To archive the doxycycline and tetracycline MIC and disk breakpoints for *Acinetobacter* spp. with a comment to indicate that they are under review and to eliminate the prediction comment.	13-0-1-0	88
15.	To add sulbactam-durlobactam I or R for *Acinetobacter baumannii* complex to Category I in Appendix A.	13-0-0-1	98
16.	To align the order of presentation of Appendix A organisms with Tables 2.	13-0-0-1	98
17.	To accept the *Burkholderia cepacia* complex ECVs for ceftazidime (16 µg/mL), levofloxacin (8 µg/mL), meropenem (16 µg/mL), minocycline (8 µg/mL), and trimethoprim-sulfamethoxazole (2 µg/mL), keep the reference method as broth microdilution, and add disclaimers	10-3-0-1	103
18.	To accept the Table 2B-3 revisions.	12-1-0-1	105
19.	To accept the Appendix F revisions.	11-2-0-1	109
20.	To approve moving exebacase to Appendix H and to accept the proposed revisions to Table 5A-1, Table 6A, and Appendix H.	12-0-1-1	124
21.	To remove the tetracycline footnotes from Tables 1, keep in Tables 2, and add a comment to the Tables 1 introduction to refer to Tables 2.	12-1-0-1	129
22.	To accept the Tables 2 tetracycline comment, “Isolates that test susceptible to tetracycline may be reported as susceptible to doxycycline or minocycline. Isolates that test intermediate or resistant to tetracycline should be tested against doxycycline or minocycline if those results are needed for reporting.” for Enterobacterales, *Salmonella* and *Shigella* spp., *Staphylococcus* spp., *Acinetobacter* spp., other Non-Enterobacterales, and *Enterococcus* spp.	11-1-0-2	130
23.	To accept the Tables 2 tetracycline comment, “Isolates that test susceptible to tetracycline may be reported as susceptible to doxycycline. Isolates that test intermediate or resistant to tetracycline should be tested against doxycycline if those results are needed for reporting.” for *Streptococcus pneumoniae*.	13-0-0-1	130
24.	To accept the Tables 2 tetracycline comment, “Isolates that test susceptible to tetracycline may be considered as susceptible to doxycycline and minocycline.” for *Haemophilus influenzae* and *Haemophilus parainfluenzae*, *Neisseria gonorrhoeae*, *Streptococcus* spp. ß-hemolytic group, and *Streptococcus* spp. viridans group.	12-1-0-1	131
25.	To change the voted on Tables 2 tetracycline comments to state “considered” instead of “reported”.	12-1-0-1	131
26.	To accept the Tables 2 oxazolidinone comment, “Isolates that test susceptible to linezolid may be considered as susceptible to tedizolid. Isolates that test intermediate/resistant/nonsusceptible to linezolid should be tested against tedizolid if that result is needed for reporting.” for *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp. ß-hemolytic group, and *Streptococcus* spp. viridans group.	12-0-1-1	133
27.	To accept the ampicillin-sulbactam direct blood disk breakpoints for *Acinetobacter* (S≥22, 17-21 I, R≤16 mm) for an 8-10h and 16-18h reading time.	13-0-0-1	139

Summary of Passing Votes			
28.	To accept the proposed revisions to the Carbapenemase Detection Table.	13-0-0-1	152

[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.

2024 JUNE AST MEETING SUMMARY MINUTES PLENARY 1: Monday, 24 June 2024 (In-person) 7:30 AM – 12:00 PM Central Standard (US) Time	
#	**Description**
1.	**OPENING REMARKS (J. LEWIS)** Dr. Lewis opened the meeting at 7:30 AM Central Standard (US) time by welcoming the participants to the hybrid CLSI meeting in Orlando, Florida.
2.	**CLSI WELCOME AND UPDATE (B. JONES)** Dr. Jones thanked the CLSI experts for their ongoing support, time, and efforts.
3.	**CLSI AWARDS (B. JONES)** Dr. Jones presented the CLSI Excellence in Member Organization Leadership Award to the Centers for Disease Control and Prevention (CDC).

4.	**CLSI M100 35TH EDITION PUBLICATION AND REMINDERS (E. GOMEZ)**
	Ms. Gomez provided an update on the M100 35th edition timeline and reminders. The main points included:
	• M100 35th Edition is publishing in January 2025.
	• CLSI Internal M100 Process Improvements
	  ○ Meetings with all applicable departments on education of timeline and significant deadlines
	  ○ Clearer and more detailed internal process steps and staff instruction
	  ○ Additional spring editing step
	  ○ Changes to Supplemental Vote comment resolution review
	  ○ Edaptive table formatting fixes for easier layout to publication
	• Ask of AST Subcommittee Subject Matter Experts
	  ○ January and June 2024 meeting decisions are incorporated into M100 35th edition
	  ○ No additional decisions after the June 2024 meeting
	  ○ Any pending M100 comments or revisions need to be provided to the TTWG Chairholders (April B. and Shelley) by EOD on Tuesday, 25 June
	  ○ Meet set review deadlines
	• Important Upcoming Dates
	  ○ Second TTWG Review: 22 July to 30 July
	  ○ AST SC Supplemental Review: 22 August – 3 September
	• Supplemental Review Reminders
	  ○ Vote and Comment: AST Subcommittee Members
	  ▪ Need 2/3 approval votes and 1 vote from each constituency to pass
	  ○ Comment Only: AST Subcommittee Chairholders, Advisors, and Reviewers
	  ▪ WG members and advisors are AST Subcommittee Reviewers
	  ○ Completed using the Edaptive Platform
	  ○ Focus on tracked changes (new revisions to 35th edition)
	  ○ Comments regarding content outside the tracked changes, email TTWG Chairholders or the applicable WG Chairholders
	• 2025 AST Subcommittee Meeting Dates
	  ○ January 2025
	  ▪ 26 – 28 January 2025 in Orlando, Florida
	  ▪ Meeting materials due 9 December 2024
	  ▪ Virtual Only Working Group Meetings in Weeks of 6 January and 13 January 2025
	  ○ June 2025
	  ▪ 31 May – 3 June 2025 in Dallas, Texas
	  ▪ Meeting materials due date TBD

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

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

- WG on VAST Breakpoints/Editorial Tables (VET01S)
  - 7th edition published in January 2024, now working on revisions for 8th edition (expected 2026)

Subgroup 1: Breakpoint Comment Review and Harmonization
- Standardize verbiage of comments throughout document

Subgroup 2: Human Breakpoint Additions and Removal (inclusion/exclusion)
- Develop criteria for evaluating if an M100 breakpoint should or should not be included in VET01S

Subgroup 3: Update Appendix A

Subgroup 4: *Staphylococcus* subgroup
- Oxacillin BP work continues for *S. pseudintermedius*-group, *S. coagulans*, and *S. schleiferi*

Subgroup 5: Table 1 Reorganization
- Will function as a standalone AHWG

- New AHWG on Disk Diffusion Breakpoint Development
  - >200 MIC BPs developed within VAST but many lack corresponding zone diameter (ZD) BPs
  - ZD BPs from M100 have been adopted in cases where M100 and VET01S MIC BPs align
  - A pilot project will be undertaken to establish ZD BPs for several vet drugs (prioritized list to be finalized)
  - Data for ≥100 isolates per bug/drug combo will be collected proactively and via published literature
  - Data will come from ≥5 labs, with initial analysis by dBETs and following VET02 criteria
  - Dry-form BMD MICs will be acceptable so long as parallel QC data is present
- VET09 Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings
  - 2nd edition published in February 2024
  - Expanded sections describing how BPs are set, importance of PK-PD, critically important agents
  - 2 new chapters:
    - Poultry-Specific Breakpoints and Factors Affecting AST Results Interpretation for Chickens and Turkeys
    - Factors Affecting AST Results Interpretation for Animals without Species-Specific Breakpoints
  - Several veterinary schools have acquired funding to provide copies to all students + faculty
  - Integrated into curriculum for 3rd-year Iowa State veterinary students
  - Potential industry sponsorships to fund distribution of copies at additional schools
- VET06 Methods for AST of Infrequently Isolated or Fastidious Bacteria Isolated From Animals
  - Ongoing discussion as to whether S/I/R interpretive categories should or should not be used
  - Challenge: Limited MIC data availability + variable quality of published data (methods, QC, etc.)
  - Currently compiling and seeking additional data for >30 genera

- Discussion on BMD method (+/- lysed horse blood) when testing *Pasteurella* spp. other than *P. multocida*
- Draft expected in winter 2025

- WG on Aquatic Animals (AWG)
  - Many ECVs presented and approved in January 2024 for inclusion in the next edition of VET04

Organism	MIC ECV	Zone Diameter ECV
Yersinia ruckeri		oxytetracycline, trimethoprim-sulfamethoxazole
Vibrio anguillarum	florfenicol, gentamicin, oxytetracycline, trimethoprim-sulfamethoxazole	enrofloxacin, florfenicol, gentamicin, oxolinic acid, oxytetracycline, trimethoprim-sulfamethoxazole
Vibrio cholerae (non-O1/ non-O139)		amikacin, amoxicillin-clavulanate, ampicillin, cefepime, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, erythromycin, florfenicol, gentamicin, imipenem, meropenem, nalidixic acid, norfloxacin, streptomycin, tetracycline, trimethoprim, trimethoprim-sulfamethoxazole
Vibrio harveyi	enrofloxacin, florfenicol, gentamicin, oxolinic acid, oxytetracycline, trimethoprim-sulfamethoxazole	

  - More to come in Fall 2024 for *Y. ruckeri,* multiple *Aeromonas* and *Edwardsiella* spp., and *Streptococcus iniae*

- Animal Health WG on Molecular AST
  - Formed December 2022
  - Initial goal: draft white paper on use of genotypic antiretroviral therapy (ART) in routine veterinary diagnostic medicine
    - J Am Vet Med Assoc. 2024 Jan 31;262(3):303-312. doi: 10.2460/javma.23.12.0687. PMID: 38295518.
  - In collaboration with VET09 WG, a new caution statement was added to each animal chapter of VET09:

> **CAUTION:** If a laboratory uses molecular methods (eg, PCR, whole-genome sequencing) to identify resistance genes, instead of using broth dilution, agar dilution, or disk diffusion AST methods as shown in Figure 5B, laboratory reports may look substantially different. The results should not be interpreted in the same manner as susceptible or resistant categorization based on dilution or disk diffusion AST methods (see Subchapter 2.5.2 for caveats to molecular methods).

  - Next task: review and adapt M100 Appendix H for veterinary application and inclusion in VET01S-Ed8

- WG on Education
  - Working with CLSI's marketing team to develop a series of short (~3 minute) videos
  - Current topics under consideration include:
    - CLSI Basics (what an MIC is, S/I/R definitions, etc.)
    - SDD Breakpoints and how to use them
    - Why labs cannot/do not perform AST (predictable "S", intrinsic "R", no approved method, etc.)
  - CLSI Vet AMR virtual training program in 2022-2023 had 42 participants from 12 Latin American countries

- Sponsored by USDA and the Inter-American Institute for Cooperation on Agriculture (IICA)
- Primary focus was small or limited-resource labs that perform food safety and surveillance testing
- 27 hours of recorded material, 11 asynchronous and 9 synchronous hours, covering 90 topics/questions
- Plans are being developed for a 2nd phase with in-person laboratory training

- Additional VAST January 2024 Educational Plenary Items
  - A plenary workshop was led by Dr. Virginia Fajt:
    - "Finding and Appraising Pharmacokinetic Data: Searching for and critically appraising pharmacokinetic (PK) data for use in the VAST SC Generic Drug Working Group and other analyses"
    - Why we care about the mathematical descriptions of antibiotic concentrations in blood and where those descriptions come from
    - How to find, access and extract those data
    - How to recognize useful and high quality PK data
  - A plenary presentation/discussion was led by Dr. Marilyn Martinez on behalf of the PK/PD WG:
    - "Factors influencing Protein Binding Estimation on Percent Target Attainment"
    - The importance and variability of protein binding was demonstrated, with data suggestive that pooled *in vitro* sampling may result in underestimation of PTA, particularly in relation to the fluoroquinolone class.
- "Improve AST" Collaborative Study Protocol
  - Joint project of the Institute of Microbiology and Epizootics - Freie Universität Berlin and the Federal Office for Consumer Protection and Food Safety, Berlin, Germany
  - Aims to develop:
    - MIC and ZD QC ranges for 6 antimicrobials licensed for treatment of bovine mastitis (amoxicillin, cloxacillin, cefapirin, cefquinome, oxytetracycline, tylosin)
    - bovine mastitis BPs for *E. coli, S. aureus, Staphylococcus* spp. other than *S. aureus* (SOSA) , *S. agalactiae, S. dysgalactiae, S. uberis*
  - Proposal brought to VAST for feedback on study design
  - Plenary discussion resulted in methodologies enabling both CLSI and EUCAST criteria to be met
  - Testing to begin during the second half of 2024

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

TIER 2 QC

RIFASUTENIZOL

- Background

Drug: Rifasutenizol	**Abbreviation (Glossary II & III)**: pending	**Previous ID**: TNP-2198
Solvent (Table 6A): DMSO	**Diluent (Table 6A):** DMSO	**Preparation (Table 6C combination agents)**: N/A
Route of administration (Glossary II): PO	**Class (Glossary I & II):** Rifamycin-nitroimidazole (drug conjugate)	**Subclass (Glossary I & II):** N/A
Study Report by: Element Iowa City (JMI Laboratories)	**Pharma Co**: TenNor Therapeutics	**Control Drugs**: Clarithromycin and Tetracycline

Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, etc): Not yet completed • **ISO/TS 16782 assessment of Tier 2 study materials**: Agar dilution only
Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):** No.
Discussion	Placement of QC range in Table 23D of CLSI M45 Testing against Helicobacter pylori ATCC 43504 by agar dilution using Mueller-Hinton Agar (MHA) media supplemented with 5% (v/v) aged (≥14 days) sheep blood Colony Counts: Average of all participating laboratories = 6.2×10^7 CFU/mL Range of all participating laboratories = 3.5×10^6 to 1.6×10^8 CFU/mL

- Proposed QC Ranges

Drug Name:	Rifasutenizol Table 23D of CLSI M45					Votes:	13/0/2/1 (For, Against, Absent, Abstain)			
QC Strain	Range	% In	Mode	Dil	Shoulder	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
Helicobacter pylori ATCC 43504	0.001-0.008	100%	0.004	4	44%@ 0.002	3@ 0.004	1@0.001 1@0.002 1@0.002 -0.004 4@0.004	0.002-0.008, 3, 85.7%	0.001-0.008, 4, 100%	No media variability. Lab variability. None @ 0.008, 14% @ 0.001

A motion to accept the rifasutenizol QC ranges for *Helicobacter pylori* ATCC 43504 (0.001-0.008 µg/mL) was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

ZOSURABALPIN

- Background

Drug: Zosurabalpin	**Abbreviation (Glossary II & III):**	**Previous ID**: RO7223280; RG6006
Solvent (Table 6A): sterile distilled water	**Diluent (Table 6A):** sterile distilled water	**Preparation (Table 6C combination agents)**: N/A
Route of administration (Glossary II): IV	**Class (Glossary I & II):** Peptide (tethered macrocyclic)	**Subclass (Glossary I & II):** N/A
Study Report by: Element Iowa City (JMI Laboratories)	**Pharma Co**: **F. F.** Hoffmann-La Roche, Ltd	**Control Drugs**: Meropenem

Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, etc): Completed (22-ROC-01 study) • **Equivalency of agar dilution to broth dilution:** In process. • **ISO/TS 16782 assessment of Tier 2 study materials**: Confirmed
Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):** No.
Discussion	Add footnotes Zosurabalpin MIC testing was conducted in cation-adjusted Mueller-Hinton broth supplemented with 20% heat-inactivated horse serum Add standard footnote that QC range was established with broth microdilution. Agar dilution equivalency has not been established Average colony counts: *A. baumannii* NCTC 13304 3.6 x 10^5

- Proposed QC Ranges

Drug Name:	Zosurabalpin	**Votes:**	13/0/2/1 (For, Against, Absent, Abstain)

QC Strain	Range	% In	Mode	Dil	Shoulder	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
Acinetobacter baumannii NCTC 13304	0.016 – 0.12	100%	0.03	4	72.5% @0.06	2@0.03, 1@0.06	4@0.03, 1@0.03-0.06, 3@0.06	0.016 – 0.12, 4, 100%	0.016 – 0.06, 3 100%	Some media and lab variability

A motion to accept the zosurabalpin QC ranges for *Acinetobacter baumannii* NCTC 13304 (0.016 – 0.12 µg/mL) with the following footnotes: 1) "Zosurabalpin MIC testing was conducted in cation-adjusted Mueller-Hinton broth supplemented with 20% heat-inactivated horse serum" and 2) the standard footnote that "QC range was established with broth microdilution. Agar dilution equivalency has not been established." was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

TIER 3 MIC QC

- No votes requested.
- Additional data requested/monitor
  - Aztreonam/avibactam data for *E. coli* ATCC 25922
  - Ceftriaxone and doxycycline data for *S. pneumoniae* ATCC 49619
  - Imipenem/relebactam data for *K. pneumoniae* ATCC BAA-1705
- Discussion and Decisions

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recommended	Concern/Analysis	Reported
K. pneumoniae BAA-1705	Imipenem/ relebactam	0.03/4-0.25/4	No change. Archive	Reports for out of QC high. Dec 2023: Added data from an additional lab, resulting in 6 total labs with Tier 3 data (3 labs reported data from multiple years for 12 total Tier 3 datasets + Tier 2). Only 2.2% out high @ ≥0.5/4 for all Tier 3 (n=1422 results). Shoulder @ 0.25/4 = 28%. June 2024: Added supplemental data provided by one lab; resulted in 2.4% out high @ ≥0.5/4 for all Tier 3 (n=1453 results). Shoulder @ 0.25/4 remained at 28%. Available data supports current range.	19-Jan
E. coli ATCC 25922	Aztreonam/ avibactam	0.03/4-0.12/4	QCWG to expand range to include 0.25/4 did not pass (5/8/3/0). Request additional data.	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 bringing Tier 3 data to n=2198, did not impact prior analysis.	21-Jun
S. pneumoniae ATCC 49619	Ceftriaxone	0.03-0.12	Request additional data	Signal reported from one lab that there may be an issue with MICs frequently observed at the upper end of the range. Data based on freeze-dried panels, need reference data to determine whether this is in fact a signal for the reference method. Dec 2023: data from 3 labs using reference method added, two show strong mode in middle of range with no appreciable shoulder and <1% out of QC high. June 2024: Added supplemental data provided by one lab bringing Tier 3 data to n=165, did not impact prior analysis.	22-Nov

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recommended	Concern/Analysis	Reported
K. pneumoniae BAA-1705	Imipenem	4-16	No new data provided for review. Request additional data.	Signal from recent Tier 2 study showed a mode at 16 (70% of total results) and out of QC results at 32 (6.7%). Dec 2023: Data from 2 additional labs added, resulting in data from 3 labs (n=318), all show similar results with a bimodal distribution or the mode at the high end of the range. Data supports expanding range to 4-32 but we are just only over the threshold of number of labs and results to make a decision (most results are from one lab).	23-Jan
K. pneumoniae BAA-2814	Imipenem	16-64	No new data provided for review. Request additional data.	Signal from Tier 2 study showed a mode at 64 (67% of total results) and out of QC results at 128 (24.8%). Dec 2023: Data from 1 additional lab added, resulting in data from 2 labs (n=656). Results from both labs are similar with the mode at the high end of the range and out of QC results high. Need data from more than 2 labs to take action.	23-Jan
S. pneumoniae ATCC 49619	Doxycycline	0.016-0.12	No new data provided for review (data provided was off-scale).	Signal from EDL 5 lab dried panel study where nearly 70% of results tested at 0.12, the high end of the range; requesting frozen reference method data to see if further monitoring or adjustment is warranted Jun 2024: no reference data submitted	23-Jun

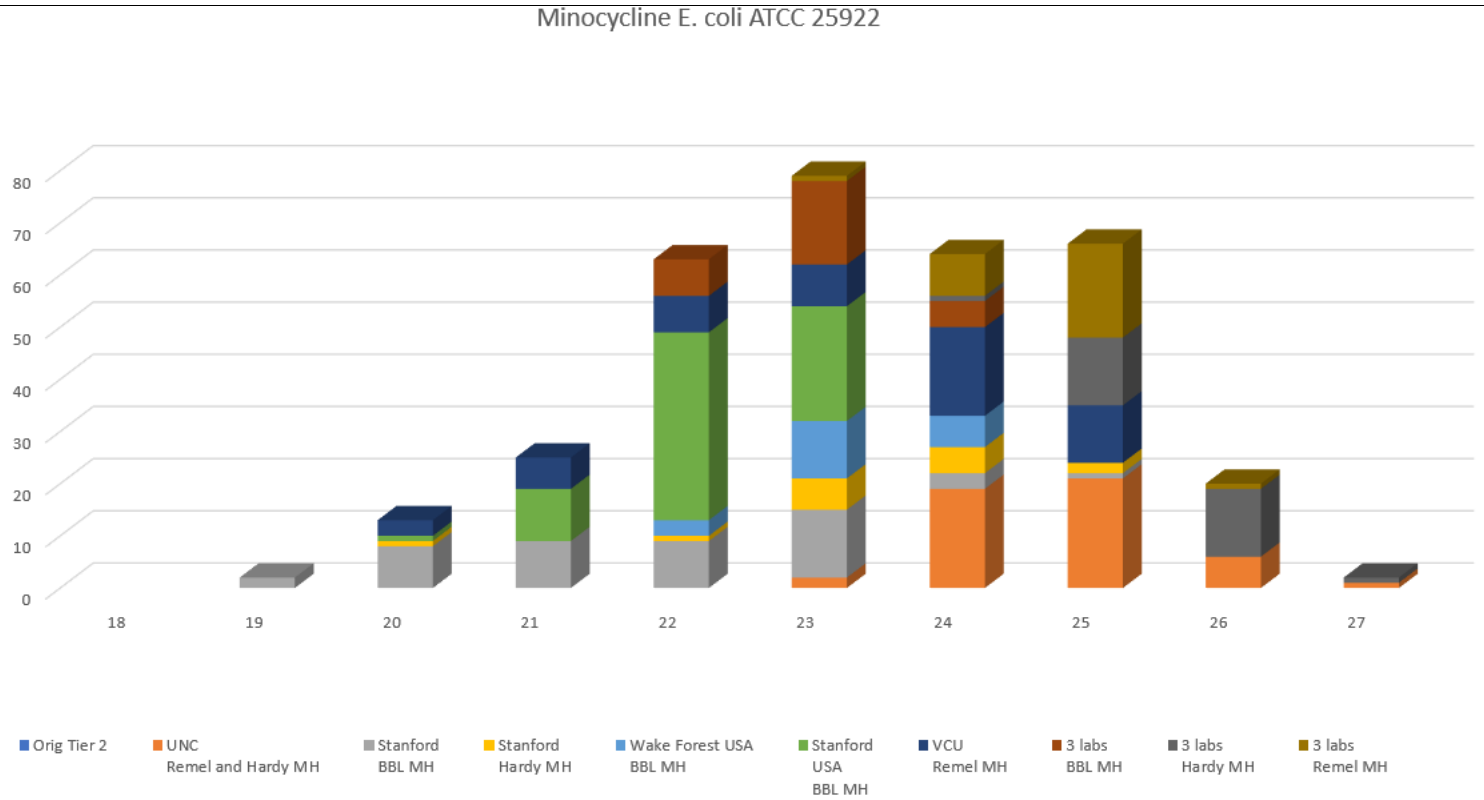
TIER 3 DISK DIFFUSION QC

- Additional data requested/monitor
  - Minocycline 30 µg for *E. coli* ATCC 25922
  - Spectinomycin 100 µg for *N. gonorrhoeae* ATCC 49226
  - Ceftibuten 30 µg for *E. coli* NCTC 13353
- Discussion and Decisions

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recmd	Concern	Update	Date Reported
E. coli ATCC 25922	Minocycline 30 µg	19-25	QCWG motion passed 13/0/3/0 Revise Minocycline disk range with E. coli ATCC 25922 to 20-26 mm.	Values at top of range and above range from one lab.	June 2024: Data from 3 labs with 2 disk manufact. and 3 media manufacturers added. Includes data generated by Stenotrophomonas AHWG 334 data points, 7% out of range.. Gavan statistics: 20-26 mm (99% within range) RangeFinder: 19-26 mm (99.5% within range) See next slide for chart	Jan-21
N. gonorrhoeae ATCC 49226	Spectinomycin 100 µg	23-29	Continue to monitor until June 2025. Request additional data.	QC study out high	June 2024: No additional data. June 2022: Observations in gentamicin QC study, especially with one lab and media	June-22
E. coli NCTC 13353	Ceftibuten 30 µg		Continue to monitor until January 2027. Request additional data.	Zone diameters in the lower part of range and out of range	June 2024: No additional data.	Jan-24

MINOCYCLINE *E. COLI* ATCC 25922

- Current range: 19-25mm, 7% out of range high
- Gavin: 20-26 mm, 99%
- RangeFinder: 19-26 mm, 99.5%
- 334 results from 5+ labs, 3 media manufacturers
- Media and lab variability



SC DISCUSSION (MAIN POINTS)

- Recognition that there is a media concern. Seeing repeated issues by media manufacturer.
- Problem with how to recognize the media differences when CLSI does not comment on specific media.
- There are things manufacturers can do. Could the Methods Development and Standardization Working Group help address why?
- The ISO standard has not kept up with current reality.
- When trying to set new disk correlates, it is important to have the QC correct first. The *Stenotrophomonas* disk diffusion breakpoints excluded data points that were out of QC, and QC range is expanded. The *Stenotrophomonas* disk diffusion breakpoints might have been different now that the QC range has changed.

A motion to accept the minocycline 30 µg disk diffusion QC ranges for *E. coli* ATCC 25922 (20 – 26 mm) was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

AST ROUTINE USER QC IMPROVEMENTS

- Background
  - Support from previous meetings to streamline CLSI QC recommendations.
    - High cost with increased number of QC strains, multiple AST methods used, etc.
    - Some bug/drugs provide little value; focus on key indicators to detect system failures.
    - Leverage existing IQCP process (individual labs define plan and document rationale). Almost 10 years experience with IQCP in US. Use to further streamline QC testing.
  - Survey from manufacturers and users confirmed high quality AST devices.
    - Very low frequency (≤0.4%) QC out of range. More often due to random errors/normal variability and issues with QC strains themselves. System issues are rare/infrequent.
  - Commercial AST manufacturers provide expected ranges for multiple strains but refer to CLSI guidelines for frequency and strain selection.
  - Actions for June 2024 meeting:
    - Finalize proposals (eg, Table 5F/4D revisions, guidance on key indicators, refer to IQCP process): Addressed in this presentation
    - Accrediting agency feedback: Confirmed support from CAP. Discussion with CMS pending
    - White paper/training: pending
- QC Testing Strategy Proposals – M100 35th Edition

M100 34th edition	Current Title	Proposed Title
Table 4C	Disk Diffusion QC Testing Frequency	Selection of QC Strains, Frequency of QC Testing, and Development of an IQCP
Table 5F	MIC QC Testing Frequency	Selection of QC Strains, Frequency of QC Testing, and Development of an IQCP

- Expand Tables 4C and 5F
  - Leverage IQCP to develop QC strategy
  - Lot/shipment QC testing with multiple QC strains to confirm quality
  - Routine QC testing with minimal QC strains throughout shelf life to detect deterioration.
- Refer to separate file with draft Tables 5F.
- Revise Tables 2 QC recommendation boxes
- Edit content throughout M100 where the proposed change would impact the instruction/comment
  - Daily or frequency determined by IQCP
- Use terminology:
  - QC strain
  - Strain for Routine QC (NOT routine QC strain)
  - Strain for Supplemental QC (NOT supplemental QC strain)

SC DISCUSSION (MAIN POINTS)

- Consensus of the discussion concluded that it is important to include this information in the M100-35th Edition.
- Texts and Tables Working Group can take this new table in 2-4 weeks, so it does not have to be done tomorrow.
- Need education sessions to explain concrete examples to labs.

- Action Item: The QC Working Group will continue to work on the table and examples with the goal of getting the information into this upcoming M100 edition.

TABLES 2 "QC BOX" CONTENTS

- Option 1: No changes proposed
  - Some support keeping QC strain information.
  - Others concerned it would be confusing.
  - Requires edits/corrections to update (previously presented, see back up slides)

Table 2A-1. Zone Diameter and MIC Breakpoints for Enterobacterales (excluding *Salmonella/Shigella*)

Testing Conditions	
Medium:	Disk diffusion: MHA Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix H)[1] Agar dilution: MHA
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [4])
Incubation:	35°C ± 2°C; ambient air Disk diffusion: 16–18 hours Dilution methods: 16–20 hours

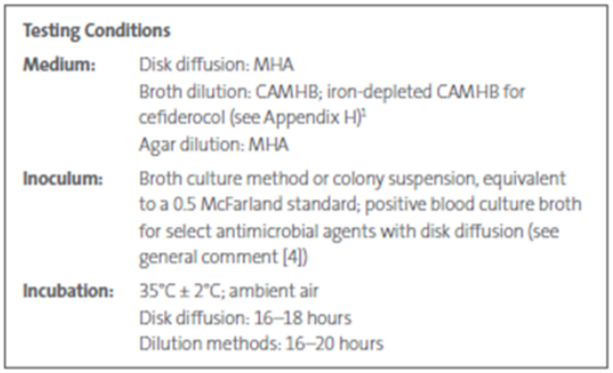
Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®[a] 25922
Pseudomonas aeruginosa ATCC® 27853 (for carbapenems)

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

- Option 2: Revisions made below
  - Remove reference to specific strains. Refer to QC tables for ranges.
  - Refer to new tables for QC strain and frequency recommendations.
  - Refine statement about commercial tests to avoid redundant/conflicting comments.
  - WG Vote: 13-0-3-0.



Testing Conditions

Medium:	Disk diffusion: MHA Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix H)[3] Agar dilution: MHA
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [4])
Incubation:	35°C ± 2°C; ambient air Disk diffusion: 16–18 hours Dilution methods: 16–20 hours

QC Recommendations
Refer to QC Tables for acceptable QC ranges applicable for each method (Tables 4A-1, 4A-2, 5A-1, 5A-2)

Refer to Table 4Dx (Disk Diffusion) and 5Fx (MIC) to develop a QC testing plan (e.g., QC strains, frequency) to ensure quality of materials for a new lot, upon receipt of a new shipment and routine testing throughout the shelf life.

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC ~~test recommendations~~ strains and QC ranges.

SC DISCUSSION (MAIN POINTS)

- Consider if future commercial companies might state QC has to be done daily, then labs need to follow the instructions for use.
- STMA is trying to provide education to companies to not be too prescriptive in the instruction for use around QC to allow labs to do an IQCP with QC at a frequency that fits their needs.

A motion to endorse the general direction the QC Working Group is going with the streamlined QC and to accept the following revisions to the Tables 2 QC Box: 1) Remove the reference to specific strains, 2) Refer to QC tables for ranges, 3) Refer to new tables for QC strain and frequency recommendations, 4) Refine statement about commercial tests to avoid redundant/conflicting comments was made and seconded. Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

TABLES 3 QC INSTRUCTIONS

- Table 3A (ESBL)
  - "Either strain, *K. pneumoniae* ATCC® 700603 or E. coli ATCC® 35212, may then be used for routine QC (eg, weekly or daily, or as determined by IQCP)."
- Table 3B (CarbaNP)
  - "Test positive and negative QC strains and uninoculated reagent control tubes each day of testing or as determined by IQCP."
- Table 3C (mCIM)
  - "In addition, perform QC of meropenem disks and test media daily or weekly or as determined by IQCP following the routine disk diffusion QC procedure, and handle disks as described in CLSI M02."
- Table 3D (ceftazidime/avibactam)
  - "QC of the method must be performed with every new lot or shipment of reagents to ensure the accuracy of results." (also see fn a that is lifted from M02 for daily or weekly)."
- Table 4C (Disk Diffusion Reference Guide to QC Frequency)

- “This table summarizes the suggested QC frequency when modifications are made to antimicrobial susceptibility test systems (refer to CLSI EP23™). It applies only to antimicrobial agents for which satisfactory results have been obtained with either the 15-replicate (3 × 5-day) plan or 20 or 30 consecutive test-day plan or as determined by IQCP. Otherwise, QC is required each test day.”

- Table 3G. Tests for Detecting B-lactamase production in *Staphylococcus* spp.
  - Footnotes:

c. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see CLSI M02[3] and CLSI M07[4])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

e. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

f. QC recommendations – supplemental

- Supplemental QC strains can be used to assess a new test, for training personnel, and for competence assessment. It is not necessary to include supplemental QC strains in routine daily or weekly AST QC programs. See Appendix C, which describes use of QC strains.

APPENDIXES (3G, 3H, 31, 3J, 3K, 3L, ETC.)

- QC recommendations - routine
- QC recommendations – lot/shipment
- QC recommendations - supplemental
- For lot/shipment - test negative and positive QC strains
- Routine - test only positive QC strain
- Appendix C. QC Strains for Antimicrobial Susceptibility Tests

NOTE 1: ~~Routine~~ QC strains recommended for routine QC ~~listed in Tables 2A through 2J (in "Routine QC Recommendations" boxes at the top of each page)~~ are tested regularly (ie, daily or weekly or as determined by IQCP) to ensure the test system is working and produces results that fall within specified ranges listed in CLSI M100. The ~~routine~~ QC strains recommended in this document should be included if a laboratory performs CLSI ~~reference~~ disk diffusion or MIC testing as described herein. For commercial test systems, manufacturer's recommendations should be followed for all QC procedures. ~~Other~~ Some QC strains are used to assess particular characteristics of a test or test system in select situations ~~or may represent alternative QC strains.~~ For example, *H. influenzae* ATCC® 10211 is more fastidious than *H. influenzae* ATCC® 49247 or *H. influenzae* ATCC® 49766 and is used to ensure HTM can adequately support the growth of patient isolates of *H. influenzae* and *H. parainfluenzae*. QC strains may possess susceptibility or resistance characteristics specific for one or more special tests listed in CLSI M02 and CLSI M07. It may be useful to include QC strains beyond those that are tested routinely ~~They can~~ be ~~used~~ to assess a new test, for training new personnel, and for competence assessment~~, and it is not necessary to include them in routine daily or weekly AST QC programs.~~

- Use terminology:
  - QC strain
  - Strain for Routine QC (NOT routine QC strain)
  - Strain for Supplemental QC (NOT supplemental QC strain

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

JOINT WORKING GROUP DOCUMENTS

- M23S (June 2020); M23S 2nd Edition (completed June 2024; publish January 2025)
  - Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria
- M23S2 (July 2021); Revision ongoing in Edaptive platform
  - Process to Submit Disk Content (Potency) Data for Joint CLSI-EUCAST Working Group Review and Approval
- M23S3 (June 2023); Consider expanding to CAMHB
  - 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
- All available with other CLSI AST free documents here: https://clsi.org/all-free-resources/ (also on EUCAST's website as SOPs 11.0, 12.0 and 13.0)

DISK CONTENT SELECTION IN PROGRESS

WG Assigned Study #	Agent	Sponsor	Notes
JWG-2022-5	Aztreonam-nacubactam (1:1) and Cefepime-nacubactam (1:1)	Meiji	Phase 2 planned for summer 2024
JWG-2022-9	Zoliflodacin	Nobelex	Phase 2 completed; will begin analysis
JWG-2023-1	BWC0977	Evopoint Biosciences [a]	Phase 1 completed
JWG-2023-1	Piperacillin-tazobactam (reassessment)	CLSI/EUCAST	Evaluating (µg) 100/10 (CLSI); 30/6 (EUCAST); 20/5 and ??? Seeking funding
JWG-2024-1	GDC0829	Genentech	Phase 1 in progress
JWG-2024-2	Ceftibuten-xeruborbactam	Qpex Biopharma	Phase 1 starting
JWG-2024-3	Meropenem-ANT3310	Antabio	Phase 1 complete

[a] formerly Sinovent Pharmaceuticals

MUELLER HINTON AGAR EVALUATIONS IN PROGRESS

WG Assigned Study #	Agent	Sponsor	Notes
JWG-2022-6	Debio 1452	Debiopharm	Still investigating disks

PRE QC OF MUELLER HINTON AGAR

- Aim
  - To confirm that the 3 Mueller Hinton agar (MHA) sources selected are acceptable prior to performance of a full QC study to avoid problems when establishing QC ranges.
  - The testing procedure is designed to minimize factors (eg, inoculum, incubation, measuring zones) other than the MHA source that might affect the results.
  - Occurs after disk content is selected, prior to establishing disk diffusion QC ranges
- M23S3 Pre-QC of MHA

Parameter	Criteria
Test strains	• QC strains representing target organisms • At least two strains with elevated MICs and reduced zone diameters
Disks	• At least 2 manufacturers • Same lots to be used in the QC study, if possible
MHA	• MHA that meets the specifications in ISO+TS+16782-2016 and with QC results within CLSI and EUCAST ranges • Strain-indicator agent combinations (listed in M23S3) can be tested (should be tested for MHA if the user has done limited testing with the media) • At least four manufacturers (two lots from one manufacturer can be used, if necessary)
Testing procedure	• Test strains in triplicate • One inoculum suspension for all MHA manufacturers • Two test disks and control disk on the same plate • Zone diameters are read by one reader

  - Strain-Indicator Agent Combinations
    - Test each QC strain-antimicrobial combination at least once for each MHA source.
    - If the reason for unacceptable performance of MHA cannot be justified, it is suggested that an alternative source/lot of MHA be used.
  - Data Analysis
    - Calculate per disk-organism combination
      - Mean value

- Standard deviation
- Range of zone diameters (smallest to largest)

- Optimally, select 3 MHA sources that demonstrate mean zone diameters within ± 1 mm for test agent.
  - If mean or median zone diameters are not within ± 1 mm for three media, perform three additional tests using the same disk-organism-media combinations.

PRE-QC OF MUELLER HINTON BROTH CONSIDERATIONS

- FDA requirements for media for AST
- Discussed previously to consider:
  - Generate a Mueller Hinton broth (MHB) Table equivalent to Table A1 in M23S3 provided for MHA.
  - Variability in AST results from manufacturer to manufacturer is greater than lot to lot variability from the same manufacturer.
- CLSI M23 6th Edition (2023)
  - Sponsors with new agents are asked to provide data to include effect of various components on MIC of new agent.
    - Stability
    - Inoculum
    - Reading
    - Incubation time and temperature
    - Cations, zinc, or surfactants
- ISO/TS 16782 includes
  - MHB Requirements
  - Minimum Organism-Drug Combinations (likely to detect problems with medium)
  - Effects of dehydrated MHB
- Next Steps
  - Draft language/procedure to address evaluation of MHB sources prior to use in M23 MIC QC studies
    - Refer to ISO 16782 only?
    - Add to M23S3?
    - Add to M23?
  - Consider requesting assurance that MIC data presented to CLSI and EUCAST were obtained using media meeting ISO 16782 specifications

SC DISCUSSION (MAIN POINTS)

- Are the ranges that are listed in the ISO document the same as CLSI QC ranges?
  - CLSI is constantly updating the QC ranges.
  - It is an issue as the ISO document has not been updated in a long time.
  - There are also issues with disk diffusion because the disk mass is different between EUCAST and CLSI in some cases.
- How should CLSI be thinking about pre-qualification of MHA before including it in re-evaluation of disk diffusion breakpoints? CLSI needs to be encouraging the revision of the ISO document.
- Need to communicate media variability to users. CLSI needs to be more proactive in understanding the media differences.
- Need to prioritize disk diffusion breakpoint re-evaluation because the disk breakpoints do not seem to be matching up with MIC methods in a recent comparison.

- Consider publishing the QC modes to help users have a more specific target with their QC.
- Do manufacturers indicate conformity for pre-poured media?
    - Yes, but the problem is that ISO standard maybe not up to date.

QC RANGES FOR Β-LACTAMASE/Β-LACTAMASE INHIBITOR COMBINATIONS

- CLSI and EUCAST QC Ranges

CLSI	EUCAST
• Test ONE QC strain: B-lactamase producer • Example: Piperacillin-tazobactam Test… E. coli ATCC 35218 • No QC range "target or mode" published	• Test TWO QC strains: - B-lactamase producer - check inhibitor - Susceptible strain - check active drug • Use "target" value within QC range as additional QC check • Example: Piperacillin-tazobactam Test… - E. coli ATCC 35218 or K. pneumoniae ATCC 700603 - E. coli ATCC 25922 or P. aeruginosa ATCC 27853 • QC range "Target" published

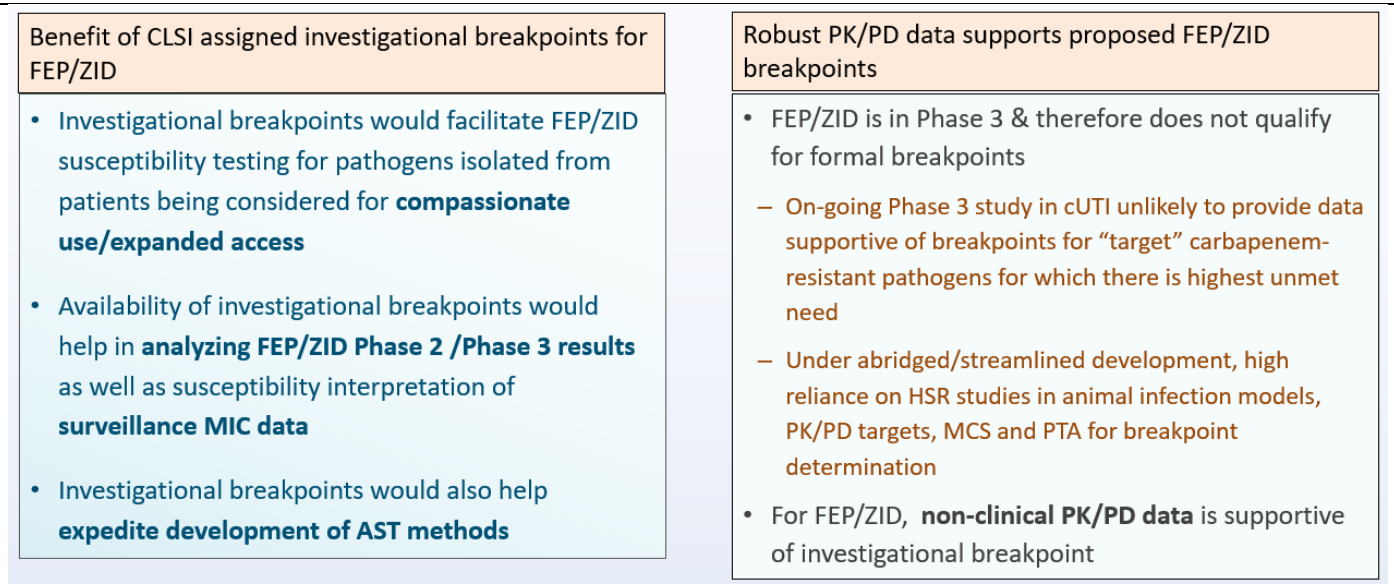
- Next Steps
    - Try to harmonize
    - There are differences between CLSI and EUCAST QC recommendations; for EUCAST:
        - Target
        - For BL-BLI combos test β-lactamase producing strain and susceptible strain
    - New BL-BLI agents - CLSI now only publishing ranges for β-lactamase producing QC strains, not susceptible QC strains
        - Value of testing non-β-lactamase producer?
    - Prepare a list of where there are differences between QC range for BL-BLI combination and parent drug alone for susceptible strains, where scientifically they should be the same

READING GUIDES

- CLSI Quick Guides 2024 for disk diffusion (M02-ED14-QG) and BMD (M07-ED12-QC)
- Differences were presented between the CLSI and EUCAST reading guides
- Next Steps

	○ Try to harmonize ○ If harmonizing QC and other testing, it is imperative to harmonize reading recommendations ○ Prepare a list of where there are differences ▪ Some real differences ▪ Sometimes instructions expressed differently **SC DISCUSSION (MAIN POINTS)** • CLSI looked at fosfomycin and determined a different interpretation than EUCAST, so the WG needs to consider during harmonization. It may be worthwhile for these cases, for CLSI to have a different reading guide. VRE might also be another example of intentional differences.

8.	**BREAKPOINTS WORKING GROUP PART 1 (N. NARAYANAN AND M. SATLIN)** **INVESTIGATIONAL BREAKPOINTS FOR CEFEPIME-ZIDEBACTAM** • M23 6th Edition (2023) Investigational Breakpoint Information **investigational breakpoint** – a breakpoint for an antimicrobial agent in development and for which there is no regulatory approval. This breakpoint is based on relevant microbiological and pharmacokinetic/pharmacodynamic data and is not published in CLSI documents. **4.3 Investigational Breakpoints** For antimicrobial agents that are in development (ie, for which registration has not yet occurred for any indication), information on zone diameter and MIC relationships, distributions of MICs for organisms relevant to the intended clinical uses, and PK/PD indices may be submitted to the relevant CLSI subcommittee and to any relevant CLSI working group (eg, the CLSI Working Group on AST Breakpoints) co-chairholders at any time. The relevant CLSI subcommittee can then assist in the selection of "investigational" breakpoints to be used by clinical investigators during clinical trials that assess efficacy. Investigational breakpoints that are not yet approved by a regulatory agency are not published in CLSI documents. If an antimicrobial agent has regulatory agency approval for some organisms but the approval does not include a specific organism, it may be published in CLSI documents as investigational for that specific organism. • Rationale for seeking investigational breakpoints for cefepime-zidebactam (FEP/ZID)



- Sponsor (Wockhardt) proposed investigational interpretative criteria for FEP/ZID and rationale

Organism/ organism group	MIC (mg/L)		
	Susceptible*	Intermediate	Resistant
A. baumannii	≤ 64/64	-	-
P. aeruginosa	≤ 64/64	-	-
Enterobacterales	≤ 64/64	-	-
FEP/ZID MICs determined in 1:1 ratio of FEP and ZID			

*Susceptible-only criteria (isolates with FEP/ZID MICs >64 mg/L are globally rare)

Rationale for identified FEP/ZID breakpoints

- ≥99% PTA of FEP and ZID **PK/PD targets**
- ≥1 $\log_{10}$-kill elicited by FEP/ZID **HSR** in neutropenic mice thigh or lung infection models
- Global **MIC distribution** for "target" carbapenem-resistant pathogens extending to far right (MICs 8 to 64 mg/L)
- **U.S. FDA** agreed to employ PK/PD breakpoint of ≤64 mg/L for *A. baumannii*, *P. aeruginosa* & Enterobacterales in Phase 3 studies of FEP/ZID

- FDA's acceptance for proposed FEP/ZID breakpoint to use in Phase 3 studies
  - An end-of-Phase 2 meeting was held with US FDA to consult on Phase 3 study plans
  - As part of this meeting, the Sponsor submitted data on PK/PD targets and their PTA
  - FDA accepted use of ≤64 mg/L as breakpoint (for Enterobacterales, *P. aeruginosa* and *A. baumannii*) in Phase 3 studies
- Summary of MIC distribution studies
  - Sizable number of isolates at the right end with MICs spanning across 4-64 mg/L, more so, in high-resistance countries
  - MIC distribution of isolates collected during the compassionate use program invariably on the right end (MICs up to 64 mg/L) – indicator of "unmet need in the real-world"
  - Various surveillance studies showed that at the PK/PD breakpoint of 64 mg/L, >90% of carbapenem-resistant *A. baumannii*, *P. aeruginosa* and Enterobacterales were inhibited
  - FEP/ZID's antibacterial spectrum includes
    - Carbapenem-resistant (KPC and/or OXA-48-like and/or MBL –producing and with non-enzymatic resistance mechanisms) Enterobacterales
    - Carbapenem-resistant *P. aeruginosa* (Hyper-efflux, Porin loss, Hyper- Amp C, MBLs, KPC-producing)
    - Carbapenem-resistant *A. baumannii* (OXA-carbapenemase-producing and with non-enzymatic resistance mechanisms)
- Probability of target attainment of FEP and ZID for *A. baumannii*
  - FEP or ZID coverage in terms of FEP/ZID MICs by applying PTA-derived FEP or ZID concentrations
  - Both the constituents jointly cover MIC of 128 mg/L which is for the patients with normal renal function, however, for other renal groups, a lower FEP/ZID MIC of 64 mg/L is covered jointly – proposed PK/PD breakpoint

	Sub-MIC providing best-fit exposure-response	T>sub-MIC duration
FEP	% $\underline{f}$ FEP T >FEP/ZID 1/4x MIC	28.4
ZID	% $\underline{f}$ ZID T >FEP/ZID 1/64x MIC	30.7

These attainments based on T>sub-MIC

→ Rounded to 30%: ≥99% PTA up to 32 mg/L

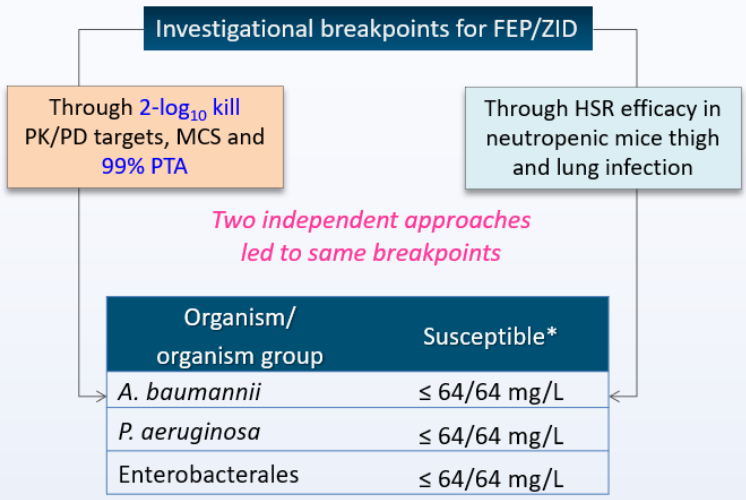
→ Rounded to 30%: ≥99% PTA up to 8 mg/L

	Sub-MIC providing best-fit exposure-response	Concentration with ≥99% PTA	In terms of FEP/ZID MIC (mg/L)
FEP	% $\underline{f}$ FEP T >FEP/ZID 1/4x MIC	32	32 x sub-MIC factor = 32 x 4 = 128
ZID	% $\underline{f}$ ZID T >FEP/ZID 1/64x MIC	8	8 x sub-MIC factor = 8 x 64 = 512

- Key aspects of FEP and ZID PK/PD targets and their PTA
  - PD-driving FEP or ZID sub-MICs are related to minimum elongation concentration (MECs) and minimum spheroplast-forming concentration (MSCs), however always not same
  - PD-driving sub-MICs are variable within pathogen group and between pathogen groups
    - Strain specific extent of partial PBP saturation varies depending on the resistance mechanisms
  - PD drivers are sub-MICs: 1/2x to 1/64x MICs (except 1X MIC for low FEP/ZID MIC Enterobacterales)
  - Probability of 1x MIC being a PD driver is associated only with strains exhibiting lowest FEP/ZID MIC (≤ 1 mg/L) – no risk for false susceptibility
  - In the face of usual strain to strain variability in PK/PD targets for all antibiotics, PTA is assessed for:
    - co-modelled target for each pathogen group (normalizes the variability) - an approach commonly used for all antibiotics eliminating bias from most stringent as well as most liberal targets
    - most conservative targets in each pathogen group
  - As PTA of FEP or ZID targets assessed for ≥99% PTA, joint PTA will be 98%
- Summary and outcome of *in vivo* human-stimulated regimens (HSR) studies
  - For *A. baumannii*, *P. aeruginosa* and Enterobacterales, translational kill of 1-log10 achieved up to an MIC of 64 mg/L
  - Thus, *in vivo* HSR studies confirmed the PK/PD breakpoints identified through PTA of *in vivo* PK/PD targets

S. no.	Carbapenem-resistant organism group	CAIRD/Sponsor	Lung/thigh	Plasma/ELF HSR	FEP/ZID MIC range (mg/L) of the isolates tested	Highest MIC with kill
1	*A. baumannii*	CAIRD	Lung	Plasma HSR	16 - 64	64
2		CAIRD	Thigh	Plasma HSR	16 - 64	64
3		Sponsor	Lung	ELF HSR	8 - 64	64
4	*P. aeruginosa*	CAIRD	Lung	ELF HSR	4 - 32	32
5		CAIRD	Thigh	Plasma HSR	4 - 32	32
6		Sponsor	Lung	ELF HSR	16 - 256	64
7		Sponsor	Thigh	Plasma HSR	16 - 32	32
8	*Enterobacterales*	CAIRD	Lung	ELF HSR	1 - 4	4
9		CAIRD	Lung	Plasma HSR	1 - 4	4
10		Sponsor	Lung	ELF HSR	4 - 64	64
11		Sponsor	Thigh	Plasma HSR	16 - 64	64

- Profile of patients treated with FEP/ZID under compassionate use was presented.
- PK/PD studies reveal the therapeutic scope of FEP/ZID in managing MDR/XDR Gram-negative infections
    - In most registrational trials, patients with carbapenem-resistant infections are not adequately represented, in such trials MICs of investigational antibiotics for trial isolates are generally low
    - However, real world use of novel antibiotic such as FEP/ZID will be for patients with serious CR-GNB infections and FEP/ZID MICs for such causative pathogens would be relatively higher, however within the therapeutic scope
    - Breakpoints should enable the use of novel antibiotics for treating the real world patients
    - In that context, for FEP/ZID, preclinical infection model data based PK/PD analyses are supportive of breakpoints commensurate to its therapeutic scope involving treatment of serious CR-GNB infections
- Summary
    - ACBN = *A. baumannii*, PSA: *P. aeruginosa* ENT: Enterobacterales



Organism/ organism group	Susceptible*
A. baumannii	≤ 64/64 mg/L
P. aeruginosa	≤ 64/64 mg/L
Enterobacterales	≤ 64/64 mg/L

FEP/ZID MIC (mg/L)	No of strains with ≥1 log_{10} kill in lung and thigh out of tested		
	ACBN	PSA	ENT
2	-	-	5/5
4	-	7/7	2/2
8	4/4	11/11	-
16	5/5	18/18	4/4
32	16/16	Cumulative: 20/16* CAIRD: 7/3**	2/2
64	9/9	2/1***	1/1

*includes 3 CAIRD isolates with -0.73, -0.77, -0.91 kill;
** 4 isolates that did not show 1 log kill: Stasis: 3, Growth: 1
*** 1 isolate that did not show 1 log kill: with 0.67 log kill

- Investigational breakpoints are supported by
  - ≥99% PTA of 1.5 to 2-log10 linked FEP and ZID targets employing CR-GNBs
  - Robust 1 to 3 log10 kill of CR-GNBs by FEP/ZID HSR in neutropenic murine thigh and lung infection models
  - MIC distribution of CR-GNBs extending to the far right
- FEP/ZID Ad Hoc Working Group Report
  - Summary of discussions
    - Novel mechanism of action: "enhancer effect" with multiple PBP inhibition
    - MIC is what we can measure, but is only a proxy
      - *In vitro*:
        - Minimum elongation concentration (MEC)
        - Minimum spheroplast-forming concentration (MSC)
        - Subinhibitory-MIC bactericidal concentrations in the presence of the combination
      - PK/PD:
        - Free drug time above "sub-inhibitory MIC"
  - High MIC Reproducibility
  - New QC organism required
  - PK/PD Modeling
    - PD driving sub-MICs are strain dependent
      - Clinical microbiology lab cannot practically determine a particular isolate's sub-MIC
    - Several approaches were taken to mitigate this:

- Co-modeling of several strains with a range of sub-MICs
- Robust PK/PD targets – 99% PTA for 2-log10 kill
- Target of one constituent determined in presence of sub-clinical exposure of other

▪ R^2 range was wider with more variation for *P. aeruginosa*
- AHWG asked for PTA of target from individual strains: 99% PTA for all except for one showing 90% PTA (for 1 log kill target)

▪ AHWG asked about correlations of 8-hour MICs (to capture 'early killing' effect) and correlation with PK/PD and sub-MICs
- Sponsor has looked at this data, and sub-MIC correlates better with PK/PD parameters

○ Animal Data
▪ > 1-$\log_{10}$ kill of *A. baumannii* up to 64 µg/mL in neutropenic mouse thigh model and lung infection model
▪ > 1-$\log_{10}$ kill of Enterobacterales up to 64 µg/mL in neutropenic mouse thigh model and lung infection model
▪ Some concerns with failure to achieve 1-$\log_{10}$ kill of *P. aeruginosa* in a neutropenic mouse lung infection model
- Single study, different (lower) HSR dosing was used

▪ Summary
- At least 1-$\log_{10}$ kill in *A. baumannii* and Enterobacterales
- Failure to achieve 1-$\log_{10}$ kill of *P. aeruginosa* in a single study neutropenic mouse lung infection model
  - Lower doses of FEP/ZID were used
  - *P. aeruginosa* isolates also with higher ZID MICs (32-64 mg/L)
- Data from neutropenic thigh model with *P. aeruginosa*
  - 18/21 strains with at least 1-$\log_{10}$ kill
  - 3/21 with stasis
  - For isolates included in both lung and thigh studies results were similar

○ Compassionate Use Data
▪ Range of organisms, majority *P. aeruginosa*
- FEP/ZID MIC range 1-64 mg/L

▪ Clinical improvement noted for all patients

○ Further Data
▪ Clinical outcomes from Phase 3 trials
- Bacterial isolates will be collected, MEC/MSC data would be helpful, especially from isolates with higher MICs
- For clinical failures, it will be important to evaluate if this correlates with elevated MEC/MSC, or higher sub-MICs (especially for MICs 32-64 mg/L)

▪ Additional testing modalities
- MIC may not be the "best" proxy
- Automated phenotypic or microscopy-based systems may provide more accurate correlate of the sub-MIC, however large-scale adoption challenging
- Mechanism for surveillance of isolates for "sub-MIC creep" (ie, increases in sub-MIC without corresponding increases in MIC)

○ AHWG Discussion and Recommendation
▪ MIC testing reproducible
▪ For MDR isolates, MIC90
- *A. baumannii*: 32-64 mg/L

- *P. aeruginosa*: 8-16 mg/L
- Enterobacterales: 2-4 mg/L

- Do not want to be so stringent that we limit drug where it could provide benefit
- Investigational breakpoint, it can be changed based on further data
- Uncertainty in the "sub-MIC" for a particular strain
  - 1/2x may be different than 1/32x
- MICs in the 32-64 mg/L common for *A. baumannii*
  - ZID MSCs lower 0.25-0.5 mg/L
  - Sub-MICs seem to be ~1/32x
- MICs for *P. aeruginosa* lower, but
  - ZID MSC trend higher in *P. aeruginosa* (4-8 mg/L)
  - More variation in Sub-MIC (some 1/2x to 1/4x)
  - Strains 32-64 mg/L with less killing in one animal model
- Considered an intermediate range for *P. aeruginosa*
  - Susceptible ≤ 16 mg/L
  - Intermediate 32-64 mg/L
- Concern there could be a resistance mechanism not identified by the MIC method for isolates with MICs of 32- 64 mg/L to explain outcome variability in one animal study
- Thought it was best to be more inclusive with the breakpoint to ensure the data is collected and evaluated in the clinical trial, rather than prematurely exclude MICs that largely look to have activity. Animal model outcome variability may be due to lower dosing used in one study.
- Motion to accept the sponsor's proposed investigational breakpoint of a FEP/ZID susceptible only criteria of ≤64/64 mg/L for *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and the Enterobacterales. AHWG Vote: 6-0-0-0.
- The AHWG specifically acknowledges this is an investigational breakpoint, with an area of uncertainty in the range of 32-64 mg/L, especially with *Pseudomonas aeruginosa*. Additional data from ongoing trials will be important in determining if a lower breakpoint may be appropriate in the future.

- BPWG Discussion and Recommendation
  - PK modeling developed with healthy volunteers, may not translate to infected patients
  - Standard β-lactam PK/PD parameter instead of novel metric
  - Timing and need of investigational breakpoints
    - Need for drug in India (compassionate use and phase 2 CR pathogen study)
  - Going forward, measuring MSC and MEC or only MIC
    - Plan to accumulate higher MIC isolates over course of phase 3 trial and continue to correlate parameters
  - Approval based on sub-MICs and concerns about higher MICs (32-64 mg/L)
  - Marginal differences in R^2 values for decisions on what PK/PD parameter to move forward (%fT>MIC vs %fT>sub-MIC)
  - Discussion on examination of data (points completely unexplained by MIC) in addition to R^2 value
  - Fixed ZID concentrations?
  - Motion to accept the AHWG recommendation for FEP/ZID susceptible only criteria of ≤64/64 mg/L for *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and the Enterobacterales. WG Vote: 6-3-1-2. Against vote reasons:

- Not sure ready and maybe do not need investigational yet
- Concerns about modeling data

SC DISCUSSION (MAIN POINTS)

- The most recent version of M23 published in 2023 states that investigational breakpoints will no longer be published in the M100. This change was implemented due to lessons learned from cefiderocol over the years.
- What is the value to the sponsor to set investigational breakpoints with CLSI?
  - This will expand testing access to the phase II and III clinical trials and compassionate use protocol.
  - India, where the clinical trials are taking place, uses CLSI guidelines.
  - The FDA rules stated it is too early to determine a susceptibility breakpoint, but it seemed acceptable to use the proposed FEP/ZID susceptibility breakpoint of 64 µg/mL for the proposed phase 3 clinical trial.
- M23 WG thought that the M100 document should be for drugs with adequate clinical data to support a breakpoint, not for investigational drugs.
- CLSI currently has drugs with investigational breakpoints listed in the M100 such as teicoplanin. Note that teicoplanin is not used in the USA, but it is in other regions, and this is meant to be an international document.
- CLSI needs to clean up the M100 for the currently investigational breakpoints, such as pefloxacin.
- Investigational breakpoints are for drugs not approved by a regulatory agency.
- The issue with the existing data on FEP/ZID is that there is not much data on negative controls because the drug worked so well across all the isolates at the HSR used in the study. It would be good to demonstrate places where the drug fails, and one way to do that would be to test lower doses. If the MSC and MEC relationship is maintained, should see efficacy of the drug drop off at lower doses.
- For *Acinetobacter*, it seems like the cefepime exposure is what is driving the MIC. Whereas with *Pseudomonas* when the zidebactam MIC is varied, the cefepime did not really change and it is the zidebactam; activity is seen once the zidebactam hit it. Should we consider a fixed does cefepime and varying zidebactam for some of these organisms? Different parameters are diving activity for different organisms. In the HSR data, zidebactam alone has activity for *Pseudomonas*, but not *Acinetobacter* and the Enterobacterales.
- Consider testing MICs in different conditions (different ratios and fixed concentrations) then take the curves to the model and fit to the different MICs.
  - The sponsor did not show the data, but when exposures fall below the MSC or the MEC there is failure of the drug. MICs >64 are rare, so they could not build a model based on that.
- CLSI needs to better define the criteria for investigational breakpoints.
- CLSI recommends that the sponsor looks at the MEC, MSC, and MIC for as many isolates as possible in the clinical trials.
  - The sponsor does plan to estimate the MSC and MEC of the isolates that will come to the clinical trials, especially those in the range of 8 to 64. They also believe that, based on the data that the MIC is a reliable indicator of susceptibility. Whenever MICs cross 64, there is an increase in the MSC as well. So, they think it is unlikely to see false resistance.
- The R^2 numbers are not actually that far apart.
- CLSI should consider putting this in as an investigational drug, then clean up the M100 for 2026. CLSI should consider writing a white paper or rationale document to help the sponsor.
- PK/PD targets were determined in BHI, which is more variable than MHB, so that is confusing.
  - There was only a minimal shift in MICs between BHI and MHB.
- Caution against using MIC or MEC, if you look at graphs showing MIC in relationship between MEC and MIC, the elongation concentration, correlates closely with the IC50 for binding PBP3, which is what cefepime is doing. IC50s usually run just slightly lower than MICs. If you look at the MSC, the

	spherical concentration is quite a bit lower than the MIC if you have a compound that binds to PBP2, so what we are seeing here is PBP2 and PBP3 binding, which is not easily tested in the clinical lab. It is a good investigational tool, but MES and MSC would be hard for labs to measure.
	A motion to accept the investigational breakpoints for cefepime/zidebactam (≤64/64 µg/mL) for Enterobacterales, *A. baumannii*, and *P. aeruginosa* to be published in the M100 35th Edition and add a comment stating, "Relevant microbiologic and pharmacokinetic pharmacodynamic data and clinical data are not yet available. The use of this breakpoint is for consideration investigational for clinical studies." the was made and seconded. Vote: 6 for, 7 against, 1 abstain, 0 absent (Fail)
	Against Vote Reasoning: • Should not contradict CLSI M23. • The data look acceptable for Enterobacterales, but there is less data for the other bacteria. Should vote on each organism group separately. • These results will drive patient testing, and there is concern over the method. • There is no satisfaction around how CLSI plans to handle. **SC DISCUSSION (MAIN POINTS)** • Consider if investigational breakpoints should go in a separate table • The reproducibility data looks good, but it has been analyzed across all organisms. Most of the data is with the Enterobacterales, not *A. baumannii*, and *P. aeruginosa*; so, that is something to consider if decision to vote on the organism groups individually. • CLSI will address how to clean up the existing investigational breakpoints in January 2025. • Consider adding to Micro Free online. CLSI will draft a document to outline these investigational breakpoints. • The general concept of the following will be conveyed in the document: relevant microbiologic and pharmacokinetic pharmacodynamic data and clinical data are not yet available. The use of this breakpoint is for consideration investigational for clinical studies.
	A motion to accept the investigational breakpoints for cefepime/zidebactam (≤64/64 µg/mL) for Enterobacterales, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* to be published in a separate document in the CLSI free resources was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)
9.	**ADJOURNMENT** Dr. Lewis thanked the participants for their attention. The meeting was adjourned at 12:00 PM Central (US) time.

2024 JUNE AST MEETING SUMMARY MINUTES PLENARY 2: Monday, 24 June 2024 (In-person) 1:00 PM – 5:30 PM Central Standard (US) Time	
#	**Description**
1.	**OPENING** Dr. Lewis opened the meeting at 1:00 PM Central Standard (US) time.

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

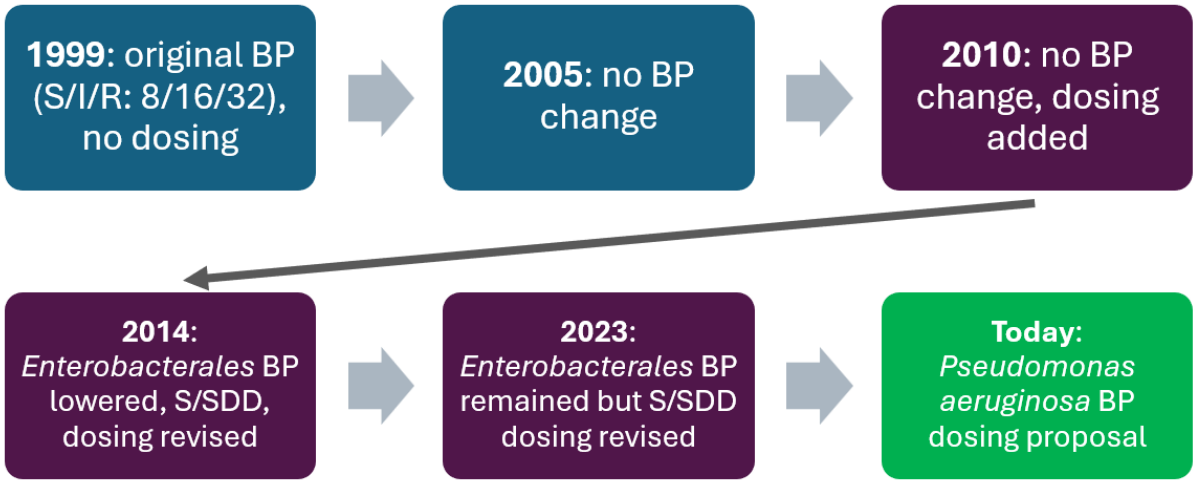
CEFEPIME BREAKPOINT DOSAGE COMMENT FOR *PSEUDOMONAS AERUGINOSA*

- Objective: Re-evaluate the breakpoint dosage comment for cefepime and *Pseudomonas aeruginosa*
- Current *P. aeruginosa* Breakpoint/Dosage

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
Cefepime	30 µg	≥ 18	15-17^	≤ 14	≤ 8	16^	≥ 32

Cefepime	1 g IV q 8 h or 2 g IV q 12 h

- Brief History of Cefepime Breakpoints



- Cefepime Enterobacterales MIC Breakpoints (mg/L)

Organization (year)	Susceptible	Intermediate		Resistant
CLSI (1999)	≤8	16		≥32
CLSI (2014)	**≤2** **1g Q12h**	**4[a]** **1g Q8h or 2g Q12h**	**8[a]** **2g Q8h**	**≥16** **-**
EUCAST (2023)	≤1 1g Q8h or 2g Q12h	2-4[b] 2g Q8h		≥8 -
FDA (2023)	≤2 -	4-8 2g Q8h		≥16 -
USCAST (2021)	≤2	4-8[b]		≥16

[a]SDD, susceptible dose-dependent
[b]SIE, susceptible increased exposure

- Cefepime Enterobacterales in M100-34th Edition

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
Cefepime	30 µg	≥ 25	19-24	-	≤ 18	≤ 2	4-8	-	≥ 16

Cefepime	S: 1 g IV q 8 h or 2 g IV q 12 h SDD: 2 g IV q 8 h over 3 h

- Cefepime Enterobacterales PK/PD
  - Cefepime-specific %fT>MIC thresholds differ between pre-clinical and clinical outcomes studies
  - Cefepime PTA is impacted primarily by %fT>MIC threshold, renal function, and dosing regimen
  - 1g Q12h over 0.5h does not achieve >90% PTA against all susceptible MICs (≤2 mg/L)
  - 2g Q12h or 1g Q8h over 0.5h achieve >90% PTA against all susceptible MICs (≤2 mg/L)
  - 2g Q8h is necessary to achieve >90% PTA against SDD MICs (4-8 mg/L)
    - Extended infusion over 3-4h necessary to ensure adequate PTA at 8 mg/L

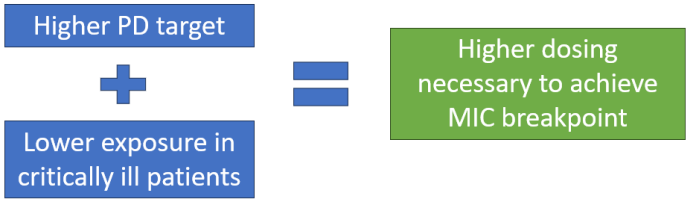
Summary of PK/PD Studies Investigating FEP Achievable MIC Targets for ≥90% PTA

Dosing regimen	%*f*T>MIC target	Infusion duration (h)	Maximum achievable MIC (µg/mL)
1g Q12h	50	0.5	1
	65		4
	100		1
	65	3	4
	100		2
1g Q8h	50	0.5	2, 8
	60		4
	65		8
	70		2, 4
	100		4
	70	2	2
	65	3	8
	100		4
	70	4	4
2g Q12h	50	0.5	2, 8
	60		0.25, 1, 4
	65		8
	70		2, 2, 2
	100		2
	60	3	0.5
	65		8
	100		4
	60	4	2

Summary of PK/PD Studies Investigating FEP Achievable MIC Targets for ≥90% PTA

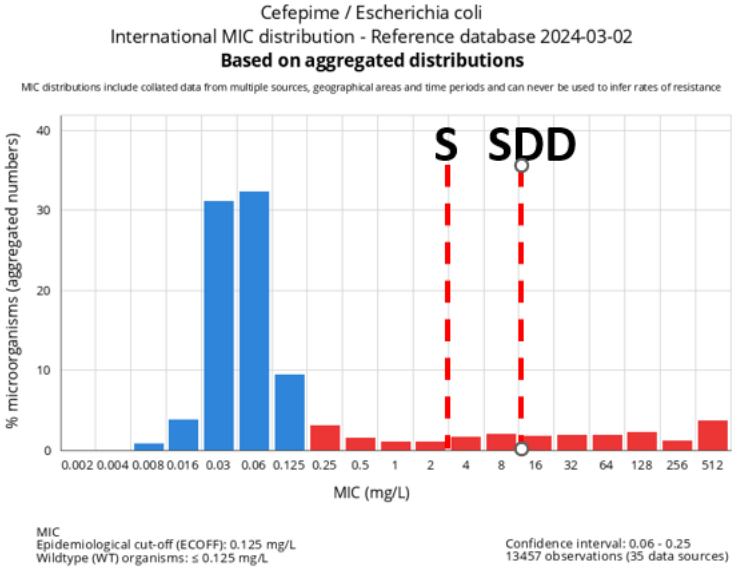
Dosing regimen	%*f*T>MIC target	Infusion duration (h)	Maximum achievable MIC (µg/mL)
2g Q8h	50	0.5	4, 8
	60		1, 4, 8
	65		16
	70		4, 8, 8
	100		2, 8
	60	2	4
	70		4
	50	3	8, 8, 8
	60		4, 8, 8
	65		16
	70		8
	100		8, 8
	60	4	8
	70		8

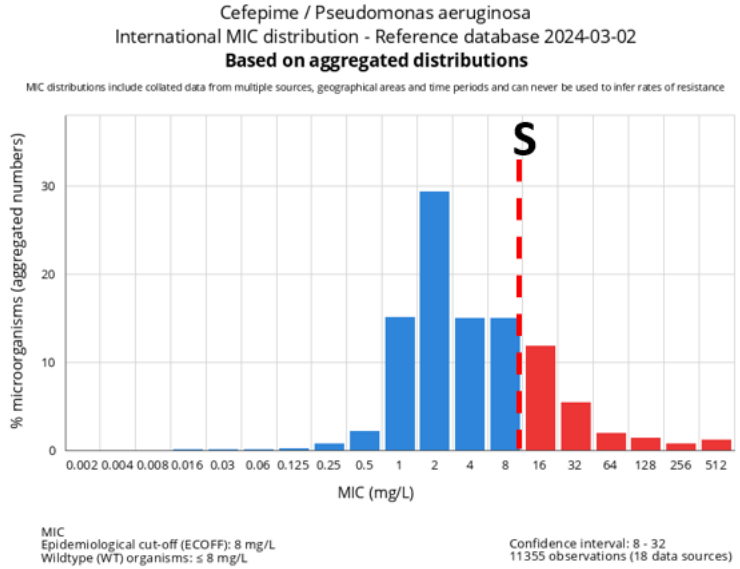
- Why does 2010 dosage (Enterobacterales and *P. aeruginosa*) and 2023 dosage (Enterobacterales) differ?
  - Since 2010, more data suggesting a clinical PD target (60-70% fT>MIC)
    - 2010 PD target = 50% fT>MIC
    - 2023 PD target = 60-70% fT>MIC
  - PK model used for Dudley et al (CID 2013) PK/PD simulations was based on healthy volunteer study (≠ PK in critically ill patients)



- Cefepime and *P. aeruginosa*

- Most extrapolated or aligned with Enterobacterales
- Crandon et al. (Hartford group), AAC 2010: clinical PD
  - Non-UTI *P. aeruginosa* infections
  - Microbiological failure was associated with a fT > MIC of ≤60%
  - Doses of at least 2 g every 8 h are required to achieve this target
- Baur et al. (Ohio State), AAC 2013: clinical PD/outcome
  - Bacteremia or pneumonia
  - 2g q8h: 30-min vs. 4-h infusion
  - Lower mortality in extended infusion group (20% versus 3%; p = 0.03)





- For *P. aeruginosa*, many more isolates are at the far end of the WT distribution -> clinically, high dose extended infusion dosing for cefepime is likely more necessary on average for *P. aeruginosa* vs. Enterobacterales
- FDA Breakpoints

	Minimum Inhibitory Concentrations (mcg/mL)				Disk Diffusion (zone diameter in mm)			
Pathogen	S	SDD	I	R	S	SDD	I	R
Enterobacterales[a]	M100 standard is recognized				M100 standard is recognized			
Pseudomonas aeruginosa [b]	≤8	-	-	≥16	≥18	-	-	≤17

S = Susceptible; SDD= susceptible-dose dependent; I = Intermediate; R = Resistant

[a] For isolates of Enterobacterales with SDD susceptibility, use a dose of 2g every 8 hours in patients with normal renal function.

[b] For *Pseudomonas aeruginosa*, use a dose of 2 g IV every 8 hours in patients with normal renal function.

- EUCAST Breakpoints (v14.0)

Cephalosporins	MIC breakpoints (mg/L)		
	S ≤	R >	ATU
Cefaclor	-	-	
Cefadroxil	-	-	
Cefalexin	-	-	
Cefazolin	-	-	
Cefepime	0.001	8	

An arbitrary "off scale" breakpoint which categorises wild-type organisms as "Susceptible, increased exposure (I)".

Cephalosporins	Standard dosage	High dosage	Special situations
Cefepime	1 g x 3 iv or 2 g x 2 iv	2 g x 3 iv	**Severe *P. aeruginosa* infections:** 2 g x 3 with extended 4-hour infusion

- CLSI Cefepime Breakpoints and Dosage

Year	*Enterobacterales*		*P. aeruginosa*	
	Breakpoint (µg/mL)	**Dosage**	**Breakpoint (µg/mL)**	**Dosage**
2010	S ≤8	1g q8h or 2g q12h	S ≤8	1g q8h or 2g q12h
2014	S ≤2 SDD 4 SDD 8	1g q12h 1g q8h or 2g q12h 2g q8h		
2024	S ≤2 SDD 4-8	1g q8h or 2g q12h 2g q8h over 3h		

- BPWG Discussion and Recommendation
  - Questions on consideration for S vs. SDD
  - Agreement with aligning with FDA
  - Question on precedence of two different doses for cefepime for different breakpoints (dose for S and Enterobacterales)
  - Motion to revise the *P. aeruginosa* susceptible cefepime dosage to 2g IV q8h over 3 hrs. WG Vote: 8-0-0-4.

SC DISCUSSION (MAIN POINTS)

- With the current dosing, cannot get to the MIC breakpoint. Need to let users know where/how the breakpoint was set.
- Altered mental status for cefepime is a concern.
- FDA STIC and CLSI *P. aeruginosa* cefepime breakpoints are not aligned because FDA does not have an intermediate range.
  - Response from the FDA: The FDA was just made aware of the issue and are going to go back and look at this.
- For non-clinical and clinical PK/PD, there is some data that cefepime PK/PD targets could be lowered.

A motion to accept the susceptible cefepime dosage for *P. aeruginosa* as 2g IV q8h over 3 hrs was made and seconded. Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

PENCILLIN RESISTANT GROUP B *STREPTOCOCCUS*

- Background
  - In May 2024, the WHO updated WHO Bacterial Priority Pathogens List. These are bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance.
  - The 2024 BPPL includes 15 families of antibiotic resistant (ABR) pathogens, grouped into critical, high and medium categories of priority for R&D and for public health measures.
  - The WHO Advisory Group on the BPPL-2024 gathered 20 experts from all continents, as well as the WHO and their regional offices representatives.

- Summary of Changes
  - Critical group: Carbapenem resistant *P. aeruginosa* was moved to high group. *Mycobacterium tuberculosis* was included in the list.
  - High group: *S. aureus* vancomycin intermediate and resistant phenotypes were removed. *H. pylori* was removed.
  - Medium group: *S. pneumoniae* penicillin –non susceptible was changed by macrolide-resistant. Group A Streptococci macrolide resistant was included. Penicillin-resistant Group B Streptococci was included.
- Concerns
  - From a public health perspective, this priority list update, and especially penicillin R group B streptococci will put the pressure on surveillance systems and reference labs. Some will ask medical institutions to report these isolates when they appear, but many clinical labs are not testing routinely for penicillin or ampicillin.
  - Furthermore, some institutions are not performing accurate ID (eg, MALDI-TOF or PCR) and are using mostly automation for AST (with variable performance).
  - Interpreting results like "nonsusceptible" is conflicting. It may be caused by real resistance mechanisms (eg, PBP mutations), misidentification, or AST errors.
  - Should penicillin "nonsusceptible" Group B streptococci isolates be considered as penicillin "resistant" for public health purposes?
- Proposal: German Esparza will check for *in vitro* and *in vivo* (case reports) data to look for bacterial species, methods used, clinical outcomes, PK/PD data, if available, to bring to the January 2025 Breakpoints Working Group meeting.

SC DISCUSSION (MAIN POINTS)

- Consensus of the Subcommittee endorses this endeavor. No vote taken.
- It would be good to get public health involved.
- If there is a lower breakpoint that is not clinically relevant, it may be helpful to call out MIC creep.

TRIMETHOPRIM-SULFAMETHOXAZOLE BREAKPOINTS FOR Β-HEMOLYTIC STREPTOCOCCI

- Current Status
  - No CLSI or FDA susceptibility breakpoints for β-hemolytic streptococci and trimethoprim-sulfamethoxazole (TMP-SMX)
  - There is a misconception that TMP-SMX is not active against *Streptococcus pyogenes*
  - This leads to physicians combining TMP-SMX with a β-lactam agent for empiric treatment of skin and soft tissue infections for coverage of β-hemolytic streptococci and MRSA (maybe only TMP-SMX is needed)
  - EUCAST has TMP-SMX breakpoints (for "*Streptococcus* groups A, B, C, and G")
    - S: ≤1 µg/mL
    - I: 2 µg/mL
    - R: ≥4 µg/mL
- Background
  - Initial reports of high rates of resistance of *S. pyogenes* to sulfa antibiotics were likely related to excess thymidine in culture media that provided the organism with a salvage pathway to make nucleic acids and survive outside of the typical folate pathway
  - Since 2006, thymidine content of MHA has become strictly regulated to maintain a low level of thymidine (CLSI M6-A2 protocol)
  - Contemporary studies using thymidine-controlled MHA show high susceptibility rates of *S. pyogenes* to TMP-SMX, according to EUCAST breakpoints
  - TMP-SMX works for *S. pyogenes* skin infections

- Current recommended testing conditions for ß-hemolytic streptococci in M100

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).

  - LHB (lysed horse blood) has thymidine phosphorylase which decreases thymidine content
  - Sheep blood does not have thymidine phosphorylase
- Why are there no FDA breakpoints for TMP-SMX and ß-hemolytic streptococci?

Rx only

BACTRIM™
sulfamethoxazole and trimethoprim DS
(double strength) tablets and tablets USP

Streptococcal Infections and Rheumatic Fever

The sulfonamides should not be used for treatment of group A β-hemolytic streptococcal infections. In an established infection, they will not eradicate the streptococcus and, therefore, will not prevent sequelae such as rheumatic fever.

- BPWG Discussion and Recommendation
  - May be a clinical need for this breakpoint
  - Concern about thymidine levels in tissue and lack of clinical data for TMP-SMX for invasive infections or pharyngitis due to ß-hemolytic streptococci
  - Prior data suggesting increased risk of recurrence with streptococcal pharyngitis treated with sulfa derivatives
  - Resistance more prevalent in other areas of the world -> breakpoints would allow detection the emergence of resistance here
  - There may be a QC strain that would allow for the evaluation of thymidine content in media
  - Motion to form an ad hoc working to develop susceptibility breakpoints for TMP-SMX and ß-hemolytic streptococci. WG Vote: 9-1-0-2

SC DISCUSSION (MAIN POINTS)

- 1970's clinical data with relapses in Group A pharyngitis is listed in the pediatric Red Book. Should relook at that data.
- Sheep blood, which is the disk method, does not have thymine phosphorylase. Need to think about what to do for a disk method.

- Amoxicillin is used for pharyngitis, but TMP-SMX is used all the time for other infections.
- Is this for just *S. pyogenes* or all β-hemolytic streptococci?
    - Most of the data is in *S. pyogenes*, but the AHWG would investigate this more.

A motion to form an ad hoc working group on trimethoprim-sulfamethoxazole breakpoints for β-hemolytic streptococci was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

CARBAPENEM RESISTANT ENTEROBACTERALES AND CARBAPENEM TESTING

- CLSI Jan 2024 meeting: Motion and Voting Results
    - Breakpoint Working Group motion: "Enterobacterales isolates that are not susceptible to ertapenem, imipenem, or meropenem (except for *Proteus*/*Providencia*/*Morganella* [PPM] that are only resistant to imipenem) should undergo testing to detect and differentiate the most common carbapenemases".
        - Passed BPWG: 11 (Yes), 0 (No)
    - AST Subcommittee motion: "Carbapenemase testing is recommended for Enterobacterales isolates that are resistant to at least one carbapenem—ertapenem, imipenem, or meropenem (except for PPM only resistant to imipenem) with a footnote that *Enterobacter cloacae* resistant to ertapenem but not other carbapenems may be resistant by other mechanisms"
        - Vote: Yes (8), No (6)
        - Reasons for No vote
            - Need to work out practicality and language—agree with direction
            - Missing the points on carbapenemase type and clinical implication
            - Concerns about testing burden on lab with inclusion of isolates such as *E. cloacae* that are resistant to ertapenem, but not other carbapenems
- Improving proposal for this meeting
    - Reviewed additional data on the frequency of carbapenemase detection in ertapenem-mono-resistant Enterobacterales isolates, in aggregate and by species:
        - Data from CDC:
            - Emerging Infections Program (EIP)
            - Antimicrobial Resistant Laboratory Network (AR Lab Network)
        - Data from IHMA
    - Clarify the type of carbapenemase test recommended and the purpose of the testing
- Rationale: Knowing the carbapenemase type could improve clinical care
    - Differences in *in vivo* responses in neutropenic mouse models for cefepime and meropenem-vaborbactam depending on the presence and type of carbapenemase -> comments added in CLSI M100 Tables 2
        - "Enterobacterales that harbor OXA-48-family enzymes may test susceptible to meropenem-vaborbactam but may not respond to this therapy *in vivo*. If OXA-48 is detected, suppress or report as resistant"
        - "Cefepime S/SDD results should be suppressed or reported as R for isolates that demonstrate carbapenemase production".
    - New β-lactam/β-lactamase inhibitor agents are developed for specific types of carbapenemase-producing CRE
    - Carbapenemase testing is now cheaper and easier, with both lateral flow and PCR
- Sensitivity

Want a CRE definition that doesn't **miss** isolates with a carbapenemase

↓

What % of carbapenemase-producing isolates would be missed with different CRE definitions?

↓

Need a dataset that evaluates for carbapenemases in both CRE and carbapenem-susceptible isolates

- SENTRY Data to determine the % of carbapenemase-producing isolates that test susceptible, intermediate, or resistant to carbapenems
  - Whole-genome sequencing of the following Enterobacterales isolates (global collection):
    - MIC values ≥2 µg/mL for at least 2 of: ceftazidime, ceftriaxone, aztreonam, or cefepime OR
    - MIC value ≥2 µg/mL for imipenem or meropenem
  - Limitations:
    - Not all Enterobacterales underwent carbapenemase testing
    - Ertapenem AST not always performed for urinary isolates

Organisms	Ertapenem			Imipenem			Meropenem		
KPC	S	I	R	S	I	R	S	I	R
All Enterobacterales (n=2409; 1272-erta)	1.4%	1.1%	97.5%	1.7%	3.3%	95.0%	4.2%	5.8%	90.0%
Klebsiella pneumoniae (n=1915; 1029-erta)	0.9%	0.5%	98.6%	1.3%	1.2%	97.6%	2.0%	3.4%	94.6%
Enterobacter cloacae (n=183; 91-erta)	1.1%	1.1%	97.8%	4.4%	8.2%	87.4%	9.3%	13.7%	77.0%
Escherichia coli (n=80; 45-erta)	8.9%	11.1%	80.0%	7.5%	20.0%	72.5%	21.2%	21.2%	57.5%
Serratia marcescens (68; 31-erta)	0.0%	0.0%	100.0%	0.0%	1.5%	98.5%	2.9%	7.4%	89.7%
Klebsiella oxytoca (n=60; 30-erta)	0.0%	0.0%	100.0%	0.0%	8.3%	91.7%	11.7%	16.7%	71.7%
OXA-48-like									
All Enterobacterales (n=861; 590-erta)	2.9%	2.7%	94.4%	8.2%	20.3%	71.4%	26.7%	9.3%	64.0%

Data courtesy of Mariana Castanheira: Presented at Jan 2024 CLSI Meeting

- SENTRY data: Carbapenem susceptibilities of Enterobacterales with MBL genes: Global

Organisms	Ertapenem			Imipenem			Meropenem		
NDM	S	I	R	S	I	R	S	I	R
All Enterobacterales (n=714; 439-erta)	0.5%	0.7%	98.9%	1.1%	1.0%	97.9%	1.8%	1.0%	97.2%
Klebsiella pneumoniae (n=485; 297-erta)	0.7%	0.0%	99.3%	1.6%	0.4%	97.9%	2.1%	0.6%	97.3%
Enterobacter cloacae (n=90; 58-erta)	0.0%	0.0%	100.0%	0.0%	2.2%	97.8%	2.2%	4.4%	93.3%
Escherichia coli (n=85; 50-erta)	0.0%	0.0%	100.0%	0.0%	2.4%	97.6%	0.0%	0.0%	100.0%
VIM									
All Enterobacterales (n=214; 128-erta)	27.3%	11.7%	60.9%	1.4%	7.5%	91.1%	26.2%	17.8%	56.1%
Klebsiella pneumoniae (n=79; 58-erta)	13.8%	12.1%	74.1%	1.3%	1.3%	97.5%	15.2%	20.3%	64.6%
IMP									
All Enterobacterales (n=80; 44-erta)	4.5%	11.4%	84.1%	11.2%	18.8%	70.0%	15.0%	8.8%	76.2%

- "Sensitivity" of different CRE definitions for detection of carbapenemase-producing Enterobacterales (CPE)
  - Conclusions:
    - Ertapenem resistance misses 5% of CPE, but misses many VIM and IMP
    - Meropenem resistance misses 18% of CPE, 10% of KPC, and 42% of OXA-48
    - Resistance to any carbapenem misses <6% of all carbapenemase types

Only JMI isolates that underwent testing to ertapenem, imipenem, AND meropenem ≥95% <90%

	KPC only (n=1255)	NDM only (n=367)	OXA-48 only (n=528)	VIM only (n=117)	IMP only (n=41)	Any of big 5 CP, including multiple (n=2395)
Ertapenem-R	97.5%	98.7%	93.8%	57.3%	82.9%	94.7%
Meropenem-R	89.7%	97.3%	57.8%	47.9%	68.3%	81.7%
Erta-R, imi-R, AND mero-R (ALL 3)	89.1%	96.6%	54.7%	43.6%	56.1%	80.2%
Erta-R, imi-R, OR mero-R (ANY of 3)*--current CDC defn	98.1%	99.2%	94.5%	94.3%	95.1%	97.3%
Ertapenem-R OR meropenem-R (for labs that don't test imipenem)	97.7%	99.2%	93.8%	66.7%	85.4%	95.5%

*Excluding *Proteus, Providencia, Morganella*

- Alternate approach: Set meropenem MIC value below resistant breakpoint to not miss CP-CRE
  - Using a definition of meropenem MIC ≥1 still misses 27% of OXA-48s

- A definition of meropenem MIC ≥0.25 misses <5% of KPC, NDM, and OXA-48
- However, this definition may not be practical because many laboratories use automated panels that do not have dilutions this low or may use disk diffusion

% of isolates with a carbapenemase missed by meropenem MIC value					
Meropenem MIC value (µg/mL)	**≥ 4 (R)**	**≥ 2 (I or R)**	**≥ 1**	**≥ 0.5**	**≥ 0.25**
KPC (n=2411)	10.0%	4.2%	1.7%	0.9%	0.8%
NDM (n=617)	6.3%	2.8%	1.8%	1.3%	1.1%
OXA-48-like (n=861)	42.0%	36.0%	26.7%	13.6%	4.2%
VIM (n=214)	61.2%	43.9%	26.2%	10.3%	3.3%
IMP (n=80)	46.2%	23.8%	15.0%	11.2%	2.5%

- Specificity

Want a CRE definition that doesn't lead to unnecessary carbapenemase testing of isolates that are unlikely to have a carbapenemase -> don't want to overburden clinical labs

↓

What % of CRE isolates, using different definitions, have a carbapenemase?

↓

Need a dataset that performs carbapenemase testing on all CRE isolates, including ertapenem-mono-resistant strains

↓

4 datasets:
1) CRACKLE-2; 2) CDC EIP; 3) IHMA; 4) CDC AR Lab Network

  - CRACKLE 2: % of CRE isolates with carbapenemases
    - CRACKLE-2 is an ARLG-funded prospective cohort of patients with CDC-defined CRE isolates between 2016-2019
    - WGS performed on 136/213 (64%) of ertapenem-mono-resistant CRE isolates and 442/643 (69%) of multi-carbapenem-resistant CRE isolates
    - Results:
      - Ertapenem-mono-resistant (n=136, 24%): 12% with a CP gene
      - Multi-carbapenem-resistant (n=442, 76%): 68% with a CP gene
  - Updated data from CDC's Emerging Infections Program (EIP)
    - 2,244 CRE (by reference BMD) isolates from multi-site EIP program from 2016-2023
    - All isolates were also considered CRE by local lab

- PCR screening for big 5 carbapenemases
- Results:
  - 1355 (61%) were ertapenem mono-R isolates
  - 10.6% of ertapenem mono-R isolates with a CP

- IHMA: % of CRE isolates with a carbapenemase in USA
  - IHMA surveillance program of 400 CRE isolates from USA from 2018-2022
  - AST by BMD
  - Molecular characterization by a random sampling of 332 (83%) isolates
  - Results:
    - 146 (44% were ertapenem mono-R isolates)
    - 6.8% of ertapenem mono-R isolates with a CP
- IHMA: % of CRE isolates with a carbapenemase in globally much higher
  - IHMA surveillance program of 10,380 global CRE isolates from 2018-2022
  - AST by BMD
  - Molecular characterization by a random sampling of 9,767 (90%) isolates
  - Results:
    - 1987 (20%) were ertapenem mono-R isolates
    - 32.3% of ertapenem mono-R isolates with a CP
- % of ertapenem-mono-resistant isolates that harbor a carbapenemase: Summary of data from 4 different sources of USA isolates

	CRACKLE-2	CDC's EIP	IHMA	CDC's AR Lab Network (biased towards CP)
Overall	11.8% (16/136)	10.6% (143/2244)	6.8% (10/146)	19.3% (1438/7466)
Klebsiella pneumoniae		19.7% (46/234)	23.1% (3/13)	28.0% (381/1359)
Escherichia coli		17.9% (46/257)	13.6% (3/22)	27.0% (296/1096)
Enterobacter cloacae		5.5% (41/747)	5.8% (3/22)	13.2% (467/3532)
Klebsiella aerogenes		2.1% (2/96)	0% (0/12)	4.1% (16/393)

- Conclusions from data re: optimal CRE definition to recommend carbapenemase testing
  - Current CRE definition (resistant to any carbapenem) captures >94% of all the big 5 carbapenemase types

        - Relying on meropenem resistance only will miss 10% of KPCs and >40% of OXA-48s (and ½ of VIMs and 1/3 of IMPs)
    - Approximately ½ of CRE isolates are ertapenem-mono-resistant in USA
        - Approximately ½ of ertapenem-mono-resistant isolates are *Enterobacter cloacae*
    - 7-12% of ertapenem-mono-resistant isolates have a carbapenemase
        - This % is higher globally, suggesting that if US epidemiology shifts to be more reflective of global epidemiology, this proportion will increase
        - % depends on species
            - *E. coli* and *K. pneumoniae*: approximately 20%
            - *Enterobacter cloacae*: 5-6%
            - *Klebsiella aerogenes*: 2%
- CLSI M100 ED34: Table 2A-1. Enterobacterales/carbapenems

Current comment:

Institutional treatment guidelines, infection prevention procedures, or epidemiological investigations may necessitate identification of carbapenemase-producing Enterobacterales. Isolates with elevated carbapenem MICs (intermediate or resistant) can be tested for carbapenemase production by a phenotypic and/or a molecular assay (refer to Tables 3B and 3C for methods). See Appendix G, Table G3 regarding suggestions for reporting when mechanism of resistance-based testing (molecular and phenotypic methods) is discordant with phenotypic AST.

Proposed revised comment:

Isolates resistant to ertapenem, imipenem, or meropenem (except *Proteus* spp., *Providencia* spp., or *Morganella* spp. only resistant to imipenem) should undergo carbapenemase testing to identify the presence of particular carbapenemases (e.g., KPC, NDM, OXA-48, VIM, IMP). These results are important for treatment decisions, and may inform infection control procedures and/or epidemiologic investigations. Depending on local epidemiology and resources, laboratories may consider omitting carbapenemase testing for *Enterobacter cloacae* complex and *Klebsiella aerogenes* isolates that are only resistant to ertapenem, because carbapenemases are 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.

- BPWG Discussion and Recommendation
    - Current tests do not detect emerging carbapenemases that are not in the “big 5” (eg, SME in *Serratia marcescens*) -> do we want to recommend that labs do additional phenotypic testing if suspect carbapenemase-producing CRE and testing for big 5 negative?
    - Although the big 5 carbapenemases are most common now, others could emerge
    - Concern that definition could be ertapenem-resistant OR meropenem-resistant in reality, since many labs do not test imipenem
    - Important to provide guidance to labs of what to do with results
    - Motion to accept the proposed revised Table 2A-1 comment with an additional comment that there may be additional carbapenemases and phenotypic testing may be warranted. WG Vote: 9-0-0-1.

SC DISCUSSION (MAIN POINTS)

- Concern for saying that *E. cloacae* are less likely to have a carbapenemase when worldwide that is not necessarily true.
- Concern for including ertapenem resistance as an inclusion criteria for carbapenemase testing.
- Consider changing the wording from "carbapenemase testing" to "test to identify carbapenemases" or "detection differentiation".
- Many urine panels only have ertapenem.
- What about coordination with ASP and based on local epidemiology?
- Consider removing "may inform". Use stronger language to inform infection control and epidemiology.
- Concern about the less sophisticated labs. Worried about the burden this puts on laboratories.
- It is wrong to discourage labs from performing a phenotypic carbapenemase test because a new carbapenemase or a SME would be missed if moved directly to an assay that looks for a limited number of known carbapenemases.
- Reference labs need a clinical indication, if CLSI says it is important clinically then all labs can figure it out because testing is easier than an mCIM. Is there a need to specify timing? It is too slow to send it to a reference laboratory.
- It is easy for labs to do the lateral flow assay.
- Is there a need to tell labs to test all three carbapenems? Is there need to give guidance that if checking ertapenem, then OK, and probably do not need to check imipenem or meropenem?
- It is easier to do lateral flow assay than mCIM. Access to these devices in resources limited settings could be a concern.
- Carbapenemase testing should be performed, preferably along the lines of an assay able to differentiate which one.
- Concern for resource limited setting and cost. Will this be misinterpreted that all three, ertapenem, imipenem, and meropenem, need to be tested?

A motion to accept the Table 2A-1 comment, "Isolates resistant to ertapenem, imipenem, or meropenem (except *Proteus* spp., *Providencia* spp., and *Morganella* spp. only resistant to imipenem) should undergo carbapenemase testing with a phenotypic and/or molecular assay to ideally identify and differentiate the presence of particular carbapenemases (eg, KPC, NDM, OXA-48, VIM, IMP). These results are important for treatment decisions and inform infection control and prevention interventions and/or epidemiological investigations depending on the local epidemiology and resources. Laboratories may consider omitting carbapenemase testing for *Enterobacter cloacae* complex and *Klebsiella aerogenes* isolates that are only resistant to ertapenem, because carbapenemases are uncommon in such isolates." was made and seconded. Vote: 5 for, 9 against, 0 abstain, 0 absent (Fail)

Against Vote Reasoning:

- This is too prescriptive.
- Want to add to comment: 1) that AST should still be performed. 2) This should be a decision made with stewardship, lab, and infection control.
- Including ertapenem alone is too much work for labs.
- Want to ensure users do not think they have to test all three carbapenems.
- If no carbapenemase genes are detected, then consider doing a more general carbapenemase test.

A motion to accept the Table 2A-1 comment, "Isolates resistant to any carbapenem tested (eg, ertapenem, imipenem, meropenem) except *Proteus* spp., *Providencia* spp., or *Morganella* spp. only resistant to imipenem, should undergo carbapenemase testing using a phenotypic and/or molecular assay to identify and ideally differentiate the presence of particular carbapenemases (eg, KPC, NDM, OXA-48, VIM, IMP). The decision of testing and reporting is best made by each laboratory in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. These results are important for treatment decisions and inform infection control and prevention interventions and/or epidemiologic investigations,

but do not replace antimicrobial susceptibly testing for new agents. Depending on local epidemiology and resources, laboratories may consider omitting carbapenemase testing for *Enterobacter cloacae* complex and *Klebsiella aerogenes* isolates that are only resistant to ertapenem, because carbapenemases are currently uncommon in such isolates. See Appendix G, Table G3 regarding suggestion for reporting when new mechanism of resistance-based testing (molecular and phenotypic methods) is discordant with phenotypic AST." was made and seconded. Vote: 12 for, 2 against, 0 abstain, 0 absent (Pass)

Against Vote Reasoning:

- The first sentence states users need to test and then the second sentence states there is a choice. It is confusing.
    - The intent of the second sentence is the way labs test and report is up to local stakeholders.

CARBAPENEM RESISTANT ENTEROBACTERALES AND CARBAPENEM TESTING CONTINUED

- CLSI M100 ED34: Tables 3B and 3C: Tests for Carbapenemases in Enterobacterales and *Pseudomonas aeruginosa*

Current comment:

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]

Proposed revised comment:

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

- BPWG Discussion and Recommendation
    - Motion to accept the proposed revised Tables 3B and 3C comment. WG Vote: 9-0-0-1.

A motion to accept the Tables 3B and 3C comment, "Institutional treatment guidelines, infection prevention procedures, or epidemiological investigations may necessitate identification of carbapenemase-producing Enterobacterales or *P. aeruginosa*. Tests that detect the type of carbapenemase are recommended to inform treatment decisions for carbapenem-resistant Enterobacterales isolates." was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

AMPICILLIN-SULBACTAM MIC BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Sulbactam and *Acinetobacter* spp. Background
    - Sulbactam is a sulfone β-lactamase inhibitor that inhibits some Class A enzymes
    - Intrinsic antibacterial activity against *Acinetobacter* spp. due to high affinity for PBP3 (and PBP1)
    - Is a substrate for β-lactamases common among *A. baumannii*
        - *Acinetobacter*-derived cephalosporinases (ADCs, class C)

        - OXA-type carbapenemases (class D) – acquired or (upregulated) chromosomal
    - Resistance related to ß-lactamases AND PBP1 and especially PBP3 mutations (may have fitness cost)
    - Fixed 2:1 combination of ampicillin-sulbactam (AMP-SUL) is the only commercially available formulation in the US.
    - Dosing, AST methods, and breakpoints for AMP-SUL originally developed based on AMP which has no role in the treatment of *Acinetobacter* spp.
    - Nonetheless, AMP-SUL in combination with ≥1 other agent is recommended as first line for treatment of CRAB by IDSA, ESCMID, SIDP, etc.
- Sulbactam Background
    - FDA-approved dosage of AMP-SUL is:
        - 1.5g (1g AMP:0.5g SUL) or
        - 3g (2g AMP:1g SUL) q6h over 0.5h
        - Not to exceed a total daily dose (TDD) of 4g of SUL
    - SUL with durlobactam (SUL-DUR) recently FDA approved for *Acinetobacter baumannii* at a dose of 2g (1g SUL:1g DUR) q6h over 3h (4 g of SUL per day)
    - IDSA/SIDP recommended dosing against susceptible CRAB isolates is SUL 6-9g TDD (as AMP-SUL 18-27g TDD) for moderate-severe infections
- Current Breakpoints

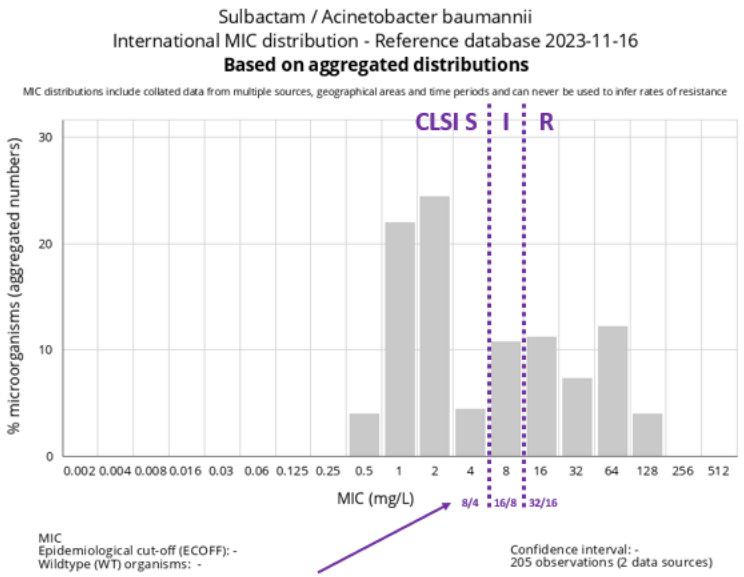
Drug	Organization (year)	MIC (mg/L)		
		Susceptible	Intermediate	Resistant
AMP-SUL	CLSI (2003)	≤8/4	16/8	≥32/16
AMP-SUL	FDA (2023)	≤8/4	16/8	≥32/16
SUL-DUR	CLSI (2023)	≤4/4	8/4	≥16/4
SUL-DUR[a]	FDA (2023)	≤4/4	8/4	≥16/4

[a]*A. baumannii calcoaceticus* complex only

- Microbiology
    - Identification of *Acinetobacter* spp. in the clinical lab
        - *Acinetobacter baumannii* complex includes *A. baumannii* and closely related organisms
        - Identification to species level by commercial biochemical systems unreliable
        - MALDI-TOF mass spectrometry is rapid and reliable
            - Can distinguish *Acinetobacter* species that are phylogenetically well-separated
            - With current databases, may or may not distinguish between closely-related species, including within the *A. baumannii* complex
        - Among genomic methods, rpoB sequencing works better than 16S rRNA
            - Can also (in general) use blaOXA-51 PCR for identification of *A. baumannii*
        - Note: CLSI M100 Table 2B-2 (and the corresponding Table 1) currently apply to *Acinetobacter* spp.

- Cefiderocol and polymyxin breakpoints have a note to "report only on *A. baumannii* complex"

- AMP-SUL broth microdilution AST
  - CLSI reference broth microdilution AST method involves a fixed ratio (not a fixed concentration) of sulbactam
    - ie, 4/2, 8/4, 16/8, 32/16, 64/32
    - Current breakpoints for *Acinetobacter* spp. ≤ 8/4 S, 16/8 I, ≥ 32/16 R (Tier 1 agent)
  - EUCAST reference broth microdilution AST method generally involves a fixed concentration (not a fixed ratio) of sulbactam
    - ie, 4/4, 8/4, 16/4, 32/4, 64/4
    - No current breakpoint for *Acinetobacter* spp. (listed as "Insufficient Evidence") and no AMP-SUL MIC distribution data posted
    - However, sulbactam (alone) MIC distribution data are posted
- AMP-SUL reference BMD reproducibility
  - Frozen BMD panels prepared at CDC were sent to five laboratories experienced with reference BMD
  - Each of the five laboratories and the CDC tested the same 9 "problem" difficult-to-read *Acinetobacter* spp. isolates
  - Variations in both the MIC results and the categorical interpretations were observed for all isolates for one or more of the β-lactam agents tested
  - Only categorical results are presented, so unable to assess MIC variation (eg, are these amp-sulbactam results all within ± 1 dilution?)
  - In this small set with a limited number of R isolates, reproducibility does not look worse than with the other β-lactam agents
- AMP-SUL reference BMD reproducibility
  - ARLN experience (Amelia Bhatnagar, verbal communication):
    - 7 regional labs using Sensititre panel that includes AMP-SUL
    - 30 *Acinetobacter baumannii* AR Bank isolates included in an evaluation that compared Sensititre MICs to AR Bank MICs, which are generated by reference BMD
    - Overall, this drug-bug combination did not appear to be a problem in the evaluation
    - For isolates where the modal MIC was on-scale, they saw a typical 3-dilution spread (only one isolate had at least 4 dilutions), with a few isolates having a tight spread of only 1-2 dilutions
    - However, many isolates had off-scale MICs (especially on the high end), so reproducibility data are limited to a subset of isolates
    - "Difficult-to-read" isolates such as the isolates with "starry growth" described in the Swenson study are encountered rarely
    - When encountered, independent readers (including highly experienced readers) read them differently
  - AR Bank experience (Maria Machado, verbal communication):
    - Customer complaints about problems with this bug-drug combination related to AR Bank isolates are rare
- EUCAST Sulbactam MIC distribution
  - 205 observations representing 2 distributions
  - Data do not meet requirements to set an epidemiological cutoff value

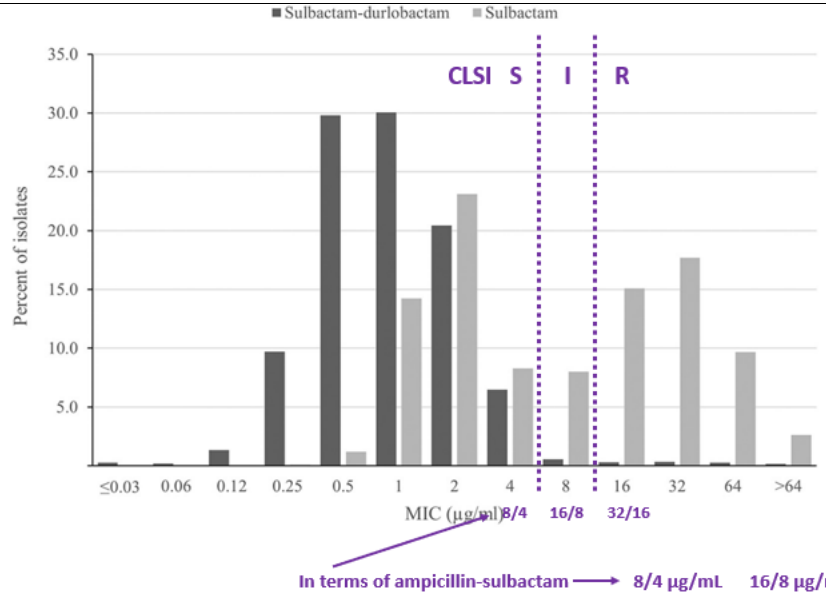


In terms of ampicillin-sulbactam → 8/4 µg/mL 16/8 µg/mL 32/16 µg/mL

Sulbactam MIC	0.5 µg/mL	1 µg/mL	2 µg/mL	4 µg/mL	8 µg/mL	16 µg/mL	32 µg/mL	64 µg/mL	128 µg/mL
Isolates (cumulative %)	8 (3.9%)	45 (25.9%)	50 (50.2%)	9 (54.6%)	22 (65.4%)	23 (76.6%)	15 (83.9%)	25 (96.1%)	8 (100%)

Data from eucast.org (accessed 11/16/2023)

- IHMA Sulbactam MIC distribution
  - 5,032 *Acinetobacter baumannii* complex
  - Collected globally 2016-2021

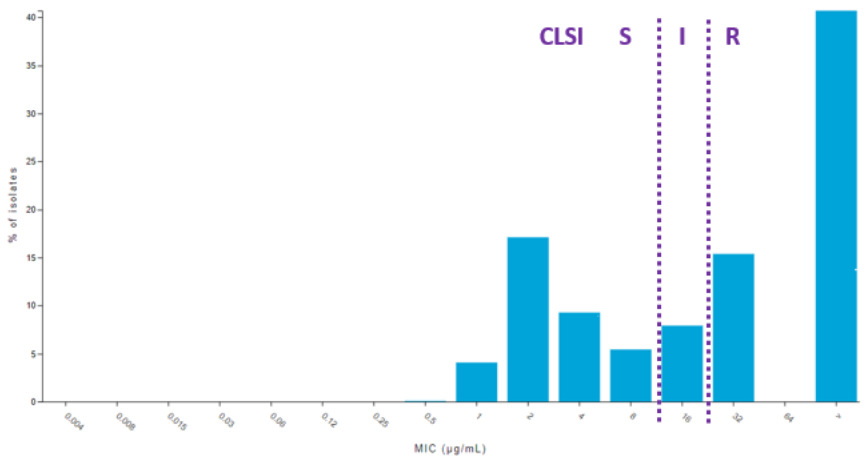


Sulbactam MIC	0.25 µg/mL	0.5 µg/mL	1 µg/mL	2 µg/mL	4 µg/mL	8 µg/mL	16 µg/mL	32 µg/mL	64 µg/mL	> 64 µg/mL
Isolates (cumulative %)	5 (0.1%)	61 (1.3%)	716 (15.5%)	1,163 (38.7%)	417 (46.9%)	403 (54.9%)	759 (70.0%)	890 (87.7%)	486 (97.4%)	132 (100%)

Data from Karlowsky JA et al. Antimicrob Agents Chemother 2022; 66: e0078122.

- JMI AMP-SUL MIC distribution
  - 9,555 *Acinetobacter baumannii* complex

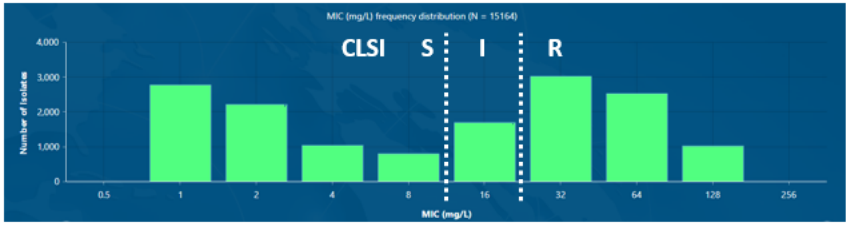
Activity of ampicillin-sulbactam (n=9,555) tested against *Acinetobacter nosocomialis*, *Acinetobacter calcoaceticus subspecies anitratus*, *Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, *Acinetobacter baumannii-calcoaceticus species complex*, and *Acinetobacter pittii* isolates in the SENTRY program



Ampicillin-sulbactam MIC	0.5/0.25 µg/mL	1/0.5 µg/mL	2/1 µg/mL	4/2 µg/mL	8/4 µg/mL	16/8 µg/mL	32/16 µg/mL	≥ 64/32 µg/mL
Isolates (cumulative %)	14 (0.1%)	393 (4.3%)	1,631 (21.3%)	884 (30.6%)	514 (36.0%)	759 (43.9%)	1,470 (59.3%)	3,890 (100%)

Data from sentry-mvp.jmilabs.com (accessed 11/16/2023)

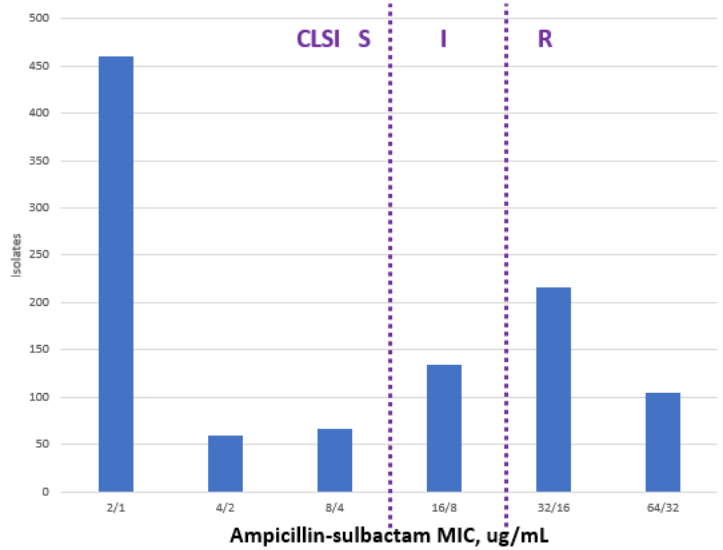
- ATLAS (Pfizer) AMP-SUL MIC distribution
  - 15,164 *Acinetobacter baumannii* complex



Ampicillin-sulbactam MIC	1/0.5 µg/mL	2/1 µg/mL	4/2 µg/mL	8/4 µg/mL	16/8 µg/mL	32/16 µg/mL	64/32 µg/mL	≥ 128/64 µg/mL
Isolates (cumulative %)	2782 (18.3%)	2223 (33.0%)	1049 (39.9%)	807 (45.2%)	1703 (56.5%)	3033 (76.5%)	2536 (93.2%)	1031 (100%)

Data from atlas-surveillance.com (accessed 11/16/2023)

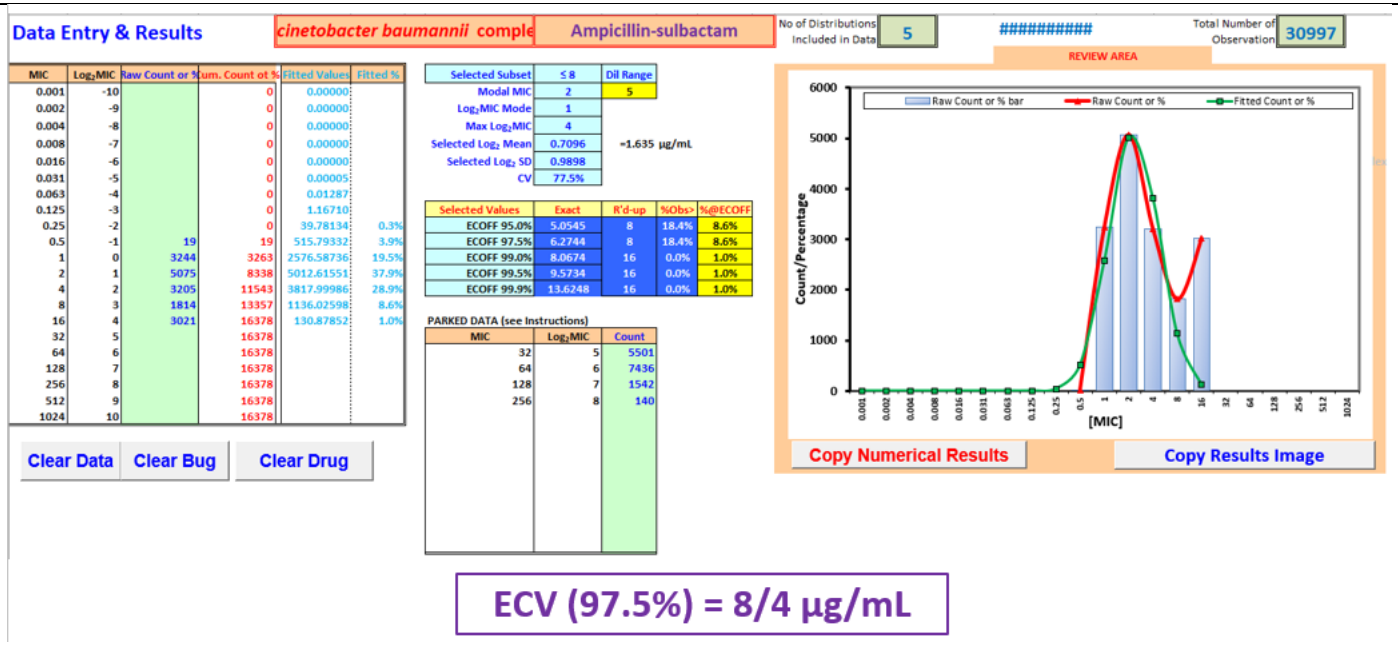
- Shionogi (via AMR Register/Viv) AMP-SUL MIC distribution
  - 1,041 *Acinetobacter baumannii* complex isolates with AMP-SUL MICs
  - From clinical laboratories (2013-2019) – collected and tested as part of SIDERO-WT surveillance studies



Ampicillin-sulbactam MIC	2/1 µg/mL	4/2 µg/mL	8/4 µg/mL	16/8 µg/mL	32/16 µg/mL	64/32 µg/mL
Isolates (cumulative %)	460 (44.2%)	59 (49.9%)	67 (56.3%)	134 (69.2%)	216 (89.9%)	105 (100%)

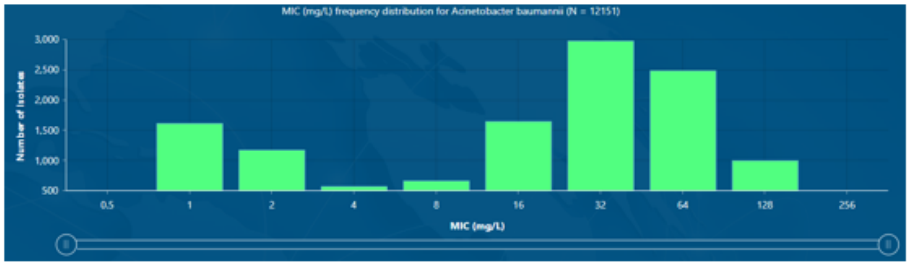
Data provided upon request from https://searchamr.vivli.org/datasetDetails/b5855796-a9ca-41a4-8a2e-e55b4ffb5b99

- ECOFF Finder for AMP-SUL and *Acinetobacter* spp.

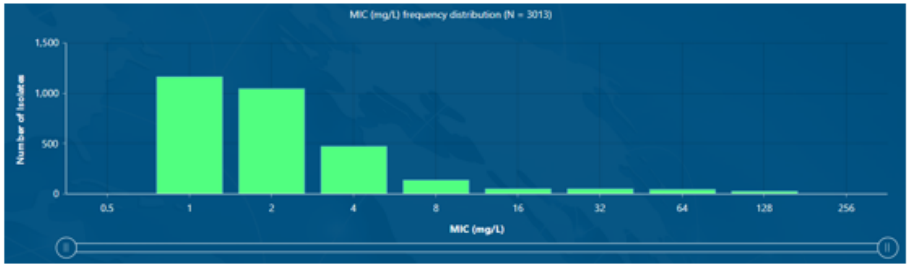


ECV (97.5%) = 8/4 µg/mL

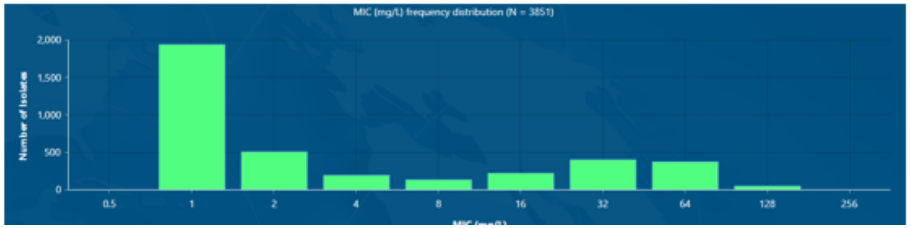
- ECV summary for AMP-SUL and *A. baumannii* complex
  - Taking the available datasets together, the *Acinetobacter baumannii* complex AMP-SUL ECV is estimated to be 8/4 µg/mL
    - aka, sulbactam 4 µg/mL
  - Note: inquiry to EUCAST (who do not have an ECOFF or breakpoint):
    - Christian Giske (verbal communication): "A technician in my lab tested 50 *Acinetobacter* many years ago vs. sulbactam alone (in-house BMD) and the tentative ECOFF/ECV was 4 mg/L"
- MIC distributions for *A. baumannii* vs. other *A. baumannii* species



12,151 *Acinetobacter baumannii*



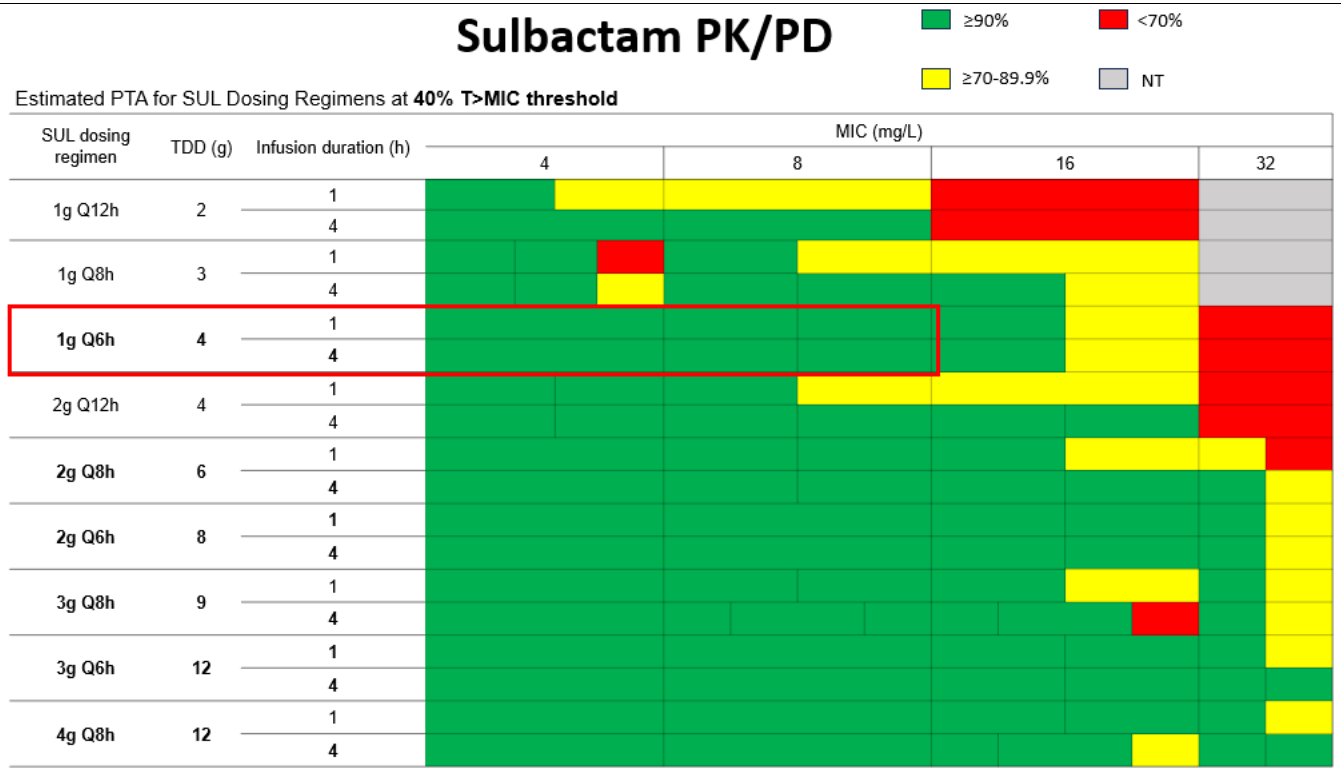
3,013 non-*baumannii A. baumannii* complex



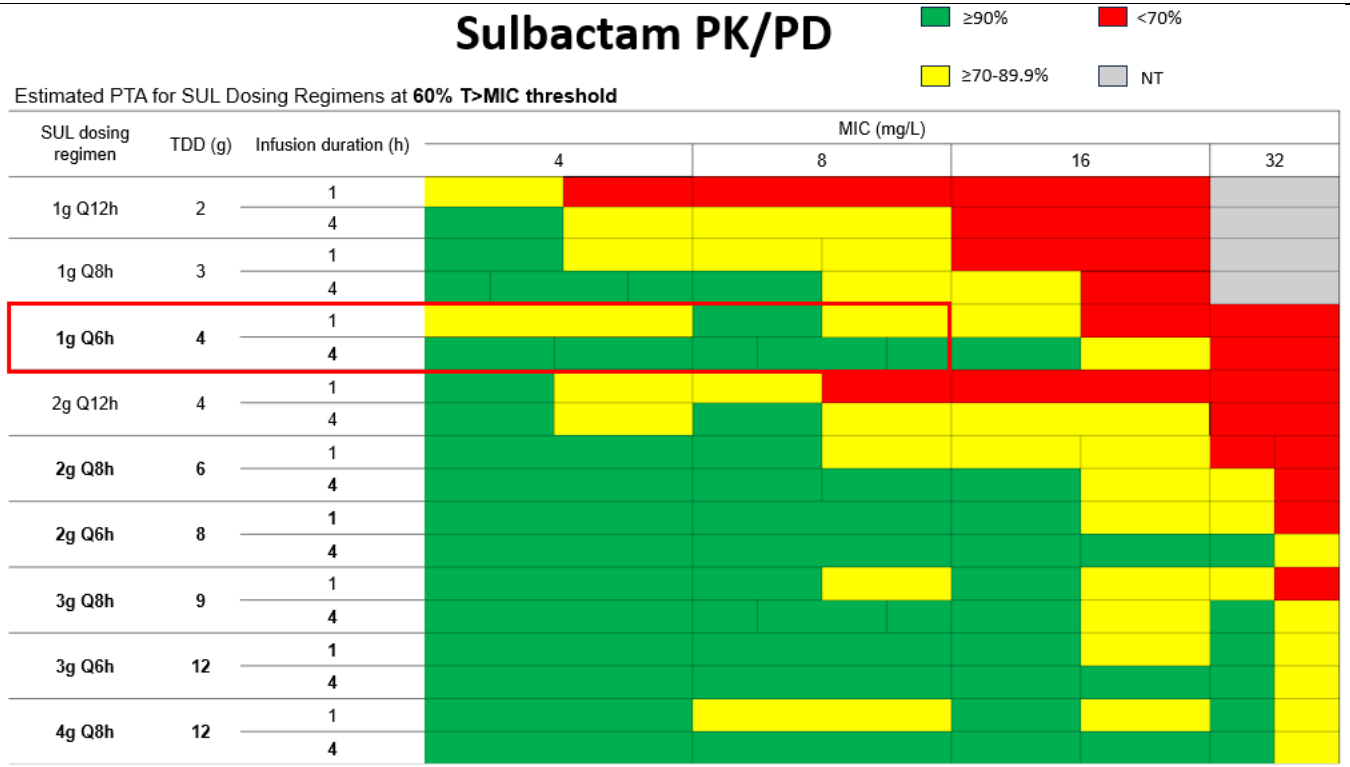
Acinetobacter spp. not in *A. baumannii* complex

Data from atlas-surveillance.com (accessed 11/16/2023)

- PK/PD
  - To summarize all 7 studies reviewed, heat map was created showing the dosing regimen on the left, the total daily dose, infusion duration of 1h or 4h, and then PTA at MICs 4-32 mg/L color coded as green for >/=90% PTA, yellow for 70-89.9%, red for <70%, and grey for not tested
  - This figure is based on a target of 40% fT>MIC and I draw your attention to the standard 1g Q6h dosing regimen where you can see either a 1h or 4h infusion appear adequate through MICs of 8 mg/L
  - Unfortunately, the studies that simulated a 3h infusion did not use a 1g Q6h dose and those that simulated a 1g Q6h dose did not use a 3h infusion so this is the best we have
  - As seen in the individual studies, doses above 1g Q6h only provide additional coverage against non-susceptible MICs



- This is the exact same figure except using a 60% fT>MIC target
- As seen the colors are shifted left as PTA at each MIC decreases in response to the higher target and shifted down as the importance of a 4h infusion becomes more obvious with a higher target



- Clinical
  - Study of 58 patients with CRAB infections, of which 22 were treated with AMP-SUL (Oliviera 2013)
    - No relationship between AMP-SUL MIC and outcomes
    - Patients who survived received higher dosages
  - RCT of COL with 9g or 12g TDD of SUL against XDR *A. baumannii* pneumonia (Ungthammakhun 2022)
    - No significant differences in outcomes by SUL MIC in patients treated with SUL
  - 2017 systematic review and meta-analysis (Chen et al 2017)
    - 12 studies - 1472 patients (1 prospective, 11 retrospective)
    - No significant difference in overall clinical/micro response or ACM for SUL vs comparators
    - In subgroup of MDR *A. baumannii*, clinical response favored SUL
    - In subgroup by SUL dose, low (3g TDD) dose had lower clinical response (1 study). Moderate (6g TDD) showed no difference. High (>/=9g TDD) favored SUL
  - Bayesian Network Meta-analysis (Jung et al 2017)
    - *A. baumannii* HAP only
    - 4 RCTs; 3 prospective obs; 16 retrospective

        - n=2118 adults
        - HD SUL = ≥9g TDD
        - Unable to assess safety
        - SUL was most effective therapy to reduce all-cause mortality in critically ill patients.
        - HD SUL had lower rates of micro cure though isolates in HD SUL group had MIC > 16 mg/L
- Summary: AMP-SUL *A. baumannii* breakpoints data
    - AMP-SUL recommended first line for treatment of CRAB by IDSA at a TDD of 6-9g SUL as an extended infusion (eg, 3g Q8h over 4h) for moderate-severe infections
    - Dosing, AST, and breakpoints for AMP-SUL against *A. baumannii* based on AMP which has no role in the treatment of *A. baumannii*
    - ECV at 4 mg/L for SUL against *Acinetobacter* based on contemporary MIC distributions
    - PK/PD data limited and highly variable but suggest high dose extended infusion regimens are needed for achieving adequate PTA at current breakpoints
        - 1g Q6h (4g TDD) over 4h for MIC ≤4 mg/L
        - 2-3g Q6-8h (6-9g TDD) over 4h for MIC 8 mg/L
    - Clinical data largely uninformative for breakpoint reevaluation
        - No clear correlation between SUL MICs and outcomes
        - Some association between higher doses of SUL and improved outcomes
- M23 cutoffs to set breakpoints AMP-SUL and *Acinetobacter* spp.

Type of cutoff	MIC (µg/mL)
Epidemiological cutoff value	8/4 µg/mL
Non-clinical PK/PD cutoff value	8/4 µg/mL (3 g q6h dosage) 16/8 µg/mL (9 g q8h dosage with prolonged infusion)
Clinical exposure response relationship cutoff value	Not available
Clinical cutoff	Not available

- June 2023 CLSI AST Subcommittee Meeting Reminders
    - Sulbactam-Durlobactam Indication and Dose
        - Indicated in adults (≥ 18 years) for the treatment of hospital- acquired bacterial pneumonia and ventilator-associated bacterial pneumonia caused by susceptible strains of *Acinetobacter baumannii-calcoaceticus* complex
        - Dose: 1.0 g sulbactam / 1.0 g durlobactam
        - Schedule: q6h administered as 3-hour IV infusion
            - Dose adjustments recommended in patients with CLCR < 45 mL/min or ≥ 130 mL/min
    - High probability of target attainment (PTA) for *Acinetobacter* at proposed MIC of ≤ 4 µg/mL
    - Mortality At Day 28 for Patients Who Received Sulbactam-Durlobactam in the CRABC m-MITT Population (Part A)

- Two patients in the m-MITT Part A population had an ABC baseline isolate with a SUL-DUR MIC = 8µg/mL. Both patients survived to day 28.
- Two patients in Part B had an ABC baseline isolate with a SUL-DUR MIC = 8 µg/mL. Both patients survived to day 28.

SUL-DUR MIC (µg/mL)	N*	Survived n (%)
0.5/4	4	3 (75%)
1/4	23	18 (78.3%)
2/4	27	21 (77.8%)
4/4	6	6 (100%)

- *Acinetobacter* AHWG Discussion and Recommendation
  - Vote 1: Susceptible and resistant breakpoints for AMP-SUL against *Acinetobacter* spp. should be ≤8/4 mg/L and ≥32/16 mg/L, respectively. AHWG Vote: 7-0-0-0.
  - Vote 2: The MIC breakpoint of 16/8 mg/L should be designated as intermediate. AHWG Vote: 7-0-0-0.
  - Vote 3: The dose associated with the susceptible breakpoint of ≤8/4 mg/L should be 1g Q6h as an extended infusion over ≥3h. AHWG Vote: 7-0-0-0.
- BPWG Discussion and Recommendation
  - Agreement that susceptible breakpoint should be ≤8/4 µg/mL and resistant breakpoint should be ≥32/16 µg/mL
  - Rationale for intermediate instead of S-DD for 16/8 µg/mL
    - Allows for technical variability and reduced activity at 16/8 µg/mL
    - Would encourage providers to use SUL-DUR instead of SUL for these organisms
    - Dosages outside of FDA label that could be used if S-DD (6-9 g SUL per day) in IDSA guidance document not widely supported by literature
    - Concern about increased PK/PD indices with SUL-resistant isolates (higher dosages may not be sufficient)
  - Which dosage?
    - 3g (1g SUL) q6h over 4h provides adequate PTA at MICs up to 8 µg/mL
    - 3g (1g SUL) q6h over 3h would fit with amount of SUL in SUL-DUR
  - OXA-23+ *A. baumannii* (where SUL ineffective *in vivo*) usually SUL-resistant
  - Motion to not change the current AMP-SUL breakpoints (S ≤8/4, I 16/8, R ≥32/16 (µg/mL) for *Acinetobacter* spp. and add on dosage of 3g AMP-SUL q6h as an extended infusion of ≥3 hours. WG Vote: 9-0-1-2.

SC DISCUSSION (MAIN POINTS)

- Add a dosage comment saying: Dosage of 3g AMP-SUL (2g AMP and 1g SUL) q6h as an extended infusion of ≥3 hours.
- This is the most challenging drug to understand when talking about the doses. With all other drugs it is the total, but here referring to sulbactam. Clarify the dose and say "3g AMP-SUL (2g AMP and 1g SUL)".
- The regular infusion seemed to look good in one paper, so why go for the longer infusion?
  - Other models suggest extended infusion is important.

A motion to not change the current ampicillin-sulbactam MIC breakpoints (S≤8/4, I 16/8, R ≥32/16 µg/mL) for *Acinetobacter* spp. and accept adding based on a dosage of 3g AMP-SUL (2g AMP and 1g SUL) q6h as an extended infusion of ≥3 hourswas made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

AMPICILLIN-SULBACTAM DISK BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Existing ampicillin-sulbactam and sulbactam-durlobactam disk correlates for *Acinetobacter* spp.
  - SUL-DUR sponsor had originally proposed disk interpretive criteria of ≥ 19 S, 15-18 I, ≤ 14 R (a 4-mm wide intermediate range)
  - FDA selected the ≥ 17 S, 14-16 I, ≤ 13 R interpretive criteria, which prioritized avoiding overcalling resistance
  - CLSI SUL-DUR AHWG had a slight preference for the sponsor's originally proposed criteria, but thought both options were acceptable -> went with FDA criteria because of the benefits of harmonization
  - Current AMP-SUL disk diffusion breakpoints same as Enterobacterales

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
Ampicillin-sulbactam	10/10 µg	≥ 15	12–14	≤ 11	≤ 8/4	16/8	≥ 32/16
Sulbactam-durlobactam	**10/10 µg**	**≥ 17**	**14–16**	**≤ 13**	**≤ 4/4**	**8/4**	**≥ 16/4**

  - Swenson dataset: CLSI reference BMD vs. CLSI standardized DD using (probably in-house prepared?) disks and BBL Mueller-Hinton agar
    - MIC ≤ 8/4 S, 16/8 I, ≥ 32/16 R
    - Disk ≥ 15 S, 12-14 I, ≤ 11 R

	N	Very Major (%)	Major (%)	Minor (%)
≥ I + 2	32	5 (15.6%)	N/A	8 (25.0%)
I + 1 to I – 1	77	14 (18.2%)	0 (0%)	23 (29.9%)
≤ I - 2	84	N/A	0 (0%)	0 (0%)

  - Viana (Brazil) dataset: CLSI agar dilution vs. CLSI standardized DD using Oxoid disks and BD Mueller-Hinton agar
    - MIC ≤ 8/4 S, 16/8 I, ≥ 32/16 R
    - Disk ≥ 15 S, 12-14 I, ≤ 11 R

	n	Very Major (%)	Major (%)	Minor (%)
≥ I + 2	20	**8 (40.0%)**	N/A	1 (5.0%)
I + 1 to I – 1	90	10 (11.1%)	0 (0%)	**55 (61.1%)**
≤ I - 2	11	N/A	0 (0%)	0 (0%)

- JMI dataset: CLSI reference BMD vs. CLSI standardized disk diffusion using BD disks and Remel Mueller Hinton agar
  - 260 clinical isolates collected during worldwide surveillance studies (2013-2015) plus 40 additional resistant isolates
  - MIC ≤ 8/4 S, 16/8 I, ≥ 32/16 R
  - Disk ≥ 15 S, 12-14 I, ≤ 11 R

MIC Range	Number	Very Major (%)	Major (%)	Minor (%)
≥I+2	128	0	N/A	9 (7.0)
I+1 to I-1	99	7 (7.1)	0	40 (40.4)
≤I-2	72	N/A	0	0
Total	299	7 (2.3)	0	49 (16.4)

- Combined Swenson, Viana, and JMI datasets
  - MIC ≤ 8/4 S, 16/8 I, ≥ 32/16 R
  - Applying existing disk correlates ≥ 15 S, 12-14 I, ≤ 11 R

	n	Very Major (%)	Major (%)	Minor (%)
≥ I + 2	180	**13 (7.2%)**	N/A	**18 (10.0%)**
I + 1 to I – 1	266	**31 (11.7%)**	0 (0%)	**118 (44.4%)**
≤ I - 2	167	N/A	0 (0%)	0 (0%)

- Some alternative disk correlate options if we keep the existing MIC BPs
  - First dBETS suggestion for optimal BPs using error-bounded method applied to full dataset (Swenson + Viana + JMI)
    - ≥ 22 S, 15-21 I, ≤ 14 R (7mm wide intermediate)
    - This would lump all isolates that previously would have been considered intermediate into the resistant category – and the whole 7mm intermediate range proposed here would have previously been considered susceptible

	n	VME	ME	mE
≥ I + 2	180	3 (1.7%)	N/A	**10 (5.6%)**
I + 1 to I – 1	266	6 (2.3%)	1 (0.4%)	74 (27.8%)
≤ I - 2	167	N/A	0 (0%)	2 (1.2%)

- If run with JMI data only, dBETS suggestion for optimal BPs using error-bounded method
  - ≥ 21 S, 16-20 I, ≤ 15 R (5mm wide intermediate)

	n	VME	ME	mE
≥ I + 2	128	0 (0%)	N/A	0 (0%)
I + 1 to I – 1	99	0 (0%)	1 (1.0%)	15 (15.2%)
≤ I - 2	72	N/A	0 (0%)	0 (0%)

- Applying the JMI-optimized dBETS suggestion to the full dataset (Swenson + Viana + JMI)
  - ≥ 21 S, 16-20 I, ≤ 15 R (5mm wide intermediate)

	n	VME	ME	mE
≥ I + 2	180	**4 (2.2%)**	N/A	6 (3.3%)
I + 1 to I – 1	266	8 (3.0%)	2 (0.8%)	76 (28.6%)
≤ I - 2	167	N/A	0 (0%)	2 (1.2%)

- dBETS suggestion for a 5mm intermediate based on the full dataset would be shifted 1mm up
  - ≥ 22 S, 17-21 I, ≤ 16 R

	n	VME	ME	mE
≥ I + 2	180	3 (1.7%)	N/A	2 (1.1%)
I + 1 to I – 1	266	6 (3.3%)	2 (0.8%)	76 (28.6%)
≤ I - 2	167	N/A	0 (0%)	2 (1.2%)

- What do data look like if we applied the sulbactam-durlobactam disk breakpoints to ampicillin-sulbactam (Swenson + Viana + JMI)?
  - ≥ 17 S, 14-16 I, ≤ 13 R

	n	VME	ME	mE
≥ I + 2	180	8 (4.4%)	N/A	8 (4.4%)
I + 1 to I – 1	266	24 (9.0%)	0 (0%)	97 (36.5%)
≤ I - 2	167	N/A	0 (0%)	0 (0%)

- Disk correlate proposal: AMP-SUL/*Acinetobacter* spp.
  - 5 mm intermediate zone that captures all data
    - S >=22, 17-21 I, <=16 R (mm)
  - Differs from the SUL-DUR disk diffusion breakpoints
    - S >=17, 14-16 I, <=13 R (mm)
  - Those were made in conjunction with the FDA to minimize any possible major errors.
  - There is precedent for disk correlates to be different even when the same active component has the same MIC (see Enterobacterales and Amp vs Amp/Sul)

	n	VME	ME	mE
≥ I + 2	180	3 (1.7%)	N/A	2 (1.1%)
I + 1 to I – 1	266	6 (3.3%)	2 (0.8%)	76 (28.6%)
≤ I - 2	167	N/A	0 (0%)	2 (1.2%)

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

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

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

- BPWG Discussion and Recommendation
  - Motion to change the AMP-SUL disk breakpoints for *Acinetobacter* spp. to S≥22, I 17-21, R≤16 mm. WG Vote: 9-0-1-2.

SC DISCUSSION (MAIN POINTS)

- CLSI should generate new data for disk diffusion given the media differences.
- Avoiding over calling resistance for AMP-SUL.
- Need to align these breakpoints with the direct from blood disk diffusion.
- Suggestion to remove the AMP-SUL direct from disk diffusion breakpoints until they can be updated.

A motion to accept the ampicillin-sulbactam disk breakpoints (S≥22, I 17-21, R≤16 mm) for *Acinetobacter* spp. and to remove the direct disk diffusion breakpoints until reviewed was made and seconded.* Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

*NOTE: In the Methods Development and Standardization Working Group report, the *Acinetobacter* direct blood disk breakpoints were reviewed and approved for M100 35th edition.

MINOCYCLINE MIC BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Minocycline Background

- 2nd-generation tetracycline first introduced in 1970s
  - Breakpoints first established in 1970s prior to modern PK/PD analyses
- Mechanism of action: bacteriostatic by binds 30S ribosomal subunit causing conformational changes to RNA
- Mechanism of resistance: primarily efflux along with ribosomal protection proteins and target site modification
- More lipophilic than tetracycline (and doxy) allowing for increased tissue penetration, longer half-life, and improved antibacterial activity
- Protein binding ~76%; PO formulation ~90% bioavailability
  - IV formulation taken off market in due to low use then reintroduced in 2015 after reformulation
- Elimination independent of hepatic or renal function
- Available PK studies besides ACUMIN (Lodise T, et al. AAC. 2021) are in healthy volunteers (Macdonald H, et al. Clin Pharm Ther. 1973) and in renal failure (Welling PG, et al. AAC. 1975; Sklenar I, et al. Agents and Actions. 1977)
- FDA approved indication for infections caused by *Acinetobacter*, including CRAB and XDR strains
- IDSA guidance: "High-dose minocycline or high-dose tigecycline can be considered in combination with at least 1 other agent for the treatment of CRAB infections. The panel prefers minocycline because of the long-standing clinical experience with this agent and the availability of CLSI susceptibility interpretive criteria".

- Tetracycline resistance in *A. baumannii*
  - Frequently due to "Tet" family of pumps
  - Tet A (most common) – doxycycline and tetracycline resistance
  - Tet B adds minocycline resistance (with presence having high PPV for resistance)
  - Tigecycline overcomes TetA and TetB but can rapidly develop resistance (TetX or RND efflux type)
  - RND efflux pumps do NOT appear to impact minocycline, therefore TetB negative RND positive strains could be susceptible to minocycline but resistant to tigecycline
- Current tetracycline breakpoints for *Acinetobacter* spp.

Drug	Organization (year)	MIC (mg/L)		
		Susceptible	Intermediate	Resistant
TET[a]	CLSI (2023)	≤4	8	≥16
DOX	CLSI (2023)	≤4	8	≥16
MIN	**CLSI (2023)**	**≤4**	**8**	**≥16**

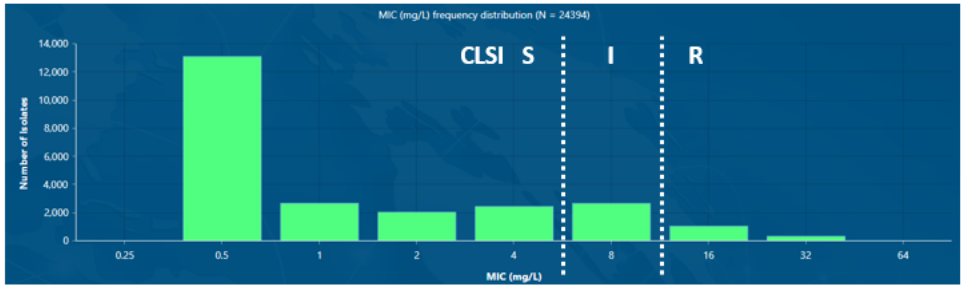
[a]Report only on organisms isolated from the urinary tract
*FDA recognizes M100; no EUCAST breakpoints

- Minocycline dosing

Reference (year)	Dosing Regimen
Package insert	PO: 200mg x 1 (loading dose), then 100mg Q12h IV: 200mg x 1, then 100mg Q12h over 1h[a]
IDSA AMR Guidance (2023)	PO/IV: 200mg Q12h
CLSI M100-S34 (2024)	PO/IV: 200mg Q12h (*S. maltophilia*)

[a]Should not exceed 400mg in 24 hours

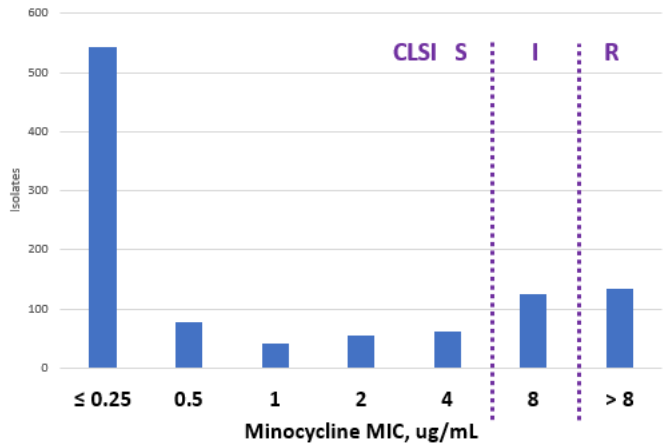
- Microbiology
  - ATLAS (Pfizer) Minocycline MIC distribution
    - 24,394 *Acinetobacter baumannii* complex isolates with minocycline MICs
    - Global collection from 2004-2017



Minocycline MIC, µg/mL	≤ 0.5	1	2	4	8	16	> 16
n (cumulative %)	13116 (53.77)	2685 (64.77)	2055 (73.20)	2459 (83.28)	2674 (94.24)	1056 (98.57)	349

Pfizer. ATLAS (Antimicrobial Testing Leadership and Surveillance). https://atlas-surveillance.com [Registration required]. Accessed 2-05-2024.

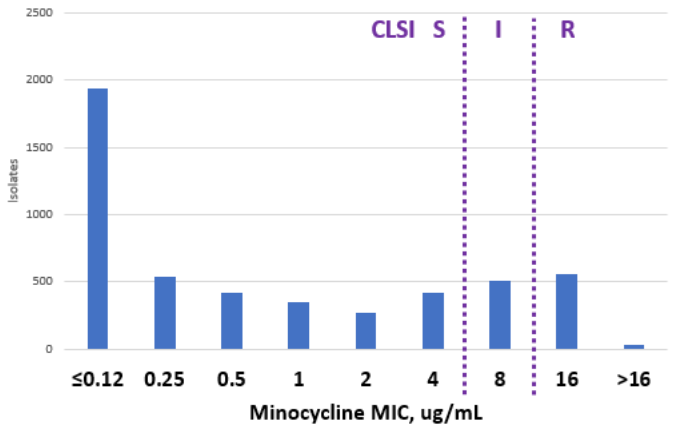
  - Shionogi (via AMR Register/VivLi)
    - 1,041 *Acinetobacter baumannii* complex isolates with minocycline MICs
    - From clinical laboratories (2013-2019) – collected and tested as part of SIDERO-WT surveillance studies



Minocycline MIC, µg/mL	≤ 0.25	0.5	1	2	4	8	> 8
n (cumulative %)	544 (52.3)	77 (59.7)	42 (63.7)	55 (69.0)	63 (75.0)	125 (87.0)	135

Data provided upon request from https://searchamr.vivli.org/datasetDetails/b5855796-a9ca-41a4-8a2e-e55b4ffb5b99

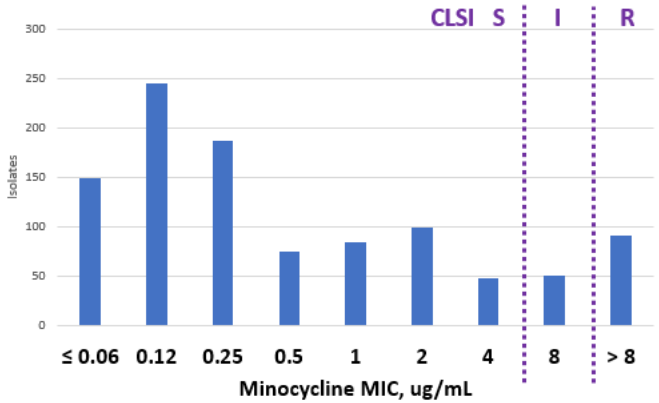
- IHMA Minocycline MIC distribution
  - 5,051 *Acinetobacter baumannii* complex isolates with minocycline MICs
  - Global collection of isolates from 2016-2021



Minocycline MIC, µg/mL	≤ 0.12	0.25	0.5	1	2	4	8	16	> 16
n (cumulative %)	1933 (38.3)	544 (49.0)	417 (57.3)	354 (64.3)	276 (69.8)	423 (78.1)	511 (88.3)	555 (99.2)	38

Data courtesy of Meredith Hackel, IHMA. Isolates described in PMID 36005804 (paper focused on sulbactam-durlobactam)

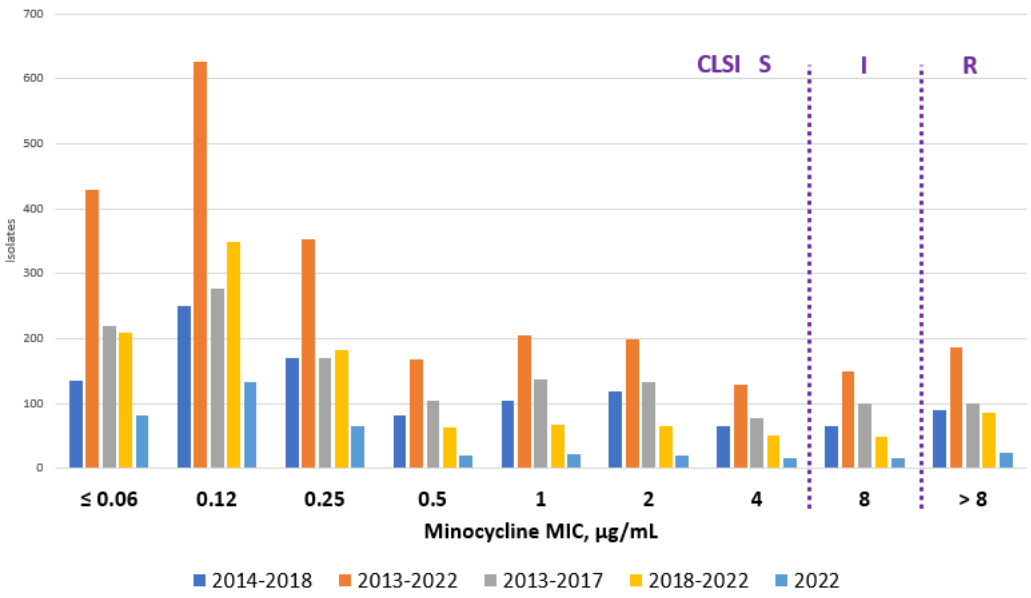
- JMI Minocycline MIC distribution
  - 1,029 *Acinetobacter baumannii* complex isolates with minocycline MICs
  - Isolates from U.S. patients with documented infections 2014-2021
  - We opted to use this recently published distribution to represent JMI data instead of the larger dataset publicly available on website



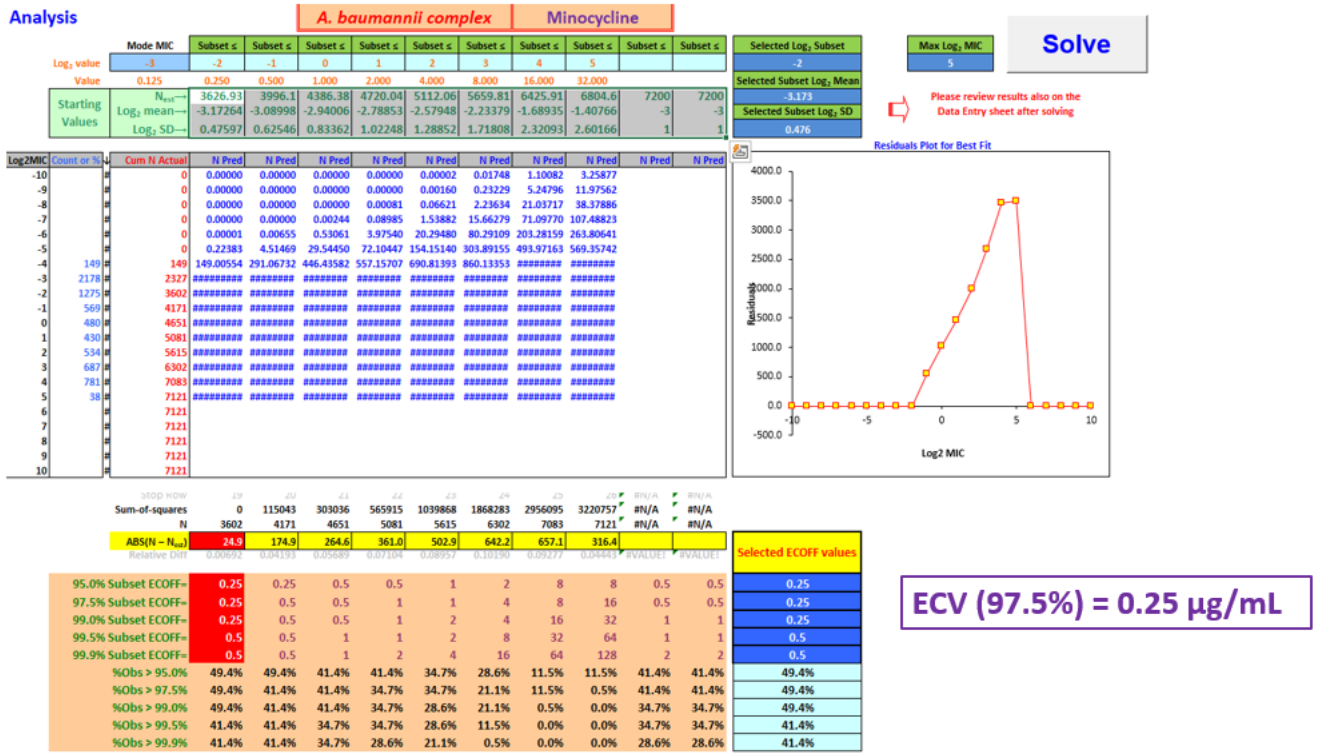
Minocycline MIC, µg/mL	≤ 0.06	0.12	0.25	0.5	1	2	4	8	> 8
n (cumulative %)	149 (14.5)	245 (38.3)	187 (56.5)	75 (63.8)	84 (71.9)	99 (81.5)	48 (86.2)	51 (91.2)	91

Pfaller et al. Microbiol Spectr 2023. PMID 37921464

- MIC data provided by Mark Redell, Melinta Therapeutics, Data Courtesy of Elements/JMI Labs
  - Isolates collected from medical centers in North America in the SENTRY program



- ECOFF Finder with Shionogi, IHMA, and JMI data (with JMI data represented by those reported in Pfaller et al. PMID 37921464)



Analysis — *A. baumannii complex* — Minocycline

Solve

Mode MIC	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤
Log_2 value	-3	-2	-1	0	1	2	3	4	5	
Value	0.125	0.250	0.500	1.000	2.000	4.000	8.000	16.000	32.000	
Starting Values N_{est}→	3626.93	3996.1	4386.38	4720.04	5112.06	5659.81	6425.91	6804.6	7200	7200
Starting Values Log_2 mean→	-3.17264	-3.08998	-2.94006	-2.78853	-2.57948	-2.23379	-1.68935	-1.40766	-3	-3
Starting Values Log_2 SD→	0.47597	0.62546	0.83362	1.02248	1.28852	1.71808	2.32093	2.60166	1	1

Selected Log_2 Subset	Max Log_2 MIC
-2	5
Selected Subset Log_2 Mean	
-3.173	
Selected Subset Log_2 SD	
0.476	

Please review results also on the Data Entry sheet after solving

Log2MIC	Count or %	Cum N Actual	N Pred	N Pred	N Pred	N Pred	N Pred	N Pred	N Pred	N Pred	N Pred	N Pred
-10	#	0	0.00000	0.00000	0.00000	0.00000	0.00002	0.01748	1.10082	3.25877		
-9	#	0	0.00000	0.00000	0.00000	0.00000	0.00160	0.23229	5.24796	11.97562		
-8	#	0	0.00000	0.00000	0.00000	0.00081	0.06621	2.23634	21.03717	38.37886		
-7	#	0	0.00000	0.00000	0.00244	0.08985	1.53882	15.66279	71.09770	107.48823		
-6	#	0	0.00001	0.00655	0.53061	3.97540	20.29480	80.29109	203.28159	263.80641		
-5	#	0	0.22383	4.51469	29.54450	72.10447	154.15140	303.89155	493.97163	569.35742		
-4	149	#	149	149.00554	291.06732	446.43582	557.15707	690.81393	860.13353	########	########	
-3	2178	#	2327	########	########	########	########	########	########	########	########	
-2	1275	#	3602	########	########	########	########	########	########	########	########	
-1	569	#	4171	########	########	########	########	########	########	########	########	
0	480	#	4651	########	########	########	########	########	########	########	########	
1	430	#	5081	########	########	########	########	########	########	########	########	
2	534	#	5615	########	########	########	########	########	########	########	########	
3	687	#	6302	########	########	########	########	########	########	########	########	
4	781	#	7083	########	########	########	########	########	########	########	########	
5	38	#	7121	########	########	########	########	########	########	########	########	
6		#	7121									
7		#	7121									
8		#	7121									
9		#	7121									
10		#	7121									

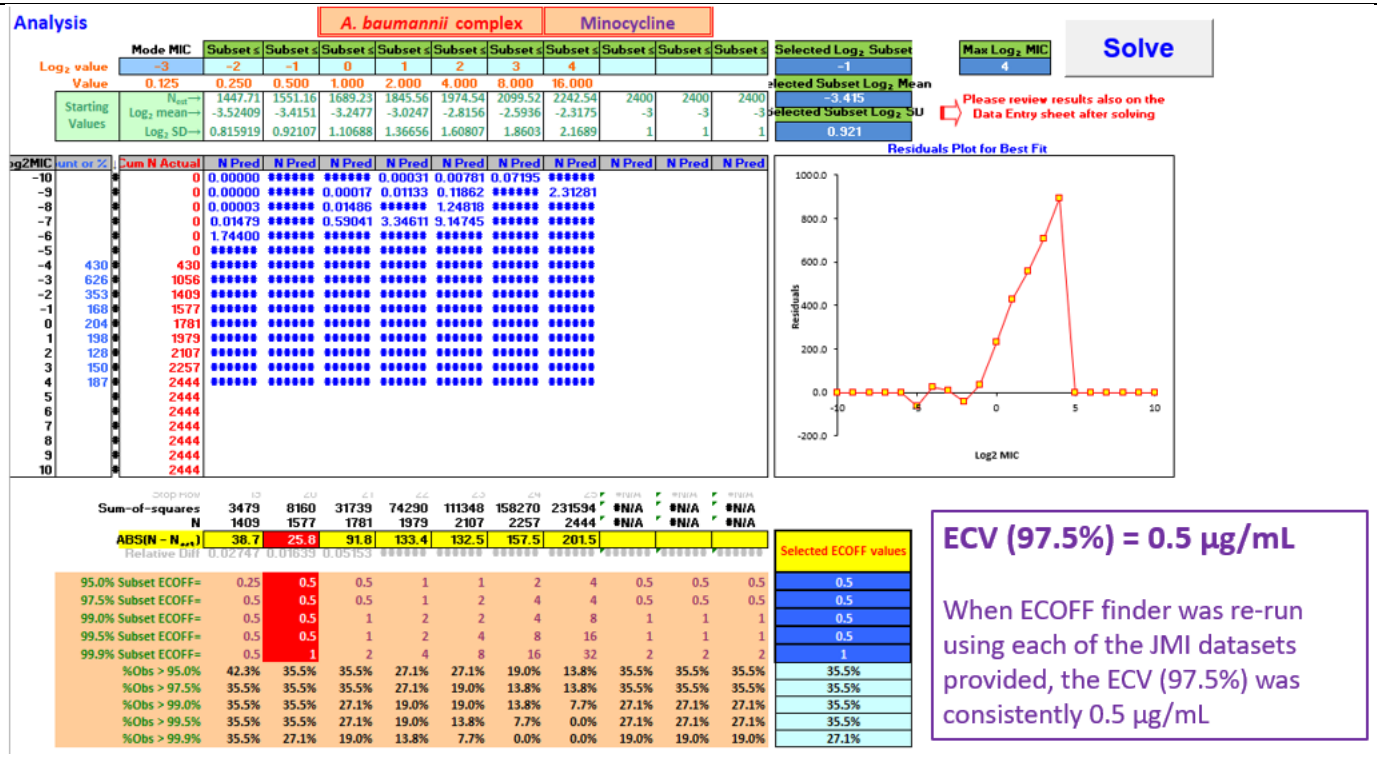
Residuals Plot for Best Fit

Sum-of-squares	0	115043	303036	565915	1039868	1868283	2956095	3220757	#N/A	#N/A	
N	3602	4171	4651	5081	5615	6302	7083	7121	#N/A	#N/A	
ABS(N – N_{est})	24.9	174.9	264.6	361.0	502.9	642.2	657.1	316.4			
Relative Diff	0.00692	0.04193	0.05689	0.07104	0.08957	0.10190	0.09277	0.04443	#VALUE!	#VALUE!	

											Selected ECOFF values
95.0% Subset ECOFF=	0.25	0.25	0.5	0.5	1	2	8	8	0.5	0.5	0.25
97.5% Subset ECOFF=	0.25	0.5	0.5	1	1	4	8	16	0.5	0.5	0.25
99.0% Subset ECOFF=	0.25	0.5	0.5	1	2	4	16	32	1	1	0.25
99.5% Subset ECOFF=	0.5	0.5	1	1	2	8	32	64	1	1	0.5
99.9% Subset ECOFF=	0.5	0.5	1	2	4	16	64	128	2	2	0.5
%Obs > 95.0%	49.4%	49.4%	41.4%	41.4%	34.7%	28.6%	11.5%	11.5%	41.4%	41.4%	49.4%
%Obs > 97.5%	49.4%	41.4%	41.4%	34.7%	34.7%	21.1%	11.5%	0.5%	41.4%	41.4%	49.4%
%Obs > 99.0%	49.4%	41.4%	41.4%	34.7%	28.6%	21.1%	0.5%	0.0%	34.7%	34.7%	49.4%
%Obs > 99.5%	41.4%	41.4%	34.7%	34.7%	28.6%	11.5%	0.0%	0.0%	34.7%	34.7%	41.4%
%Obs > 99.9%	41.4%	41.4%	34.7%	28.6%	21.1%	0.5%	0.0%	0.0%	28.6%	28.6%	41.4%

ECV (97.5%) = 0.25 µg/mL

- ECOFF Finder with 2013-2022 SENTRY North America data



- Conclusion: ECV: Minocycline and *Acinetobacter* spp.
  - For the purpose of breakpoint setting, the ad hoc working group recommends using an ECV of 0.5 µg/mL for *Acinetobacter* spp. and minocycline (no EUCAST data)

- PK/PD
  - Summary of minocycline PK/PD for Acinetobacter spp.
    - PD data exploring plasma fAUC/MIC targets against Acinetobacter limited to Tarazi and USCAST studies
      - fAUC/MIC values associated with 1-log kill vastly different at 21.1 (Tarazi) and 2 (USCAST)
    - Contemporary human PK data limited to The Medco poster (healthy volunteers) and ACUMIN study (ICU patients)
      - fAUC values associated with 200mg Q12h dose vastly different at 80.7 (Medco) and 7.18 (ACUMIN) mg · h/L
    - PTA analysis from ACUMIN used low fAUC (7.18 mg · h/L) and higher PD target (21.1) resulting in maximum achievable MIC of 0.5 mg/L for 1-log kill at 200mg Q12h dose
    - PTA analysis from USCAST used ACUMIN popPK and lower PD target (2) resulting in maximum achievable MIC of 1 mg/L for 1-log kill at 200mg Q12h dose
    - Pre-clinical *in vitro* and/or *in vivo* work demonstrates adequate antibacterial activity (stasis to ≥1-log kill) and efficacy (60-100% survival) against MICs ≤2 mg/L

- Bacterial growth/regrowth and poor efficacy (20-40% survival) observed at MICs 4-32 mg/L
  - Medco data suggests doses >200mg Q12h have poor tolerability and unpredictable PK
- Clinical
  - 7 retrospective studies of PO or IV MIN alone or in combo against MDR *A. baumannii* from 1998-2015 including 126 patients
    - 74.6% pneumonia, 18% blood
    - 12 patients monotherapy (no BSI)
  - Only 1 study (Goff et al. CID 2014) reported outcomes by MIC (Etest)
    - 73% clinical success with MIN alone or in combo
    - 78% microbiologic eradication
    - Three (7%) patients with eradication had subsequent clinical failure, all 3 had MIC of 3 mg/L
  - A Review of Intravenous Minocycline for Treatment of Multidrug Resistant *Acinetobacter* Infections (Ritchie et al 2014)
    - No adverse events (AEs) among 24 severe non-*Acinetobacter* infections
    - No AEs among 8 patients with MDR GN infections, including 5 *Acinetobacter* spp.
    - IV doses up to 10 mg/kg/day for 72h were safe and well tolerated in 60 patients with acute ischemic stroke
- Summary of data for minocycline/*Acinetobacter* breakpoints
  - ECV at 0.25-0.5 mg/L against *Acinetobacter* based on contemporary MIC distributions
  - Available PK/PD data demonstrate poor antibacterial activity, *in vivo* efficacy, and PTA values against MICs >2 mg/L
  - Higher 200mg Q12h dose is necessary to achieve ≥90% PTA at MICs ≤1 mg/L
    - Dosage aligns with package insert, IDSA AMR guidance, and CLSI breakpoints against *S. maltophilia*
  - Limited safety data in healthy volunteers and in patients suggests doses >200mg Q12h associated with poor tolerability and unpredictable PK
  - Clinical outcomes data largely unhelpful
    - Potential association between MICs of 3-4 mg/L and clinical failure/death in Goff et al.
- M23 cutoffs to set breakpoint Minocycline and *Acinetobacter* spp.

Type of cutoff	MIC (µg/mL)
Epidemiological cutoff value	0.25-0.5 µg/mL
Non-clinical PK/PD cutoff value	0.5 µg/mL (100 mg q12h) 0.5-1 µg/mL (200 mg q12h)
Clinical exposure response relationship cutoff value	Not available
Clinical cutoff	Not available

- *Acinetobacter* AHWG Discussion and Recommendation
  - Current S/I/R breakpoint of 4/8/16 mg/L is too high and needs to be revised
  - Option 1: revise to S/I/R at 0.5/1/2 mg/L (S breakpoint based on 100mg Q12h dose)
  - Option 2: revise to S/SDD/R at 0.5/1/2 mg/L (S breakpoint based on 100mg Q12h dose and SDD breakpoint based on 200mg Q12h dose)
  - Option 3: revise to S/I/R at 1/2/4 mg/L (S breakpoint based on 200mg Q12h dose)

        - Matches breakpoint and dosing for *Stenotrophomonas*
        - AHWG Vote: 7-0-0-0
    - Option 4: Something else?
- BPWG Discussion and Recommendation
    - No bimodal distribution of minocycline MICs (like with B-lactams) -> more challenging to separate isolates with MICs of 0.5 and 1 µg/mL (an argument against an S-DD approach)
    - Concern that clinicians will use the 100 mg q12h dosing when the breakpoint proposal is based on 200 mg q12h
    - Sponsor noted 83% of IV minocycline was used at the 100 mg q12h dosage in a survey done a few years ago
    - Safety and tolerability of 200 mg q12h dosage discussed
    - Motion to change the minocycline breakpoints for *Acinetobacter* spp. to S≤1, I 2, R≥4 µg/mL based on a dosage of 200mg q12h. WG Vote: 8-1-1-2.

SC DISCUSSION (MAIN POINTS)

- Concern that users are using a lower dose and should set a breakpoint to the doses users are using.
- Is the tetracycline prediction breakpoint comment accurate if minocycline breakpoint is lowered?
- This is minocycline systemic *Acinetobacter* breakpoint being discussed.
- What about S and SDD to cover the two dosing options?
    - The IDSA guidance is specifically calling out the 200mg dose and users are getting used to the 200mg doses for *Stenotrophomonas*. The dosing is less confusing now because of that.
- Why pick I vs. SDD?
    - If S and SDD, then the SDD would be at 1µg/mL, whereas with I it is 2 µg/mL. Wanted to be consistent with *Stenotrophomonas* and always want an I for testing variability.
- The ECV is 0.5. Not looking at MICs based on meropenem resistant or not. The MIC distributions are different between CRAB vs. non-CRAB.
- Is the notion that TetA does not affect minocycline wrong?
- The *Stenotrophomonas* PD data suggest 0.5 mg/L but had to go with a stasis MIC of 1 µg/mL because would split the wild type with MIC of 0.5.
- The variation in MIC is based on efflux pumps. The true genetically wildtype strains are probably close to 0.25 mg/L and anything above that probably has some efflux pump.
- There is support for the 200 mg dose. About 1/3 of meropenem resistant isolates should still be susceptible to minocycline with the newly proposed breakpoint.
- Concerns that 100 mg dose is not enough for *Acinetobacter*.

A motion to accept the minocycline MIC breakpoints (S≤1, I 2, R≥4 µg/mL) for *Acinetobacter* spp. based on a dosage of 200 mg q12h was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

MINOCYCLINE DISK BREAKPOINTS FOR *ACINETOBACTER* SPP.

- History of minocycline disk correlates for *Acinetobacter* spp.
    - Pre-1980, only tetracycline disk correlates were listed in CLSI (then NCCLS) documents
        - Not organism-specific
        - ≥ 19 mm S (MIC correlate at ≤ 4 µg/mL), ≤ 14 mm R (MIC correlate at ≥ 16 µg/mL)

- In 1981, only tetracycline was listed, but a comment was added to note that tetracycline was the class disk for all tetracyclines, while acknowledging that some *in vitro* data showed that certain organisms may be more susceptible to doxycycline and minocycline than to tetracycline
- In 1982, interpretive criteria for doxycycline and minocycline were added to the document
  - Doxycycline ≥ 16 mm S, ≤ 12 mm R
  - Minocycline ≥ 19 mm S, ≤ 14 mm R
- With increased use of tetracyclines over time, MIC-disk discrepancies were observed
  - "The *Acinetobacter* and Polymyxin Working Group of the CLSI Antimicrobial Susceptibility Testing Subcommittee addressed these concerns via a structured, multicenter comparison of three tetracyclines tested by reference MIC and standardized disk diffusion methods against contemporary strains of Enterobacteriaceae."

- From January 2006 Minutes

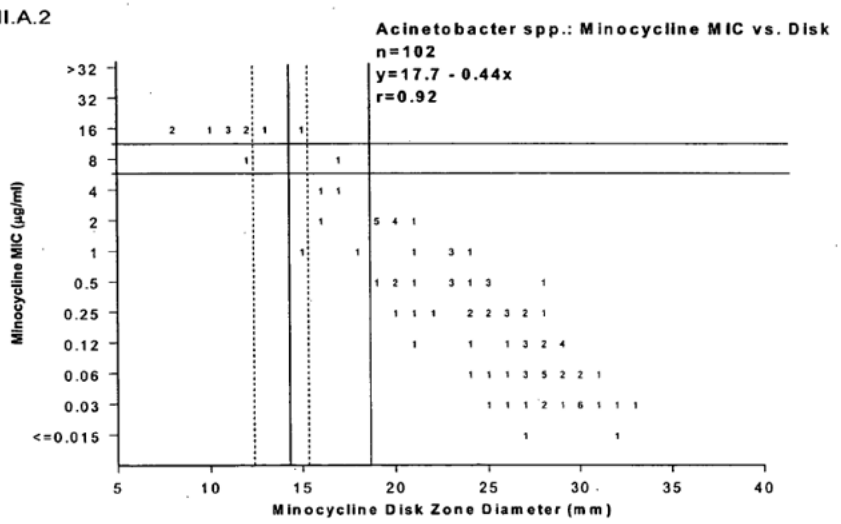
A study was conducted to address the issue of the current disk diffusion breakpoints in M100 that do not work well for *Acinetobacter* spp. for tetracycline, doxycycline, and minocycline (e.g., for the current breakpoints for tetracycline, the error rates: VM 0, M 9, Mi 23; if breakpoints shifted VM 0, M 5, Mi 12). The following changes were proposed **for Table 2B-2 in the M2 part of M100 (Approved 10-1; 1 absent):**

Table 2B-2 in M2 part of M100 for *Acinetobacter* spp.

Antimicrobial Agent	S	I	R
Tetracycline	≥15	12-14	≤11
Minocycline	≥16	13-15	≤12
Doxycycline	≥13	10-12	≤9

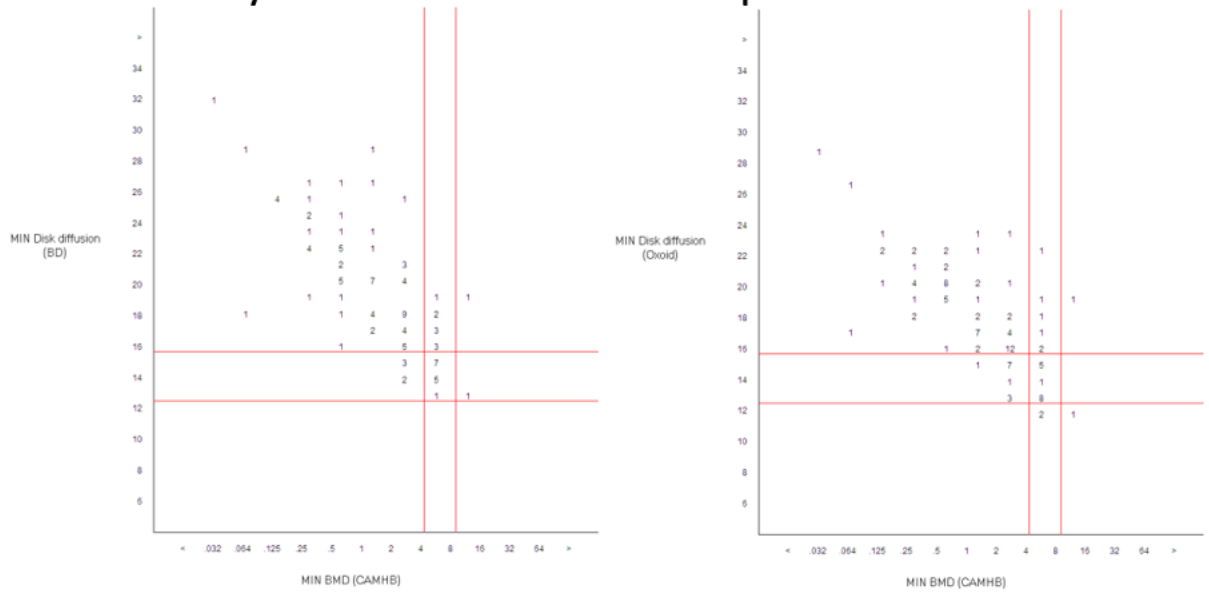
- From January 2006 Agenda Book

III.A.2



- Recent minocycline disk correlate data for carbapenem-resistant *A. baumannii*
  - 107 carbapenem-resistant *A. baumannii* clinical strains from hospitals in PA, MO, NY, NV, CA, and FL between 2009-2015
  - Reference BMD (multiple broth manufacturers), disk diffusion (single disk manufacturer but two media manufacturers), gradient diffusion (single strip manufacturer but two media manufacturers), and Sensititre
  - Testing performed by single lab (biological duplicates by blinded operators on separate days)
  - Note the axes: the presentation of these data are different than what we typically see

Recent minocycline disk correlate data for carbapenem-resistant *A. baumannii*



Wang et al. J Clin Microbiol 2016. PMID: 27629901

- Need additional data for minocycline/*A. baumannii* disk correlates
  - Any other ideas for other data sources?
    - Checked with Mariana Castenheira at JMI, who also checked with the group that developed the more recent formulation of IV minocycline (Olga Lomovskaya/Mike Dudley) – no disk data available
    - Checked with Meredith Hackel at IHMA – no disk data available
    - Are there any minocycline disks that are FDA cleared specifically for use with *Acinetobacter* spp., and if so, are the data supporting clearance available?
  - Reassessment of disk correlates depends on first knowing where the MIC breakpoints will be set (dBETs on AHWG proposed breakpoints as part of backup material)
- Questions for AST subcommittee to move forward
  - What do we do about minocycline/*A. baumannii* disk correlates?
  - What about tetracycline and doxycycline breakpoints that already exist in M100?

TETRACYCLINES							
(13) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.							
Doxycycline	30 µg	≥ 13	10–12	≤ 9	≤ 4	8	≥ 16
Minocycline	30 µg	≥ 16	13–15	≤ 12	≤ 4	8	≥ 16
Tetracycline (U)[b]	30 µg	≥ 15	12–14	≤ 11	≤ 4	8	≥ 16

- Do we publish new minocycline breakpoints first or wait until we've finished with doxycycline or tetracycline?
- Do we need doxycycline or tetracycline breakpoints?

SC DISCUSSION (MAIN POINTS)

- There are media differences.
- Trying to make the 30 µg disk work is probably not possible. The disk mass is likely too high because it was designed for a higher MIC breakpoint, so additional testing will not fix this problem.
- Proposed to change disk breakpoints to S≥22, I 18-21, R≤ 17 mm. Current breakpoints are S≥16, I 13-15, R≤ 12 mm. This data was presented in the *Acinetobacter* AHWG presentation.
- Could add a comment that isolates that test intermediate to perform confirmatory MIC testing.
- This is a CRAB heavy dataset, so more challenging than routine methods.
- Voting on the concept to resolve the disk intermediates with BMD.

A motion to accept the minocycline disk breakpoints (S≥22, I 18-21, R≤17 mm) for *Acinetobacter* spp. with a comment to test the MIC with an intermediate result was made and seconded. Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

SC DISCUSSION (MAIN POINTS)

- Suggestion to archive the doxycycline and tetracycline breakpoints.
- Is doxycycline used in the world outside the US for *Acinetobacter*?
  - South Africa uses doxycycline.
- Tetracycline is a urine breakpoint.
- There are limited data for doxycycline or tetracycline.
- Need to remove the comment on using doxycycline to predict minocycline.
- The old oral tetracycline used to be excreted from the urine. This is different for doxycycline and minocycline.
- The AHWG did pull the doxycycline and tetracycline MIC distributions and the ECV is probably similar to minocycline.
- Add a comment in M100 explaining the breakpoints are archived and under review.
- The AHWG will look at this topic again in January.

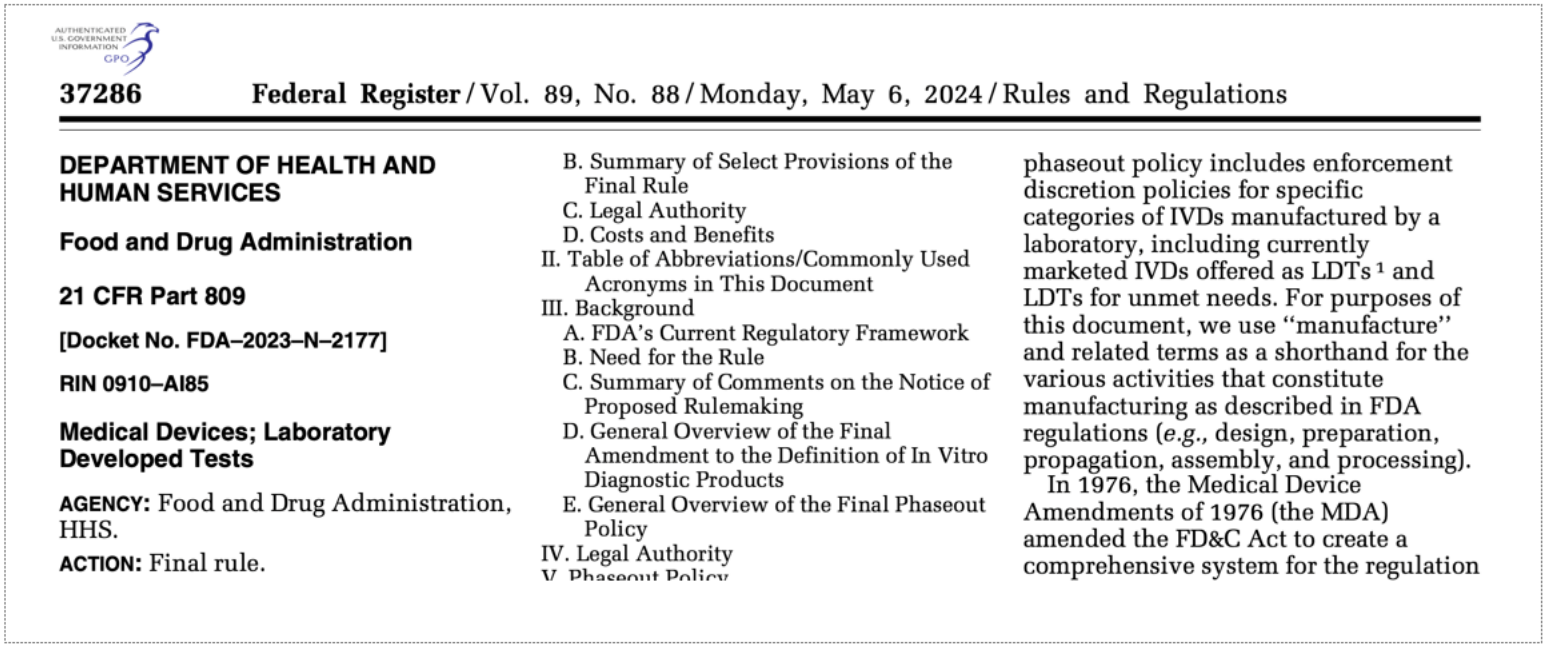
A motion to archive the doxycycline and tetracycline MIC and disk breakpoints for *Acinetobacter* spp. with a comment to indicate that they are under review and to eliminate the prediction comment was made and seconded. Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

3. **CLSI, BREAKPOINTS, AND FDA (R. HUMPHRIES)**

Dr. Humphries provided an update on the future of CLSI and FDA breakpoints with the LDT ruling. The main points included:

- Pre-May 2024
  - FDA breakpoints = "STIC", listed on website
  - CLSI breakpoints = listed in M100, M45
    - CLSI process to submit rationale for breakpoint to FDA for recognition via 21st Century-Cures defined process
  - Manufacturers must use STIC breakpoints when clearing devices
    - No current requirement for a manufacturer to update the STIC applied to their device when an update is made to STIC by FDA
    - Predetermined Change Control Plans (PCCP) finalized in 2023 reduces burden to update breakpoints on IVDs by manufacturer
  - Vast majority of laboratories in USA use FDA cleared IVD ASTs, if available
    - May modify to apply current CLSI breakpoints (= LDT)
    - Labs are required, if CAP-accredited, to update to most current FDA breakpoints, within 3 years of their publication, even if their device is not FDA-cleared for the most up to date FDA STICs
    - Labs may, per CAP, use CLSI or EUCAST breakpoints
  - If no FDA STIC, No FDA-cleared test possible
    - Examples: Some M100 breakpoints, nearly all M45 breakpoints
    - Laboratories use LDTs, either in-house or offered by a reference laboratory, to fill this gap
- What are examples of "no FDA STIC"?
  - *Acinetobacter* spp.
    - Cefepime
    - Polymyxin B/colistin
  - *Salmonella, Shigella*
    - Azithromycin
  - *E. faecium*
    - Daptomycin
  - *N. gonorrhoeae*
    - Azithromycin
  - *N. meningitidis*
    - Ciprofloxacin
    - Levofloxacin
  - *Staphylococcus*
    - Doxycycline
    - Trimethoprim-sulfamethoxazole
  - *S. maltophilia*
    - No breakpoints
  - Nearly all M45 organisms
  - "Non-Enterobacterales"
- Examples of impact: SXT and Doxycycline, *S. aureus*
  - IDSA treatment recommendations:
    - Empiric therapy, diabetic foot infections if MRSA risk

        - SSTI with surrounding cellulitis
        - Osteomyelitis (in combination with rifampin)
        - Alternatives: clindamycin, linezolid (cost, high resistance rates, side effects and drug interactions)
        - 7.5 million prescriptions for SXT and 4.5 million prescriptions for tetracyclines for SSTI ambulatory visits in US, 2011-2016
    - Currently tested on "legacy" devices using old clearance by FDA
    - No FDA STIC
- Examples of impact: M45 organisms
    - "infrequently isolated" in clinical labs
    - Cause serious infections, esp. immunocompromised patients
    - Example: *Aerococcus* spp.
        - ARUP performs ~380 isolate tests per year (data from M45 development)
        - VUMC, 257 unique patients, including 11 with serious infections (endocarditis, bacteremia)
        - Resistance is not predictable
    - No FDA STIC
    - M45 Update planned next year
- May 6



AUTHENTICATED U.S. GOVERNMENT INFORMATION GPO

37286 **Federal Register** / Vol. 89, No. 88 / Monday, May 6, 2024 / Rules and Regulations

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 809

[Docket No. FDA–2023–N–2177]

RIN 0910–AI85

Medical Devices; Laboratory Developed Tests

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

phaseout policy includes enforcement discretion policies for specific categories of IVDs manufactured by a laboratory, including currently marketed IVDs offered as LDTs [1] and LDTs for unmet needs. For purposes of this document, we use "manufacture" and related terms as a shorthand for the various activities that constitute manufacturing as described in FDA regulations (*e.g.*, design, preparation, propagation, assembly, and processing).

In 1976, the Medical Device Amendments of 1976 (the MDA) amended the FD&C Act to create a comprehensive system for the regulation

    - Key Points
        - Change to FDA's oversight of laboratory developed tests as outlined in Food, Drug and Cosmetics Act: "*In vitro* diagnostic products are those reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of

the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body. These products are devices as defined in section 201(h)(1) of the Federal Food, Drug, and Cosmetic Act (the act) and may also be biological products subject to section 351 of the Public Health Service Act, including when the manufacturer of these products is a laboratory."
        - Phased implementation, 2024 – 2028
        - For some LDTs, FDA will continue enforcement discretion of some components:
            - LDTs offered before May 6, 2024
            - LDTs for unmet need, if offered by a laboratory to patients within an integrated healthcare system
            - Still requires MDR correction, removal and reporting, registration, listing and labeling
- Serious Concerns
    - If there is no FDA STIC, there is no FDA cleared test.
    - LDTs have met this gap to date.
    - If LDTs must seek FDA clearance, where does that leave us?
    - Questions:
        - Is use of CLSI breakpoints an "unmet need"?
        - What if there is a STIC, but it differs?
        - How will reference laboratories offer these unmet need tests?
        - What does this mean for innovation?
        - What will this mean for patient care?
- FDA response to concerns
    - FDA has cleared hundreds of ASTs and ensured most up to date STIC are used
    - More than 60 ASTs with breakpoint change protocols, allowing rapid adoption of updated breakpoints without further FDA review
    - Recent final guidance on AST System Devices – Updating Breakpoints in Device Labeling = least burdensome approaches to update device labeling with updated FDA STICs
    - Disagrees that there are no FDA breakpoints for CDC urgent and serious threats -> disconnect between clearance of tests for organisms with specific resistance qualifiers (eg, distinct STIC for MRSA vs. MSSA) versus the tests used to define CDC threats or test antimicrobials used to treat the threats
- Examples of "no FDA STIC" – Urgent AR Threats

Threat Level	Antibiotic Without FDA Breakpoints	Current Testing Method and Gaps Imposed by FDA Proposed Rule
Urgent threat		
Carbapenem-resistant *Acinetobacter*	Polymyxins	Currently tested using an LDT. No testing possible under FDA proposed rule.
Candida auris	All antifungal agents	Currently tested using an LDT and interpreted using epidemiological cutoff values available from CDC and CLSI. No testing possible under FDA proposed rule.
Carbapenem-resistant Enterobacterales	Carbapenems	FDA breakpoints available for some members of the Enterobacterales group, but not all. Key exceptions include meropenem for *Klebsiella aerogenes* (ie, no FDA indication for meropenem against *K. aerogenes*). Currently tested using legacy devices or LDTs. Significantly reduced testing available under FDA proposed rule.
Drug-resistant *Neisseria gonorrhoeae*	Azithromycin	FDA rejected CLSI breakpoints. Testing not routinely performed outside public health laboratories, which use LDTs. No testing possible under FDA proposed rule.

- FDA response to concerns continued
  - Disagree that most AST are LDTs
    - Referenced Simner et al showing 95.3 -98.8% of CAP labs use IVD for AST
    - Note: the tests chosen for Simner et al were specifically those that had an IVD available, to evaluate distribution of laboratories using outdated STICs despite an IVD being available
    - Disagreed that Simner et al show that IVDs are being modified to current STICs (this was not discussed in the paper)
  - Disagree that there is a lack of FDA-cleared tests for uncommon organisms
    - FDA STIC for organism groups for Table 1 of M100
    - Some lack FDA breakpoints, due to lack of adequate data (clinical, PK/PD or *in vitro* data)
    - "In many cases, there are no breakpoints established by CLSI / EUCAST either"
    - "It is important to note that any stakeholder, including a test manufacturer, also has the ability to submit a request to FDA for recognition of a particular breakpoint"
- More comments from FDA final rule
  - "Generally, updating the STIC could significantly affect the safety and effectiveness of the AST system device and would therefore require a 510(k) submission prior to updating the device labeling."
  - Can follow the PCCP to avoid a full submission
  - For laboratories that are already offering AST devices as LDTs, FDA intends to exercise enforcement discretion and generally not enforce premarket review and QC requirements
  - Future updates to breakpoints of currently marketed ASTs offered as LDTs are within the scope of this enforcement policy, provided that such update is validated, does not change indications for use, does not alter operating principle does not include significantly different technology, does not adversely change the performance or safety specification of the AST.
  - For a modification to the breakpoint of an IVD currently offered as an LDT to be considered clinically validated, FDA expects the updated breakpoint to reflect that identified on the STIC website.

- Risks
  - Clinical validation requires use of FDA STIC
  - If FDA and CLSI do not align, must laboratories use FDA STIC?
  - As devices are updated, loss of claims for common drug/bugs (eg, *S. aureus* and doxycycline; *S. maltophilia*, etc.)
  - Impact on innovation (rapid ASTs)
  - If no FDA STIC, may fall under "unmet need"
    - However, reference laboratories would not be able to offer these tests to patients outside their own integrated healthcare systems if developed after May 6, 2024
    - What does this mean for updates to M45?
- One solution: continued collaboration between CLSI and FDA CDER

Document	Breakpoint Submitted	FDA decision	Rationale
MR01-Ed1 MR01-Ed2	Colistin/PMB for Enterobacterales *P. aeruginosa, Acinetobacter*	Not recognized	PK/PD concerns
MR02-Ed1	Fluoroquinolones	Recognized	
MR04-Ed1	NG-Azithromycin	Not recognized	Relevance to current treatment needs unclear
MR03-Ed1	Meropenem/ Acinetobacter spp.	Recognized	
MR06-Ed1	Daptomycin / Enterococci	Partial	Do not recognize *E. faecium*
MR05-Ed1	Ceftaroline / *S. aureus*	Not recognized	Dose
MR07-Ed1	Cefazolin/ Enterobacterales (systemic)	Recognized	
MR08-Ed1	Cefazolin uUTI	Not recognized	Point to STIC for individual drugs
MR14-Ed1	TZP / Enterobacterales	Partial	Do not recognize SDD
MR15-Ed1	TZP / *P. aeruginosa*	Not recognized	Disagree on analysis

- How do we move forward in CLSI AST SC?
  - Ongoing disconnect between FDA and AST SC on breakpoints is an even more pressing concern with FDA regulation of LDTs
  - Need improved communication, joint decision making
  - Alignment, when possible, is critical as we move forward
  - Recommendation: Ad hoc working group to evaluate these concerns

SC DISCUSSION (MAIN POINTS)

- Need to come up with a plan for organism name changes and naming organism groups/complexes because it can affect if an organism is covered in the package insert for a given drug.

	• For LDTs implemented before the May 6th deadline, even though they do not need a 510K, there are still steps that lab must go through for those existing LDTs. This will be a significant amount of work.
	• Can CLSI help define "unmet need"?
	  ○ The FDA needs to define "unmet need".
	• There is an opportunity for CLSI and FDA to work together.
	• Is agar dilution an LDT?
	  ○ The FDA answered yes.
	• CLSI should submit a formal member/liaison request from the FDA Standards Organization (CDRH) to request for FDA and CDRH be a part of the CLSI committee to work on harmonizing with the FDA.

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

2024 JUNE AST MEETING SUMMARY MINUTES PLENARY 3: Tuesday, 25 June 2024 (In-person) 7:30 AM – 12:00 PM Central Standard (US) Time	
#	**Description**
1.	**OPENING** Dr. Lewis opened the meeting at 7:30 AM Central Standard (US) time.

2. **METHODS APPLICATION AND INTERPRETATION WORKING GROUP (K. JOHNSON)**

APPENDIX A REVISIONS

- Suggestions
  - Adding sulbactam-durlobactam for *Acinetobacter* (category I vs. II?)
  - How are the organisms organized, should it be same as Tables 2?
  - Review categorization of cefiderocol for Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter* (should it be in II instead of I for any of these organisms? Probably still appropriate as a I for *Stenotrophomonas maltophilia*)
  - Review categorization of colistin/polymyxin B – does this still belong in category I?
  - For *Salmonella*, if the fluoroquinolone criterion is I/R (versus R), maybe more appropriate as a category III?
  - For *Shigella*, both fluroquinolone and azithromycin R have become more common – still category II, or move to III?
  - For *Pseudomonas aeruginosa* and AG criteria, do we still want to list amikacin, or do we want to just focus on tobramycin now that amikacin BP is for urine only?
  - For pneumococcus, look at cephalosporin III non-meningitis R versus carbapenem I/R positions; at least in some data sets, meropenem I/R is more common than ceftriaxone non-meningitis R, but the existing categorization may suggest otherwise
  - For viridans group streptococci, should carbapenem NS be moved out of category I?
- Discussion: What is the difference between Category I and II?
  - What do labs do differently?
  - Category 1 is sometimes an indicator for labs to look at to ensure their system/process for testing are working (is this a testing error or true resistance).
  - Labs use this table routinely
  - Resistance could vary by region or country
  - Category 1-Resistance is rare vs Category 2-Resistance is rare at one's institution
  - This would lead to most drug/bug combinations to be Category II
  - How would we specifically define each category?
  - Should Category I and 2 be combined?
  - Appendix A needs an introduction to clarify
- Future work from MAIWG
  - Introduction paragraph
  - Clear definitions
  - Reevaluate current resistance categorization
  - Bring back to January 2025 CLSI Meeting
  - Other AST groups
    - VETWG-Robert Bowen
    - Fungal-Tanis Dingle
- MAIWG Discussion and Recommendation
  - Motion to add sulbactam-durlobactam I or R for *Acinetobacter baumannii* complex to Category I in Appendix A. WG Vote: 8-0-0-3.

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	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	
Acinetobacter baumannii complex	β-Lactam combination agents	Sulbactam-durlobactam-I or R	X		
	Cephems	Cefiderocol – I or R	X		
	Carbapenems	Any carbapenem[c] – I or R			X
	Lipopeptides	Colistin/polymyxin B – R			

- Motion to align Appendix A organisms with Tables 2. WG Vote: 8-0-0-3.

- Any *Enterobacterales*
- *Acinetobacter baumannii* complex
- *Pseudomonas aeruginosa*
- *Stenotrophomonas maltophilia*
- *Haemophilus influenzae*
- *Neisseria gonorrhoeae*
- *Enterococcus* spp.
- *Staphylococcus aureus*
- *Staphylococcus* spp. other than *S. aureus*
- *Streptococcus pneumoniae*
- Streptococcus, β-hemolytic group
- Streptococcus, viridans group
- *Neisseria meningitidis*
- *Bacteroides* spp. and *Parabacteroides* spp.

- Any *Enterobacterales*
- *Pseudomonas aeruginosa*
- *Acinetobacter baumannii* complex
- *Stenotrophomonas maltophilia*
- *Staphylococcus aureus*
- *Staphylococcus* spp. other than *S. aureus*
- *Enterococcus* spp.
- *Haemophilus influenzae*
- *Neisseria gonorrhoeae*
- *Streptococcus pneumoniae*
- Streptococcus, β-hemolytic group
- Streptococcus, viridans group
- *Neisseria meningitidis*
- *Bacteroides* spp. and *Parabacteroides* spp.

SC DISCUSSION (MAIN POINTS)

- Public health uses the category 1 as a decision making point for which isolates should be submitted to public health.
- What about *Acinetobacter* NDM? They are seen outside of the US.
- There is concern that this table could be too specific to the US.

A motion to add sulbactam-durlobactam I or R for *Acinetobacter baumannii* complex to Category I in Appendix A was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

A motion to align the order of presentation of Appendix A organisms with Tables 2 was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

BURKHOLDERIA CEPACIA COMPLEX UPDATE

- Previous recommendations
  - January 2024: CLSI AST Subcommittee voted to remove *Burkholderia cepacia* complex (BCC) breakpoints based on data showing that reference broth microdilution and agar dilution do not correlate
  - Follow-up for BCC AHWG:
    - Obtain feedback from cystic fibrosis (CF) and lung transplant providers on how removal of AST breakpoints will impact their practice
    - Develop a guidance document for providers and laboratories
    - Determine Epidemiological cut-off value (ECVs) for BCC-potential drugs will be listed in Tables 1 of M100 and labs can direct clinicians to the ECV for guidance
- Feedback from lung transplant providers
  - Removing breakpoints will make labs more reluctant to do any testing. Not great from my perspective. *B. cepacia* complex colonization is a relative contra-indication for transplant at our center, irrespective of MICs. If someone does move forward, we use MIC to guide peri-transplant antibiotics.
  - We will transplant lungs with *B. cepacia* IF we think that there are active agents. We no longer do the checkerboard testing we used to do for *Pseudomonas*, but need some guidance (even if imperfect) for these patients. We do care about exact species also. Often this is the only option for young CF patients. The goal should be better testing rather than none I think.
  - While we usually decide based on species, we do consider whether the organism is susceptible or resistant – without a specific MIC cutoff – but requiring some active drugs. If pan-resistant or unobtainable MIC this is a contraindication in general to lung transplant. We historically have viewed *Burkholderia cenocepacia* and *Burkholderia dolosa* as contraindications and have transplanted *Burkholderia multivorans, gladioli, vietnamiensis*. We have also used the LiPuma lab at UMichigan for identification – they may provide added information.
  - I also share the concerns from others here of not having enough data to guide treatment decisions for not only lung transplant candidates but also recipients who may unfortunately come down with a *Burkholderia* infection. We rely on those MICs for all the situations you mentioned. I suppose removing breakpoint interpretations does not preclude micro labs from performing susceptibility testing but I think there is definitely a risk of labs, particularly the more satellite ones, of not performing it in some situations.
  - Conclusion: Decisions are based on species and all mention they do look at susceptibility.
- Feedback from CF providers
  - We don't have a lot of *B. cepacia* in our clinic but I have always taken an empirical approach to treatment rather than a susceptibility-guided one. This is similar to my approach for people infected with *P. aeruginosa*, where there is a similar phenomenon. I'd be in favor of ending routine susceptibility testing for *B. cepacia*.
  - The North American experts on have warned us about these issues. There's a bigger question here, which is whether there is any utility of *in vitro* susceptibility testing for any bacteria in chronic CF respiratory infections in general. There are good data showing little clinical

utility for either *P. aeruginosa* or *S. aureus*. For *Burkholderia*, there are additional reasons not to do it. But many of us feel that: A demonstration of high-level *in vitro* resistance is meaningful. Susceptibility, not so much, whether intermediate or complete. But resistance is probably a good reason to avoid an antibiotic if one is certain about what the target is. If someone just isn't responding to an antibiotic either by microbiologic or clinical criteria, we tend to ask for both standard and extended testing for less common agents (like beta-lactam combinations or beta lactam-lactamase inhibitor combinations).
    - Dr. John LiPuma: As you likely know, there has been an effort during the past several years to dissuade CF care centers from using routine AST in caring for pwCF…there is the broader issue of why using AST to guide antibiotic therapy in CF has limited utility in predicting treatment outcomes…the ID doc in me can't entirely let go of AST. My sense is that *in vitro* AST is more likely to underestimate *in vivo* resistance. So, I'm inclined to take R more seriously than S. Put another way, when I have a patient who is doing very poorly (eg, end stage disease or bacteremic), I may look at AST. If I see a bunch of Rs and maybe an I or S (or a relatively low MIC), I find it hard not to at least try the latter drug(s). There is admittedly some ID magic thinking here, but when the patient's back is to the wall - magic may be the only thing left. About transplant eligibility…I shudder to think about patients who were denied transplant in the past on the basis of AST.
    - Conclusion: Mixed responses from CF providers, some in favor of not testing. Knowing resistance may be helpful.
- Guidance document
    - How will these changes impact clinical microbiology laboratories and providers?
    - These changes will have a significant impact on clinical microbiology laboratories and providers, specifically those that treat people with CF and perform lung transplant. CLSI recommends that due to the problems with BCC AST, clinical microbiology laboratories should not perform routine AST and only perform testing upon request by the provider. If AST is requested, reference BMD (frozen) is the recommended method; AD, DD, gradient diffusion, and some commercial methods are unreliable. Reference BMD (frozen) should be reported without interpretations of susceptible (S), intermediate (I), or resistant (R), and providers should refer to the BCC epidemiological cut-off value (ECV) for guidance in interpreting minimal inhibitory concentrations. For providers, AST is not routinely recommended due to the issues described above.
    - Where will this document go? Clinical Microbiology Review article on CF Microbiology or Outreach Working Group for education
- What about commercial BMD?
    - One lab (UNC) compared subset of isolates 42 isolates from the study to manually read Trek panel
        - Split CF vs Non-CF
    - In general there was a bias towards lower MICs with the commercial panel
    - Essential agreement was as follows
        - Ceftazidime - 90.5% (38/42)
        - Meropenem – 85.7% (36/42)
        - Minocycline – 76.2% (32/42)
        - Levofloxacin – 85.7% (36/42)
        - TMP/SMX – 97.6% (41/42)
    - Several limitations to the study (single lab, single inoculation, single manufacturer and type of plate)
- *Burkholderia cepacia* complex (BCC) Epidemiological Cutoff Values
    - Materials and methods
        - MIC data for ceftazidime (CAZ), levofloxacin (LVX), meropenem (MEM), minocycline (MIN), and trimethoprim-sulfamethoxazole (TMP-SMX)
        - CLSI BCC AHWG studies:

- 100 cystic fibrosis (CF) isolates
- 105 non-CF isolates
- Used mode MIC value; otherwise use single replicate value

▪ EUCAST:
- 159 CF isolates
- Mode MIC value of ISO BMD performed in triplicate

▪ MIC values for BCC generated at IHMA, JMI, and Microbiologics using CLSI reference BMD
▪ Used ECOFFinder to determine ECVs:
- https://clsi.org/meetings/susceptibility-testing-subcommittees/ecoffinder/
- Entered data following the user instructions
- Truncated data (eg, >64 became 64)

o BCC species by laboratory/study
o Distribution of isolates by laboratory/study
▪ 50% isolates came from one lab for MIN and TMP-SMX
▪ Data not weighted as recommended in M23 due to lack of instructions/knowledgeable person to do this.
o Epidemiological cut-off values
▪ ECVs weren't too far off from the BCC MIC breakpoints.
▪ ECV are so high, that may not be truly a wildtype.
▪ The dilution ranges presented in the tables are the ranges tested.
o BCC ECV Limitations
▪ Wild-type versus non-wild-type population not defined
▪ ECV is at the complex level
- Not enough isolates by species to create an ECV for each drug
- 78% isolates not identified to the species-level
- Not all species represented

▪ EUCAST data is not CLSI reference BMD
- Removing EUCAST Data did not change the ECVs except TMP-SMX.

▪ Majority of data is from 2 laboratories
- > 50% of MIC data was provided by one laboratory for MIN and TMP-SMX

- Feedback from reference labs
o Asked for feedback from reference labs on how they will approach not having breakpoints for BCC
▪ Quest
- Poor performance of Vitek2. Will continue offering testing on Vitek2 until new M100 is released and then stop testing with Vitek2.
- SJC location: continue to offer testing and report MIC only without interpretation once the new M100 is released (Trek panel).

▪ LabCorp Seattle:
- Currently use Microscan but if No AST for BCC is recommended; When identified, add a report comment that no AST is performed.

- ARUP:
  - Providers still rely on MICs for dosing
  - Likely continue BMD and just report MICs

- Summary
  - Provider feedback:
    - Lung transplant providers would still prefer to have AST and use the results to guide lung transplant eligibility
    - CF providers want AST for difficult cases but understand that it does not predict clinical outcomes nor is reliable
  - Current guidance document recommends:
    - AST only upon provider request
    - Perform reference broth microdilution (frozen)
    - Reference labs will differ on how they are handling these requests
  - ECV
    - Limitations to data
    - Discussion among AHWG on whether it will be useful, confusing, and/or encourage testing
    - Should it be moved to M45 with a comment on testing?
  - BCC AHWG recommendation not to publish ECVs.
- MAIWG Discussion and Recommendation
  - Concerns about reporting MIC-only
  - One method is reproducible but correlating to clinical outcomes is unknown.
  - How does this impact how providers interpret MIC only?
  - Will people set their own "breakpoints"?
  - Move it to M45
  - Take it out completely from M100. Eventually, there may be some drugs that have activity for BCC in the pipeline.
  - Reinstitute breakpoints with caveats or answer their questions of ECV.
    - International perspective: BCC is not only a concern for CF patients as South America has an outbreak of BCC with contaminated medical products. Also, South America doesn't have access to newer drugs that may have activity.
    - If we were starting from scratch, and trying to add these BCC BPs to M100, would we be able to do it? No. So why are we leaving it in? It will be painful for providers and transplant centers, so we need education and guidance.
    - Clinical Microbiology Review could be an aspect to help promote the messaging. Labs will need to come up with a report comment.
    - IF we remove from M100, we need a rationale in its place. We can state that ref BMD is reproducible and that's it (leaving out reporting an MIC only). However, this could still be misinterpreted.
    - Suggestion: Holly publish data and CLSI can cite it.
    - Worried if we pull it out, then are asked later, will they test and use another BP?
    - Keep rationale for a few years then reevaluate the landscape.
    - M45 is in final draft status and these changes wouldn't make this revision cycle. Keep in M100 a rationale for removal in M100 then move to M45 during next update cycle.
  - Motion to not include ECVs for *Burkholderia cepacia* complex for CAZ, LVX, MEM, MIN, SXT in M100. WG Vote: 7-1-0-3 (Does not pass).
    - No Votes: Expressed concerns about what labs/clinicians will do without context or rationale as why no BPs or ECVs are provided

- If Subcommittee agrees with not adding ECVs:
  - Remove breakpoints-add frozen BMD conditions to Tables 2 with a comment
  - Remove breakpoints-add comment to Tables 2.

SC DISCUSSION (MAIN POINTS)

- Concern of being too harsh on the ECV data. Suggestion to publish the ECV and complete future work/studies to look at reproducibility BMD.
- It is hard to differentiate the species in the complex, so the ECV could look different between species.
- For antifungal AST documents, there is a footnote stating this is a high ECV and treat it with caution.
- ECVs are method dependent, and labs need to validate the ECV.
- Concern that providers will use an ECV. Do not have PK/PD data. There are new drugs like cefiderocol or BLBIs that have activity (no breakpoints). Suggestion to not include the ECV because they will be treated as a breakpoint.
- Have not done enough work with reference method BMD vs. lyophilized panels to say the method needs to be further studied/compared.
- Need to provide clear guidance to labs.
- The current language states to manually read BMD.
- If AST is not done routinely, labs need to collect these isolates. Maybe there is a role for public health here. Does not want all testing to go away.
- Inoculum matters, did the UNC/BMD study use different inoculums?
  - UNC used 30uL (the fastidious volume).
- The AST Intrinsic Resistance AHWG is not developing ECVs for clinical labs to report. This is different for *Burkholderia* where the plan is to give it out clinically. The Antifungal Intrinsic Resistance AHWG is intending for ECVs to be used clinically.
- See *Burkholderia* in bacteremia or contaminated products, so it is clinically relevant not just in the CF community.
- Physicians still want AST for specific cases. Concern that people will use ECVs as a breakpoint.
- For CF patients, it is easier to not be beholden to AST; however, for a bacteremia outbreak and physicians would not want to be blind and taking a swing in the dark for the bacteremia.
- If no guidance is given to providers, they will investigate the literature and find a poorly done gradient diffusion study. This is the best data that is out there, so ECV should be published.
- Cefiderocol and ceftazidime/avibactam requests are common on this organism group.
- For Tables 2, leave in the table and state there are no breakpoints.
- Need to delete in Tables 1.
- EUCAST is the ISO method.
- The ECVs are 1 to 2 dilutions above the current breakpoints.
- These ECVs really should be evaluated by the ECV working group. Not comfortable with the ECVs until they are reviewed.
- Stating it is tentative is not helpful, should be more specific.
- What parameters are not met with the ECV?
  - Weighting of data.
    - Weighting is the main criteria that is not met.
    - It is only off by ~4% and weighting the data does not really change much.
  - Wild-type vs. non-wild type is not defined.
  - Do not have specific species.

- Labs cannot get to the species level.
- The ECOFF finder stays if species cannot be differentiated, it is acceptable to group organisms in a complex.
- If *B. multivorans* is in the data, it is more resistant and will skew the ECV.

- Action Item: To review *Burkholderia* by the ECV Working Group.

A motion to accept the *Burkholderia cepacia* complex ECVs for ceftazidime (16 µg/mL), levofloxacin (8 µg/mL), meropenem (16 µg/mL), minocycline (8 µg/mL), and trimethoprim-sulfamethoxazole (2 µg/mL), keep the reference method as broth microdilution, and add disclaimers was made and seconded. Vote: 10 for, 3 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:
- Not ready to use ECVs clinically.
- Many more isolates are going to be interpreted as susceptible than with existing breakpoints.
- The M100 says ECVs need to be by species
- Could add a comment about the ECV for the lab report. Say these should never be reported with interpretations.
- The ECV instructions say to report as "wild type" or "non-wild type"
- If using old breakpoints, that is more stringent. Having an ECV could be worse because now it will give people a false of sense of security.
- Can there be a *Burkholderia* folder at CLSI to put all this data? Maybe each organism in M100 should have a folder for data to go. Make it easier to find data.
- Could add a comment that says the ECVs are in the appendix, but do not indicate susceptibility. Report MIC only.

BURKHOLDERIA CEPACIA COMPLEX TABLE 2B-3 REVISIONS

- MAIWG Discussion and Recommendation
  - In the most recent edition of the CLSI M100, DD breakpoints for BCC organisms were removed based on data showing that DD is not reproducible and correlates poorly with reference BMD (5,10). In January 2024, the CLSI AST Subcommittee voted to remove MIC breakpoints for BCC organisms. This decision was based on data showing that two CLSI reference AST methods, BMD and AD, do not correlate. These data agree with previous studies from EUCAST and a Brazilian study published by Fehlberg et al. demonstrating problems with BCC AST (11,12). Because AST results can significantly impact patient care, including people with CF who are eligible for lung transplant, it was voted that the breakpoints be removed. These changes will go into effect in January 2025.
  - CLSI is recommending that BCC organisms not undergo AST as methods are unreliable and should not be used to guide patient therapy or eligibility for lung transplant.
  - Data shows that reference BMD (frozen) is the only reproducible method to determine MICs but the correlation of MICs with clinical outcomes is unknown
  - Motion to present above comments in M100 in place of breakpoints. WG Vote: 8-0-0-3.
- Proposed Plenary Revisions by Romney Humphries and Virginia Pierce (Version shown below is after wordsmithing by the Subcommittee)

Table 2B-3. Zone Diameter and MIC Breakpoints for *Burkholderia cepacia* complex

Testing Conditions

Medium: ~~Disk diffusion: MHA~~
Broth dilution: CAMHB
~~Agar dilution: MHA~~

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C±2°C; ambient air; 20-24 hours~~, all methods~~

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®[a] 25922 (for chloramphenicol, minocycline, and trimethoprim-sulfamethoxazole)
Pseudomonas aeruginosa ATCC® 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of ß-lactam combination agents.

~~When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.~~

General Comments

(1) MIC and DD breakpoints for BCC organisms were removed based on data showing that two CLSI reference AST methods, BMD and AD, do not correlate. These data agree with previous studies from EUCAST and a Brazilian study published by Fehlberg *et al*. demonstrating problems with BCC AST (11,12). Epidemiological cutoff values are available, in Appendix XX, which are for epidemiological use only. In several cases, ECV are above MICs typically achievable by routine dosing for similar organisms.
(2) Laboratories may consider adding the following comment to the clinical report: "Antimicrobial susceptibility testing is not routinely performed for *Burkholderia cepacia* complex due to the lack of accurate test methods. Wild-type MICs are high and may be above MIC typically achievable by routine antimicrobial dosing."
(3) If testing is performed, reference BMD is the only reproducible method and include the comment, "Correlation of MIC values with clinical outcome is not known."

~~(4) Refer to Table 1E for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.~~

~~(5) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[1] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see the *M02 Disk Diffusion Reading Guide*[2]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a~~

SC DISCUSSION (MAIN POINTS)

- The "intrinsic resistance" should be removed. Have not reviewed it.
- Intentionally removed a lot of intrinsic resistance wording for *Burkholderia*.
- Need to harmonize the antimicrobial resistance table as well.

- Suggestion to remove the word “intrinsic resistance” and state “limited susceptibility”.
- There was agreement that there would be a warning comment.
- Suggestion to change “limited susceptibility” to “limited clinical activity”.
- There is an inconsistency earlier in the document saying that ECVs are for specific species.
    - This table will be under review. The ECV working group can clarify.
- Action Item: Look at intrinsic resistance for *Burkholderia* in January.

A motion to accept the Table 2B-3 revisions was made and seconded. Vote: 12 for, 1 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:

- Not enough data.

BURKHOLDERIA CEPACIA COMPLEX APPENDIX F REVISIONS

- Proposed Plenary Revisions by Romney Humphries and Virginia Pierce (Version shown below is after wordsmithing by the Subcommittee)

Ignore that this says "G" (it's now F) since this is from 33rd edition

Appendix G. Epidemiological Cutoff Values

Abbreviations for Appendix G

ECV epidemiological cutoff value
MIC minimal inhibitory concentration
NWT non-wild-type
WT wild-type

G1 CLSI Epidemiological Cutoff Value Additions/Revisions Since 2015

Antimicrobial Agent	Date of Addition/Revision (M100 edition)	Comment
***Burkholderia cepacia* complex**		
Ceftazidime Meropenem Minocycline Levofloxacin Trimethoprim-sulfamethoxazole	January 2025 (M100-S35)	
Anaerobes		
Vancomycin	January 2015 (M100-S25)	For use with *Cutibacterium* (formerly *Propionibacterium*) *acnes*.

G2 Defining Epidemiological Cutoff Values

G2.1 Definitions

epidemiological cutoff value (ECV) - the minimal inhibitory concentration (MIC) or zone diameter value that separates microbial populations into those with and without phenotypically detectable resistance (non-wild-type [NWT] or wild-type [WT], respectively). The ECV defines the highest MIC or smallest zone diameter for the WT population of isolates.

EXAMPLE:

	ECVs	
Interpretive Category	MIC, µg/mL	Zone Diameter, mm
Wild-type	≤ 4	≥ 20
Non-wild-type	≥ 8	≤ 19

- **wild-type (WT)** - an interpretive category defined by an ECV that describes the microbial population with no phenotypically detectable mechanisms of resistance or reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.
- **non-wild-type (NWT)** - an interpretive category defined by an ECV that describes the microbial population with phenotypically detectable mechanisms of resistance and reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.

Appendix G. (Continued)

G2.2 Epidemiological Cutoff Values vs Clinical Breakpoints

ECVs are based on *in vitro* data only, using MIC or zone diameter distributions. ECVs are not clinical breakpoints, and the clinical relevance of ECVs for a particular patient has not yet been identified or approved by CLSI or any regulatory agency. By contrast, clinical breakpoints are established using MIC distributions, pharmacokinetic/pharmacodynamic data, and clinical outcome data, when available (as described in CLSI document M23[1]).

"Caution": Zone diameter (disk diffusion) and MIC values for which ECVs are defined are not to be interpreted or reported as susceptible, intermediate, or resistant but rather as WT or NWT. The ECVs should not be used as clinical breakpoints.

G2.3 Establishing Epidemiological Cutoff Values

ECVs are determined by collecting and merging MIC distribution data obtained by testing microbes from a variety of sources and then applying statistical techniques for estimating the MIC at the upper end of the WT distribution. Subsequently, corresponding zone diameter data from disk diffusion testing are examined and a disk diffusion ECV is determined, when appropriate. To ensure reliability, ECVs are estimated while accounting for both biological (strain-to-strain) variation and MIC/disk assay variation within and between laboratories. They are based on the assumption that the WT distribution of a particular antimicrobial agent-organism combination does not vary geographically or over time.

Several conditions must be fulfilled to generate reliable ECVs. The most important are:

- An ECV can be determined only within a single species for a single agent because of the genetic diversity between species within a genus.
- All MIC values included in the dataset must have been determined using a standard reference method (eg, the CLSI MIC broth dilution method as described in M07,[2] which is also the method outlined in an international reference standard[3]). Similarly, the standard reference disk diffusion method as described in M02[4] must be used when zone diameter ECVs are defined.
- Data must be sourced from at least three separate laboratories and at least 100 unique isolates must be included in the merged dataset.

Appendix G. (Continued)

- MIC values contributed from an individual laboratory dataset should be "on scale" (ie, the MIC is not below the lowest or above the highest concentration tested), whenever possible. This is particularly important for MICs of the presumptive WT strains. Before merging data from individual laboratories, the MIC distribution from each laboratory must be inspected, and if the lowest concentration tested is also the mode, the data must be excluded.
  - Once acceptable data are merged, there are several methods that can be used to estimate the ECV.
    - Visual inspection is the simplest method and is generally acceptable for MIC distributions when there is clear separation of WT and NWT strains. When there is obvious overlap between WT and NWT strains, visual inspection is too subjective to set a reliable ECV.
    - Statistical methods are preferred because they remove potential observer bias from the estimation. The two most widely referenced statistical methods are those described by Turnidge et al.[5] and by Kronvall.[6]
  - Establishment of ECVs from MIC distributions may be supplemented with molecular tests for known resistance genes. The detection of a resistance gene per se in strains with MICs at or below the ECV does not necessarily contradict the choice of ECV, unless it can be accompanied by evidence that the gene is being expressed. In such cases, the ECV may need to be reassessed.

G2.4 Epidemiological Cutoff Value Use by the Medical Microbiology Laboratory

The need for testing and interpreting drug and organism combinations with an ECV but no clinical breakpoint must be discussed with appropriate clinical specialists (eg, antibiotic stewardship, infectious diseases, and pharmacy). While ECVs do not predict clinical outcome, laboratories may consider noting WT or NWT MIC (or zone diameter) interpretations on laboratory reports. Many physicians may choose not to consider using antimicrobial agents with an NWT interpretation, if other therapeutic options are available. However, it is critical that laboratories refrain from reporting report WT as susceptible, or NWT as resistant, as there are insufficient clinical data to support this practice. ECVs may be used to signal the emergence of resistance, although this application for ECVs is best suited to public health laboratories and surveillance studies.

Appendix G. (Continued)

References for G2

1 CLSI. *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*. 5th ed. CLSI guideline M23.Clinical and Laboratory Standards Institute; 2018.

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

3 ISO. *Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices - Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases*. ISO 20776-1. International Organization for Standardization; 2019.

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

5 Turnidge J, Kahlmeter G, Kronvall G. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. *Clin Microbiol Infect*. 2006;12(5):418-425.

6 Kronvall G. Normalized resistance interpretation as a tool for establishing epidemiological MIC susceptibility breakpoints. *J Clin Microbiol*. 2010;48(12):4445-4452.

G3 Epidemiological Cutoff Value Tables

"Caution": Zone diameter (disk diffusion) and MIC values for which ECVs are defined are not to be interpreted or reported as susceptible, intermediate, or resistant but rather as WT or NWT. The ECVs should not be used as clinical breakpoints.

ECVs listed in Table G1 are applicable only to the species indicated. Currently, there are insufficient data to support their use with other species.

Table G1. ECVs for Specific Anaerobic Species

Antimicrobial Agent	MIC ECV, µg/mL		Comment
	WT	NWT	
Vancomycin	≤ 2	≥ 4	For use with *Cutibacterium* (formerly *Propionibacterium*) *acnes*[1-4] and *Clostridioides* (formerly *Clostridium*) *difficile*.[5-7]

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

Table G2. ECVs for *Burkholderia cepacia* complex[a]

Antimicrobial Agent	MIC ECV, µg/mL		Comment
	WT	NWT	
Ceftazidime	≤ 16	≥ 32	
Meropenem	≤ 8	≥ 16	
Minocycline	≤ 16	≥ 32	
Levofloxacin	≤ 8	≥ 16	
Trimethoprim-sulfamethoxazole	≤ 2	≥ 4	

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type.
[a]Insufficient data were available to establish ECVs for individual species within the complex. Although more than 50% of the data were contributed by a single laboratory for minocycline and trimethoprim-sulfamethoxazole, the data were not weighed before pooling and analysis. The ECVs are under review and will be updated if appropriate.

References for Table G2

Placeholder to add a reference to Holly Huse's forthcoming publication describing the work done to set the ECVs

A motion to accept the Appendix F revisions was made and seconded. Vote: 11 for, 2 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:

- Not enough data.

ANAEROBE AD HOC WORKING GROUP REPORT

- M100 Comments
  - Comment #1:

Comment #	Location	Comment	Proposed Change
8625	Table 2J Testing Conditions Box, comments (5) and (6)	There have been more nomenclature changes and species formerly known as Bacteroides should be included. The current wording stresses only Bacteroides and Parabacteroides. This may be easier than an extended list which may continue to grow.	Replace "*Parabacteroides* spp. only" with "associated genera" in various locations

    - AHWG Vote: 0-11-0-0 (Not approved)
    - The AHWG feels "associated genera" is too vague.
    - The AHWG is looking into the original data behind this comment before voting on alternative wording.
  - Comment #2:

Comment #	Location	Comment	Proposed Change
8625	Table 2J Testing Conditions Box, comments (5) and (6)	There have been more nomenclature changes and species formerly known as Bacteroides should be included. The current wording stresses only Bacteroides and Parabacteroides. This may be easier than an extended list which may continue to grow.	Replace "*Parabacteroides* spp. only" with "associated genera" in various locations

Testing Conditions	
Medium:	Agar dilution (for all anaerobes): Brucella agar supplemented with hemin (5 µg/mL), vitamin K_1 (1 µg/mL), and laked sheep blood (5% v/v) Broth microdilution (for ~~*Bacteroides* spp. and *Parabacteroides* spp.~~*Bacteroides fragilis* and *Bacteroides thetaiotaomicron* only): Brucella broth supplemented with hemin (5 µg/mL), vitamin K_1 (1 µg/mL), and LHB (5% v/v)
Inoculum:	Broth culture method or colony suspension, equivalent to 0.5 McFarland suspension Agar: 10^5 CFU per spot Broth: 10^6 CFU/mL
Incubation:	36°C ± 1°C, anaerobically Broth microdilution: 46–48 hours Agar dilution: 42–48 hours

- The AHWG investigated the original data behind this comment: "Based predominantly on *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*. The remaining *Bacteroides fragilis* group species were less than 2 to 5 isolates per species."
- Anaerobe AHWG recommends "for *Bacteroides fragilis* and *Bacteroides thetaiotaomicron* only"

- Comment #3:

Comment #	Location	Comment	Proposed Change
8623	Table 1J fn c	B-lactamases have been reported in several species of this genus. Although the current text does say 'most', I think that this should also be added to explain/strengthen the fact that it is not 100% for any species.	(c) Penicillin retains good *in vitro* activity against most *Fusobacterium* spp. and may be considered for primary testing and reporting with this genus. Note: presence of β-lactamases have been reported in this genus.

- AHWG Vote: 10-0-0-0.
- MAIWG Vote: 9-0-0-2.

- Comment #4:

8624	Table 1J	Although clindamycin is in the first column, reports of resistance are increasing across number of different genera, including Fusobacterium and Cutibacterium.	Should a note of caution be added, with particular regard to induced resistance? (similar to above)

- Rather than add a footnote the AHWG would like to move the clindamycin to Tier 4. Looking at the increase and current prevalence of anaerobe resistance (reference Manual of Clinical Microbiology – Anaerobe AST chapter – {Schuetz and Carpenter}), the AHWG feels it is no longer appropriate in Tier 1. The AHWG is not aware of inducible clindamycin resistance in anaerobes.
- MAIWG no vote, agreed to not change.

- Comment #5:

8580 and 8581	Table 1J fn e and Table 2J comment (12)	It is odd to refer to Appendix D, since the only non spore-forming gram-positive rod in that table is C. acnes. The comment in this table is referring to many non spore-forming gram-positive rods. I suggest deleting the referral to Appendix D, as it's not really helpful.	(e) Many non-spore forming, gram-positive anaerobic rods are resistant to metronidazole ~~(see Appendix D)~~.

    - AHWG Vote: 10-0-0-0.
    - MAIWG Vote: 9-0-0-2.
  - Table 1J Changes
    - Remove footnote "c" from penicillin (GP Anaerobes).
      - AHWG Vote: 10-0-0-1.
      - MAIWG Vote: No Vote.
    - Footnotes with revisions:
      - (c) Penicillin retains good *in vitro* activity against most *Fusobacterium* spp. and may be considered for primary testing and reporting with this genus. Note: presence of B-lactamases has been reported in this genus.
        - MAIWG Vote: 9-0-0-0.
      - (e) Many non-spore forming, gram-positive anaerobic rods are resistant to metronidazole ~~(see Appendix D).~~
        - MAIWG Vote: 9-0-0-0.
      - (f) [New comment for clindamycin induced resistance]
        - MAIWG Vote: No vote.
    - Text and Table Working Group to revise wording/guidance for footnote (c) and (f) before sending to AHWG
- Pilot validation of disk diffusion method
  - Three sites (Mayo, IHMA and Public Health Wales)
    - Complete before October
    - Present results at January 2025 CLSI meeting
  - Methods
    - Disk diffusion - Fastidious Anaerobe Agar and Brucella Blood Agar (Read at 18+ 2hrs.)
    - Agar Dilution - Brucella Blood Agar (Read 42-48 hrs.)
  - Organisms
    - 27 clinical isolates (10 from Public Health Wales Challenge Set)
      - *Bacteroides* spp., *Prevotella* spp., *Fusobacterium necrophorum*, *Cutibacterium acnes* and *Clostridioides difficile*
    - 3 QC organisms
      - *Bacteroides fragilis* ATCC 25285, *Clostridium perfringens* ATCC 13124, *Clostridium perfringens* DSM 25589 (anaerobic conditions),
  - Antibiotics
    - Meropenem (10 mg) – 0.015 – 32 mg/ml
    - Metronidazole (5 mg) – 0.03 - 64 mg/ml
    - Clindamycin (2 mg) – 0.06 – 16 mg/ml

SC DISCUSSION (MAIN POINTS)

- The consensus of the Subcommittee agreed with the proposed M100 changes. No vote taken.

NOVEL/EMERGING AST METHODS AD HOC WORKING GROUP PROPOSAL

- Recruiting Members
  - Co-Chair: Joe Kuti (MAIWG)
  - Co-Chair: Kevin Alby (MDSWG)
  - Interested Members:
  - Darcie Carpenter
  - Trish Simner
  - Holly Huse
  - Davina Campbell
  - ???
- Proposed Initial Charge
  - Novel AST methods during new drug development
    - What do sponsors need to bring to CLSI when developing a new method?
      - Plastic concerns – compounds sticking to the plastic
      - Zinc concentration in MHB – impact on metallo-ß-lactamases
      - Testing bacteria in RPMI (eg, fungal AST testing medium)
      - Cas Amino Acids (CAA)
      - M9 media
      - Pyridoxal isonicotinyl hydrazone (PIH) for rifabutin agar dilution against *Acinetobacter baumannii*

SC DISCUSSION (MAIN POINTS)

- Need to be thoughtful about making AHWGs. Make sure there something actionable.

THERAPY AGAINST METALLO-ß-LACTAMASES-PRODUCING ENTEROBACTERALES

- Metallo-ß-Lactamases (MBL): Role of Zinc Atoms
  - Zinc is essential to ß-lactam binding and hydrolysis.
  - Metal loss from the active site of NDM-1 under extracellular Zn(II) restriction generates a degradation-prone apo-enzyme which is targeted by periplasmic proteases catalyze the hydrolysis of ß-lactams, required for protein folding and apo-stabilization in the periplasm.
- Background
  - *In vitro*/*In vivo* discordance in cefepime activity against metallo-ß-lactamases-producing Enterobacterales
  - Meropenem shows *in vivo* activity against Metallo-ß-lactamase-producing *P. aeruginosa*
- Study Objectives
  - To assess the clinical outcome of non-MBL-active ß-lactam therapy (carbapenem or ceftazidime/avibactam) among patients with BSI due to MBL-producing Enterobacterales
  - To assess the *in vivo* activity of clinical exposure of meropenem against the isolates using a translational murine infection model
  - To identify the proper *in vitro* susceptibility testing conditions that can yield MICs predictive of the *in vivo* outcome of meropenem therapy against MBL-producing Enterobacterales
- Assessment of the clinical outcome of non-MBL-active therapy among patients with BSI due to MBL-producing Enterobacterales

- Patients with BSI due to MBL-producing Enterobacterales from different hospitals in Northern Italy in 2018-2021 (n=101 cases identified)
- Cases that received empiric ceftazidime/avibactam + aztreonam (MBL-active) or carbapenem or ceftazidime/avibactam (non-MBL-active) were identified (58 cases total)
- NDM-producing *Klebsiella pneumoniae*
- Empiric and Directed Therapy

	MBL-Active BL	Non-MBL Active BL
Total Patients	29	29
Empiric β-Lactam Treatment (number of patients)	Ceftazidime/avibactam + aztreonam (29)	Meropenem, extended infusion (24) Ceftazidime/avibactam (3) Imipenem/cilastatin (2)
Median duration of empiric treatment in days (IQR)	Treatment continued as targeted therapy	4 (3-5)
Directed Therapy	Ceftazidime/avibactam + aztreonam (29)	Ceftazidime/avibactam + aztreonam (12)* Colistin containing regimen (8)* Tigecycline containing regimen (8)* Fosfomycin containing regimen (6)* Gentamicin containing regimen (1)* Meropenem containing regimen (11)*

*most patients received combination regimens

- Clinical outcomes

Outcome	MBL-Active BL	Non-MBL-Active BL
14 Day All-Cause Mortality	4 (14%)	6 (21%)
30 Day All-Cause Mortality	9 (31%)	11 (38%)
Microbiologic Failure	4 (14%)	1 (3%)

- Meropenem *In Vivo* Activity
  - Treated with 2g as 3h infusion q8h
  - Control where OXA-48 and KPC-3. These mice die quickly.
  - Model shows a correlational with clinical outcomes and meropenem is working.

	%fT>MIC for a MIC (mg/L) of:				
Species	4	8	16	32	64
Human	100%	78%	50%	19%	0%
Mouse	91%	75%	53%	23%	0%

- *In vitro* Susceptibility Testing in Zn-Adjusted CAMHB

	Human		Murine	
	Mean ± SD	Range	Mean ± SD	Range
Total Zinc (ng/mL)	690 ± 112	581-806	952 ± 42	907-991
Free Zinc (ng/mL)	69 ± 38	36-110	41 ± 9.8	33-52
% Bound	89 ± 7.3	81-95	96 ± 1.2	94-97

- Conclusions and Future Directions
  - There was a mismatch in the conventional MIC and efficacy of β-lactam therapy
  - Physiological bioactive (free) zinc <<< zinc in CAMHB
  - MICs of MBL-producers in Zn-adjusted broth were more consistent with the *in vivo* killing with β-lactams
  - Additional studies are needed to assess the free zinc concentrations in patients
    - Nutritional immunity
    - Variability
    - Different biological matrices (eg, ELF)
  - Zn concentrations assessments and standardization can play an important role in modifying the *in vitro* test algorithms to improve the ability of the test to predict the outcome of β-lactam therapy against MBL-producers
- MAIWG Discussion and Recommendation
  - Guidance would be helpful on development side so sponsors know how to show effectiveness of drug and testing method when presented to CLSI.
  - Should also consider how to provide guidance to clinical labs (ie, how will they test?).
  - The WG needs to determine where the "guidance" would go—Standalone, supplement, or M23. Perhaps M23 would be the best place.
  - Next Steps: Will come back to the subcommittee in January will an objective for the AHWG and a list of members.

SC DISCUSSION (MAIN POINTS)

- Suggestion to place this information in a M02, M07, M23, or trouble shooting guide.
- CLSI should be better about defining/understanding what is causing media differences and then decide what to do about that.

- Seeing more startups come up with new creative ways for their compounds to work, and CLSI needs to come up with guidance on how/if these deviations are allowed from the standard.
- Some of the newer alleles need less zinc to be active, has this been reviewed? Has this been seen in other animal models?
    - Some of those alleles have been included in the data. No difference was seen. Need to work on data for other species.
- Who is the best equipped to address these media specific issues? Need guidance on who needs to be involved.
- Metallo-ß-lactamases do not use just zinc, they can use the other divalent cations. Need to control those too.
- Need to be realistic, what is the next reference method? Need to think about what the future reference method will be as technology updates.

INTRINSIC RESISTANCE AD HOC WORKING GROUP REPORT

- Several discussions circulating during the past few months around the definition of intrinsic resistance. The concepts of "reduced susceptibility" and "elevated MICs" as additional categories/concepts are also being explored. (eg, clinical resistance).
- Examples include issues categorizing gentamicin with *Pseudomonas aeruginosa* or imipenem with *Morganellaceae*.
- Representatives of the three AST subcommittees had a conference call on April 23rd. Items discussed:
    - What are the definitions of intrinsic resistance, reduced susceptibility, and elevated MICs (consider as categories?)?
    - Could we standardize these definitions/concepts across antifungal/bacterial/vet SCs?
- Proposed Path Forward: The creation of an Intrinsic Resistance Definition AHWG, with representatives from each subcommittee to specifically address the definitions and come back in January 2025 with proposals to be presented and discussed.

SC DISCUSSION (MAIN POINTS)

- For the new edition of M45, there is the concept of “reduced susceptibility”. The AHWG should work together on the definitions with M45.

3.	**TEXT AND TABLES WORKING GROUP (S. CAMPEAU)** **MINOCYCLINE AND ACINETOBACTER SPP. COMMENT** • Behalf of Breakpoints Working Group • New Comment for Table 2B-2 Minocycline: Isolates that test intermediate by disk diffusion, broth microdilution should be performed if needed for treatment. **PLACEMENT OF PROCEDURES WITH MODIFICATIONS TO THE REFERENCE METHOD** • January 2024 Minutes PLACEMENT OF PROCEDURES WITH MODIFICATIONS TO THE REFERENCE METHOD • Appendix I. Cefiderocol Broth Preparation and Reading Microdilution Minimal Inhibitory Concentration End Points o Originally used this Appendix as a temporary placeholder until cefiderocol could be added to M07 • Exebacase Procedure and Reading Instructions o Exebacase content in footnotes of Table 5A-1, 6A • Decision from the TTWG: Single Appendix for non-standard methods o Combined into 1 common Appendix for modified methods (e.g. Appendix I-1, I-2, etc) o Mockup the Appendix for June • Current Exebacase Content in 34th Edition

Exebacase content in footnotes of QC tables & diluent tables

Table 5A-1. (Continued)

m. QC ranges reflect MICs obtained when CAMHB is supplemented with **horse serum (25% v/v) and** 0.5 mM DL-dithiothreitol (pH 7.2-7.4) **(CAMHB-HSD). This medium is used for testing exebacase against *S. aureus* and β-hemolytic streptococci. CAMHB-HSD does not require addition of lysed horse blood when β-hemolytic streptococci is tested. *S. aureus* ATCC® 29213 is the recommended QC strain for testing both *S. aureus* and β-hemolytic streptococci. *E. faecalis* ATCC® 29212 is also recommended for testing *S. aureus*. Agar dilution is not recommended for exebacase testing. Most end points will be clear (see Figure 1). In some cases, there may be a faint haze or tiny buttons of growth where the MIC should be read as the first well where growth is significantly reduced (see Figure 2).**

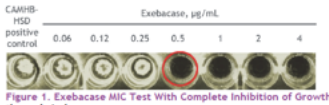


Figure 1. Exebacase MIC Test With Complete Inhibition of Growth Compared With Growth Control. **The exebacase MIC (0.5 µg/mL) is shown in the red circle.**

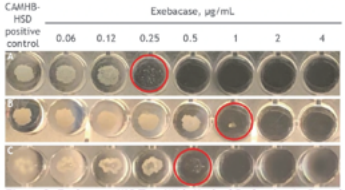


Figure 2. Exebacase MIC Test With Marked Reduction in Growth Compared With Growth Control. **The exebacase MIC is shown in the red circles (A = 0.25 µg/mL, B = 1 µg/mL, and C = 0.5 µg/mL).**

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Ceftolozane	Water or saline[d]	Water or saline[d]
Ceftriaxone	Water	Water
Cefuroxime	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Cephalexin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephalothin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephapirin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephradine	Phosphate buffer, pH 6, 0.1 mol/L	Water
Chloramphenicol	95% ethanol	Water
Cinoxacin	1/2 volume of water, then add 1 mol/L NaOH dropwise to dissolve	Water
Ciprofloxacin	Water	Water
Clarithromycin	Methanol[a] or glacial acetic acid[a,c]	Phosphate buffer, pH 6.5, 0.1 mol/L
Clavulanate	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Clinafloxacin	Water	Water
Clindamycin	Water	Water
Colistin[g]	Water	Water
Dalbavancin	DMSO[a]	DMSO[a,h]
Daptomycin	Water	Water
Delafloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Dirithromycin	Glacial acetic acid[c]	Water
Doripenem	Saline[d]	Saline[d]
Doxycycline	Water	Water
Durlobactam	Water	Water
Enoxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Enmetazobactam	Water	Water
Eravacycline	Water	Water
Ertapenem	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L
[illegible]	[illegible]	[illegible]
Exebacase	Supplied as a frozen stock in a buffer containing 20 mM L-histidine and 5% D-sorbitol, pH 7[i]	CAMHB with horse serum **(25% v/v) and** 0.5 mM DL-dithiothreitol **(DTT)** (pH 7.2-7.4) **(CAMHB-HSD)[j]**

Table 6A. (Continued)

h. Starting stock solutions of dalbavancin and telavancin should be prepared at concentrations no higher than 1600 µg/mL. Intermediate 100× concentrations should then be diluted in DMSO. Final 1:100 dilutions should then be made directly into CAMHB supplemented with 0.002% (v/v) polysorbate-80, so the final concentration of DMSO in the wells is no greater than 1%. See also Table 8B.

i. Exebacase is an enzyme that requires special handling. Frozen stock solutions should be thawed in a 25 °C water bath with gentle mixing every 30 seconds. Thawing should not take more than five minutes. The thawed stock solution and any subsequently prepared dilutions in CAMHB supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (DTT; pH 7.2-7.4) (CAMHB-HSD) should be kept chilled in an ice bucket or refrigerated at 2 to 8 °C for no more than one hour while broth microdilution MIC panels are prepared. MIC panels should be frozen within 15 minutes of preparation. Any remaining unused stock solution should be discarded.

j. To prepare one liter of CAMHB-HSD, 250 mL horse serum is added to 750 mL sterile CAMHB. Next, 500 µL CAMHB is removed, and 500 µL DTT is added. CAMHB should be prepared according to manufacturer instructions.

- Expansion and revision of Appendix H

Appendix A.	Suggestion for Confirming Antimicrobial Susceptibility Test Results and Organism Identification for Agents Approved by the US Food and Drug Administration for Clinical Use
Appendix B.	Intrinsic Resistance
Appendix C.	QC Strains for Antimicrobial Susceptibility Tests
Appendix D.	Anaerobe Cumulative Antibiogram
Appendix E.	Susceptible-Dose Dependent Interpretive Category
Appendix F.	Epidemiological Cutoff Values
Appendix G.	Using Molecular Assays for Resistance Detection
Appendix H.	**Modifications of the Minimal Inhibitory Concentration Method for Testing Select Antimicrobials** ~~Cefiderocol Broth Preparation and Reading Broth Microdilution Minimal Inhibitory Concentration End Points~~

Appendix H1 for cefiderocol
Appendix H2 for exebacase

- Proposed edits to Table 5A-1

Proposed edits to Table 5A-1
(pages 1-11 in Supplemental File)

Current Table 5A-1 exebacase footnote

o. QC ranges reflect MICs obtained when CAMHB-HSD is incubated in ambient conditions for 16–20 hours or in 5% CO_2 for 20–24 hours. Data based on incubation for 5% CO_2 and 20–24 hours were collected with limited Mueller-Hinton media manufacturers. Agar dilution is not recommended for exebacase testing. ~~This medium is used for testing exebacase against staphylococci and β-hemolytic streptococci. CAMHB-HSD does not require addition of LHB when β-hemolytic streptococci are tested.~~ *S. aureus* ATCC® 29213 is the routine QC strain; testing may use either incubation protocol described ~~below~~in Appendix H2, but MIC end points should be read as ~~shown in Figure 1~~described in Appendix H2. *E. faecalis* ATCC® 29212 is provided as a supplemental QC strain. ~~Protocols for testing and reading end points for clinical isolates are:~~

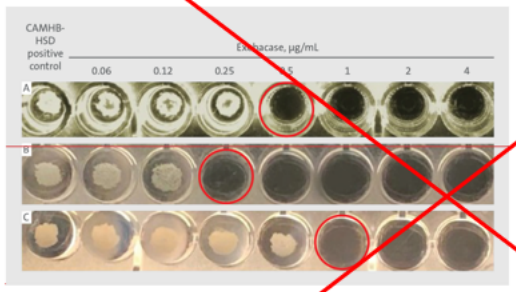
~~• For *S. aureus*, incubate in ambient conditions for 16–20 hours and read MIC end points as shown in Figure 1.~~

~~• For *Staphylococcus* spp. other than *S. aureus*, incubate in 5% CO_2 for 20–24 hours and read MIC end points at complete inhibition (see Figure 2).~~

~~• For β-hemolytic streptococci, incubate in ambient conditions for 20–24 hours and read MIC end points at complete inhibition.~~

~~Figure 1. Exebacase MIC Test: *S. aureus* Incubated in Ambient Conditions for 16–20 Hours, With MICs Shown in Red Circles.~~

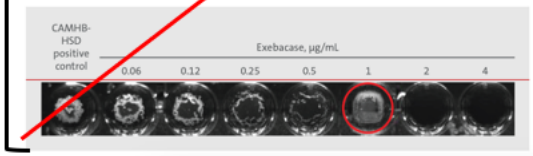
~~Most end points are read at complete inhibition of growth compared with the GC well; row A shows the MIC at 0.5 µg/mL. In some cases, a marked reduction in growth compared with GC is observed; row B shows the MIC at 0.25 µg/mL and row C shows the MIC at 1 µg/mL.~~



~~Abbreviations: CAMHB-HSD, cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4); GC, growth control; MIC, minimal inhibitory concentration; pH, negative logarithm of hydrogen ion concentration.~~

~~Figure 2. Exebacase MIC Test: *Staphylococcus* spp. Other Than *S. aureus* Incubated in 5% CO_2, With MICs Shown in Red Circles.~~

~~For staphylococci other than *S. aureus*, a marked reduction in growth compared with GC is frequently observed and MICs should be read at complete inhibition; the figure shows the MIC at 1 µg/mL.~~



Proposed update to footnote

o. QC ranges reflect MICs obtained when CAMHB-HSD is incubated in ambient conditions for 16–20 hours or in 5% CO_2 for 20–24 hours. Data based on incubation for 5% CO_2 and 20–24 hours were collected with limited Mueller-Hinton media manufacturers. Agar dilution is not recommended for exebacase testing. *S. aureus* ATCC® 29213 is the routine QC strain; testing may use either incubation protocol described in Appendix H2, but MIC end points should be read as described in Appendix H2. *E. faecalis* ATCC® 29212 is provided as a supplemental QC strain.

- Proposed edits to Table 6A exebacase footnote

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Enoxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Enmetazobactam	Water	Water
Eravacycline	Water	Water
Ertapenem	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L
Erythromycin	95% ethanol or glacial acetic acid[a,c]	Water
Exebacase	Supplied as a frozen stock in a buffer containing 20 mM L-histidine and 5% D-sorbitol, pH 7[i]	CAMHB-HSD[j]

i. Exebacase is an enzyme that requires special handling,[a,c] see Appendix H2. ~~Frozen stock solutions should be thawed in a 25°C water bath with gentle mixing every 30 seconds. Thawing should not take more than 5 minutes. The thawed stock solution and any subsequently prepared dilutions in CAMHB-HSD should be kept chilled in an ice bucket or refrigerated at 2 to 8°C for no more than 1 hour while broth microdilution MIC panels are prepared. MIC panels should be frozen within 15 minutes of preparation. Any remaining unused stock solution should be discarded.~~

j. To prepare 1 liter of CAMHB-HSD, see Appendix H2. ~~250 mL horse serum is added to 750 mL sterile CAMHB. Next, 500 µL CAMHB is removed, and 500 µL DTT is added. CAMHB should be prepared according to manufacturer instructions.~~

- Proposed template for expanded Appendix H
  - Generally follows the outline used for cefiderocol and format of M02/M07

TEMPLATE FOR NEW MODIFIED METHOD APPENDIX

Appendix HX. [Drug] Broth Preparation and Reading Broth Microdilution Minimal Inhibitory Concentration End Points

Abbreviations for Appendix HX

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

HX.1 Supplements

HX.1.1 Calcium and Magnesium Stock Solutions *(or remove if not relevant)*

Refer to CLSI M07[1] for cation stock solution preparation.

HX.1.2 [Other] Stock Solution *(those not in M02 or M07)*

The steps for preparing [other] stock solution are listed below.

Step	Action	Comments
1		
2		
3		

Abbreviations: *(add relevant abbrev.)*.

HX.2 [Drug-specific description] Mueller-Hinton Broth[a]

The steps for preparing [drug-specific description] Mueller-Hinton broth are listed below.

Step	Action	Comments
1		
2		
3		
4		
5		

Abbreviations: *(add relevant abbrev.)*.

HX.3 Determining Broth Microdilution End Points

The steps for reading and interpreting broth microdilution end points for [drug] when tested with [drug-specific broth] are listed below.

Step	Action	Comments
1		
2		
3		

Abbreviations: *(add relevant abbrev.)*.

Figure HX.1. [Drug]I MIC Test With a Clear End Point.

Add relevant images and/or reading instructions

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

References for Appendix HX

[1] Add relevant references

- Mock Up of Appendix H2

Appendix H2. Exebacase Broth Preparation and Reading Broth Microdilution Minimal Inhibitory Concentration End Points

Abbreviations for Appendix H2

CAMHB cation-adjusted Mueller-Hinton broth
CAMHB-HSD cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2-7.4)

H2.1 Supplements

H2.1.1 Calcium and Magnesium Stock Solutions

Refer to CLSI M07[1] for cation stock solution preparation.

H2.1.2 Exebacase Stock Solution

The steps for preparing exebacase stock solution are listed below.

Step	Action	Comments
1	Thaw frozen stock solutions in a 25°C water bath with gentle mixing every 30 seconds.	Thawing should not take more than 5 minutes. The thawed stock solution and any subsequently prepared dilutions in CAMHB-HSD should be kept chilled in an ice bucket or refrigerated at 2 to 8°C for no more than 1 hour while broth microdilution MIC panels are prepared.
2	Discard any remaining unused stock solution.	

Abbreviations: MIC, minimal inhibitory concentration

H2.2 Exebacase CAMHB-HSD

The steps for preparing 1 liter of CAMHB-HSD are listed below.

Step	Action	Comments
1	Prepare or obtain 750 mL sterile CAMHB.	CAMHB should be prepared according to manufacturer instructions or according to CLSI M07[1].
2	Add 250 mL horse serum to sterile CAMHB	Final 25% v/v horse serum.
3	Remove 500 µL CAMHB.	
4	Add 500 µL DL-dithiothreitol (pH 7.2-7.4).	
5	Store the media at [X°C] for up to [X months]. *(need to check with Jane if this can be stored or if it must be used immediately)*	Prepared MIC panels with CAMHB-HSD and exebacase should be frozen within 15 minutes of preparation. This medium is used for testing exebacase against staphylococci and β-hemolytic streptococci. CAMHB-HSD does not require addition of LHB when β-hemolytic streptococci are tested.

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; CAMHB-HSD, cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2-7.4)

H2.3 Determining Broth Microdilution End Points

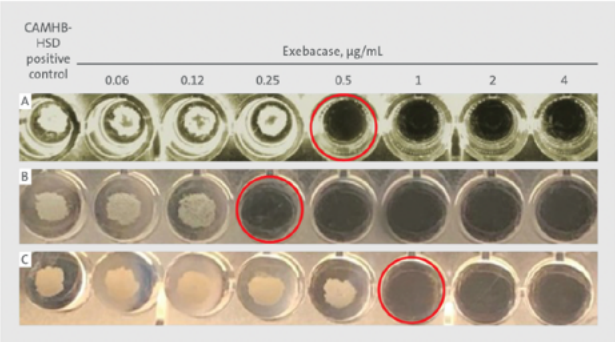
The protocols for testing and reading end points for exebacase when tested with CAMHB-HSD are listed below. *S. aureus* ATCC® 29213 is the routine QC strain; testing may use either incubation protocol described below, but MIC end points should be read as shown in Figure H2.1. *(Include mention of E. faecalis strain and how to read??)*

Organism Group	Incubation	End Points
S. aureus	Ambient conditions for 16–20 hours	Read MIC end points as shown in Figure H2.1
SOSA	5% CO_2 for 20–24 hours	Read MIC end points at complete inhibition as shown in Figure H2.2
β-hemolytic streptococci	Ambient conditions for 20–24 hours	Read MIC end points at complete inhibition as shown in Figure H2.1, row A *(confirm with Jane if this is accurate or get new figure)*

Abbreviations: SOSA, staphylococci other than *S. aureus*.

Figure H2.1. Exebacase MIC Test: *S. aureus* Incubated in Ambient Conditions for 16–20 Hours, With MICs Shown in Red Circles.

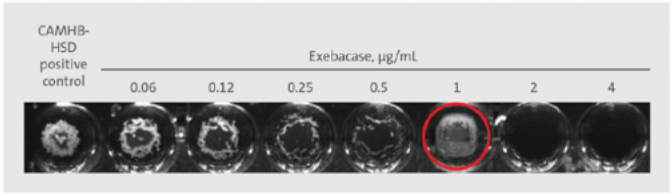
Most end points are read as complete inhibition of growth compared with the GC well; row A shows the MIC at 0.5 µg/mL. In some cases, a marked reduction in growth compared with GC is observed; row B shows the MIC at 0.25 µg/mL and row C shows the MIC at 1 µg/mL.



Abbreviations: CAMHB-HSD, cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4); GC, growth control; MIC, minimal inhibitory concentration; pH, negative logarithm of hydrogen ion concentration.

Figure H2.2. Exebacase MIC Test: SOSA Incubated in 5% CO_2, With MICs Shown in Red Circles.

For SOSA, a marked reduction in growth compared with GC is frequently observed and MICs should be read at complete inhibition; the figure shows the MIC at 1 µg/mL.



Abbreviations: CAMHB-HSD, cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4); GC, growth control; MIC, minimal inhibitory concentration; pH, negative logarithm of hydrogen ion concentration.

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

References for Appendix H2

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

- Proposed intro text to 'new' Appendix H

To precede the tables (similar to Appendix B & G):

Appendix H. Modifications of the Minimal Inhibitory Concentration Method for Testing Select Antimicrobials

Modifications to the CLSI reference broth microdilution MIC method (see M07[1]) are required for certain antimicrobial agents for MIC testing.

The following appendixes describes these modifications to include the preparation of supplements, stock solutions and/or Mueller-Hinton broth composition needed to test these antimicrobial agents.

Additionally, information for incubation conditions (e.g., incubation temperature, atmosphere, time) and guidance for endpoint determination are also provided.

- TTWG Recommendation: To implement revision/expansion (with intro to Appendix H) as proposed. WG in agreement.

A motion to approve moving exebacase to Appendix H and to accept the proposed revisions to Table 5A-1, Table 6A, and Appendix H was made and seconded. Vote: 12 for, 0 against, 1 abstain, 1 absent (Pass)

STAPHYLOCOCCUS CONTENT

- Terminology of 'SOSA' and Taxonomy of *S. aureus* complex
  - SOSA = *Staphylococcus* other than *S. aureus*
  - Terminology now used in recently published editions of M02/M07
  - January Plenary – agreed to make updates for this

- SOSA edits for 35th edition

(6) Historically, for *S. aureus* and staphylococci other than *S. aureus* (SOSA) resistance to the penicillinase-stable penicillins (see Glossary I) has been referred to as "methicillin resistance" or "oxacillin resistance." MRS are strains that express *mecA* (or its homologue, *mecC*) or another mechanism of resistance, such as changes in affinity of penicillin-binding proteins for oxacillin (eg, modified *S. aureus* strains).

Most methicillin (oxacillin) resistance is mediated by *mecA*, encoding PBP2a (also called PBP2′). Tests for *mecA* and PBP2a are the most definitive tests for detection of methicillin (oxacillin) resistance for *Staphylococcus* spp. Mechanisms of methicillin (oxacillin) resistance other than *mecA*, such as *mecC*, are rare.[5] MICs for strains with *mecC* are typically cefoxitin resistant and oxacillin susceptible; *mecC* resistance cannot be detected by tests directed at *mecA* or PBP2a.

Isolates that test positive for ***mecA, mecC,*** or PBP2a or resistant by any of the recommended phenotypic methods should be reported as methicillin (oxacillin) resistant (see the table below and Appendix G).

MRS are resistant to currently available β-lactam agents, with the exception of ceftaroline **(see comment 12)**. This is because most documented cases of MRS infections have responded poorly to β-lactam therapy or because convincing clinical data that document clinical efficacy for those agents have not been presented.

Detection of methicillin (oxacillin) resistance in staphylococci is achieved by using specific methods as listed in this table and further described in Table 3H.

	Methods or Targets for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp.							
		Disk Diffusion		MIC				
	Organism	Cefoxitin	Oxacillin	Cefoxitin	Oxacillin	*mecA*	PBP2a	Oxacillin Salt Agar
	S. aureus	Yes (16–18 h)	No	Yes (16–20 h)	Yes (24 h)	Yes	Yes	Yes (24 h)
SOSA	***S. lugdunensis***	Yes (16–18 h)	No	Yes (16–20 h)	Yes (24 h)	Yes	Yes	No
SOSA	***S. epidermidis***	Yes (24 h)	Yes (16–18 h)	No	Yes (24 h)	Yes	Yes	No
SOSA	***S. pseudintermedius***	No	Yes (16–18 h)	No	Yes (24 h)	Yes	Yes	No
SOSA	***S. schleiferi***	No	Yes (16–18 h)	No	Yes (24 h)	Yes	Yes	No
SOSA	***Staphylococcus* spp. (not listed above or not identified to the species level)**	Yes, with exceptions[a] (24 h)	No	No	Yes (24 h)	Yes	Yes	No

Abbreviations: h, hour(s); MIC, minimal inhibitory concentration; PBP2a, penicillin-binding protein 2a; SOSA, staphylococci other than *S. aureus*.

- *S. aureus* complex edits for 35th edition

(3) *S. aureus* complex consists of the coagulase-positive species *S. aureus*, *Staphylococcus argenteus*, ~~and~~ *Staphylococcus schweitzeri*, and other species not listed[1,2,3]. At this time, CLSI has not evaluated the methods described herein on species other than *S. aureus*. If *S. argenteus* is identified by MALDI-TOF MS or sequencing, it is recommended that it be reported as "*S. aureus* complex (*S. argenteus*)," and *S. aureus* phenotypic testing method recommendations, breakpoints, and interpretive categories should be used. Human infections with *S. schweitzeri* have yet to be reported.[3]

New references for *S. aureus* complex

[1] Becker K, et al. Implications of identifying the recently defined members of the Staphylococcus aureus complex S. argenteus and S. schweitzeri: a position paper of members of the ESCMID Study Group for Staphylococci and Staphylococcal Diseases (ESGS). Clin Microbiol Infect. 2019. PMID: 30872103.

[2] Schutte AHJ, et al. Characterization of *Staphylococcus roterodami* sp. nov., a new species within the *Staphylococcus aureus* complex isolated from a human foot infection. Int J Syst Evol Microbiol. 2021. PMID: 34582327.

[3] Chew KL, Octavia S, Lai D, Lin RTP, Teo JWP. *Staphylococcus singaporensis* sp. nov., a new member of the *Staphylococcus aureus* complex, isolated from human clinical specimens. Int J Syst Evol Microbiol. 2021. PMID: 34698625.

- *Staphylococcus* – Sulfisoxazole
  - 34th edition comment:

FOLATE PATHWAY ANTAGONISTS											
Trimethoprim-sulfamethoxazole	All staphylococci	1.25/23.75 µg	≥16	-	11-15	≤10	≤2/38	-	-	≥4/76	
Sulfonamides (U)[b]	All staphylococci	250 or 300 µg	≥17	-	13-16	≤12	≤256	-	-	≥512	(24) Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
Trimethoprim (U)[b]	All staphylococci	5 µg	≥16	-	11-15	≤10	≤8	-	-	≥16	

  - Is this comment still true and should it be retained?
  - January 2024:
    - Presented at Plenary to ask if drug is still being used or if comment still necessary
    - VAST: "Does have an old FDA approval for use in dogs but it doesn't seem to be currently marketed in the US. No word yet on international approval/use…"
    - Manufacturers: "Not requested for MicroScan inclusion for a long long time anywhere, including Asia" and "Used for MHA manufacturers but this is outlined in ISO document"
    - EUCAST: "No, EUCAST does not know who is using this drug"
  - TTWG Recommendation: Remove comment and drug from Tables 2 and QC Tables. WG in agreement.

SC DISCUSSION (MAIN POINTS)

- Consensus from the Subcommittee to remove sulfisoxazole and comment in Tables 2 and the QC tables. No vote taken.
- ISO is in discussion about if they are going to swap out sulfisoxazole for SXT because sulfisoxazole is to be removed from the document.

mCIM and eCIM TABLE TITLE UPDATE

- Update to "Tests Used for Carbapenemase Detection"

Proposed change

Introduction to Tables 3B and 3C. (Continued)

Tests Used for ~~Epidemiological or Infection Prevention-Related~~Carbapenemase Detection

	CarbaNP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Organisms	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales that are positive by mCIM	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by CarbaNP 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
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.	Requires overnight incubation	Requires overnight incubation	Special reagents and equipment are needed. Specific to targeted genes; false-negative result if specific carbapenemase gene present is not targeted.

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]

Carbapenemase-producing isolates of Enterobacterales usually test intermediate or resistant to one or more carbapenems using the current breakpoints as listed in Table 2A (**NOTE:** Testing not susceptible to ertapenem is often the most sensitive indicator of carbapenemase production) and usually test resistant to one or more agents in cephalosporin subclass III (eg, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone). However, some isolates that produce carbapenemases such as SME or IMI often test susceptible to these cephalosporins.

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

SC DISCUSSION (MAIN POINTS)

- Consensus from the Subcommittee to update the mCIM and eCIM table title. No vote taken.
- Does a limitation need to be added to the methods that clearly states eCIM/mCIM do not differentiate carbapenemases?
    - Virginia Pierce is checking if a comment already exists.

COMMENT CONSISTENCY

- Comments are of various varietals and vintages, often with inconsistent wording or structure, despite similar intent
- Many comments also exist in multiple locations
- Long-term TTWG project is to harmonize and/or clean up comments throughout M100
- Started with oxazolidinones, tetracycline, and penicillin comments

TETRACYCLINE PREDICTION COMMENTS

Organism Group	Breakpoints		Table 1 (footnote)	Table 2 (comment)	Organism included in FDA Label
	CLSI	FDA			
Enterobacterales	DO, MI, TE	DO, MI, TE	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline or minocycline, or both.		DO**, MI**, TE
Salmonella and *Shigella* spp.	DO, MI, TE				DO, MI (*Shigella*)
Staphylococcus spp.	DO, MI, TE	MI, TE			MI**, TE (*S. aureus*)
Acinetobacter spp.	DO, MI, $TE_{(U)}$	DO, MI, TE			DO**, MI**, TE
Other Non-Enterobacterales	DO, MI, $TE_{(U)}$				
Enterococcus spp.	DO, MI, $TE_{(U)}$	TE			DO, MI, TE *for QC only*
Streptococcus pneumoniae	DO, TE	DO, TE	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline or minocycline, or both.	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline. However, resistance to doxycycline cannot be inferred from tetracycline resistance.	DO**, MI*, TE
Haemophilus influenzae and *Haemophilus paroinfluenzae*	TE	TE	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, resistance to doxycycline and minocycline cannot be inferred from tetracycline resistance.	DO*, MI*, TE (*H. influenzae*)
Neisseria gonorrhoeae	TE	TE	None	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.	DO*, MI, TE
Streptococcus spp. β-Hemolytic Group	TE	TE	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline or minocycline, or both.	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, resistance to doxycycline cannot be inferred from tetracycline resistance.	TE (*S. pyogenes*)
Streptococcus spp. Viridans Group	TE		None		

DO – doxycycline; MI – minocycline; TE – tetracycline *FDA label only includes TE STIC; **FDA label includes statement indicating that TE susceptibility predicts DO or MI

- TTWG Recommendation: To remove the tetracycline footnotes from Tables 1. WG in agreement.

Organism Group	Breakpoints		Table 1 (footnote)	Table 2 NEW Comment Template	Organism included in FDA Label
	CLSI	FDA			
Enterobacterales	DO, MI, TE	DO, MI, TE	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline or minocycline, or both.	Isolates that test susceptible to tetracycline may be reported as susceptible to doxycycline or minocycline. Isolates that test intermediate or resistant to tetracycline should be tested against doxycycline or minocycline if those results are needed for reporting.	DO**, MI**, TE
Salmonella and *Shigella* spp.	DO, MI, TE				DO, MI (*Shigella*)
Staphylococcus spp.	DO, MI, TE	MI, TE			MI**, TE (*S. aureus*)
Acinetobacter spp.	DO, MI, $TE_{(u)}$	DO, MI, TE			DO**, MI**, TE
Other Non-Enterobacterales	DO, MI, $TE_{(u)}$				
Enterococcus spp.	DO, MI, $TE_{(u)}$	TE			DO, MI, TE *for QC only*
Streptococcus pneumoniae	DO, TE	DO, TE	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline or minocycline, or both.	Isolates that test susceptible to tetracycline may be reported as susceptible to doxycycline. Isolates that test intermediate or resistant to tetracycline should be tested against doxycycline if those results are needed for reporting.	DO**, MI*, TE
Haemophilus influenzae and *Haemophilus parainfluenzae*	TE	TE	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.	Isolates that test susceptible to tetracycline may be reported as susceptible to doxycycline and minocycline.	DO*, MI*, TE (*H. influenzae*)
Neisseria gonorrhoeae	TE	TE	None		DO*, MI, TE
Streptococcus spp. β-Hemolytic Group	TE	TE	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline or minocycline, or both.		TE (*S. pyogenes*)
Streptococcus spp. Viridans Group	TE		None	See upcoming slide	

- TTWG Recommendation: To update tetracycline comments in Tables 2 for the groups listed above. WG in agreement.

SC DISCUSSION (MAIN POINTS)

- Want to remove the tetracycline footnotes from Tables 1 to align with the oxazolidinone.
- All this information is in Tables 2, and it will continue to exist. Refer for Tables 2.
- A comment will be added to the introduction to Tables 1 referring to Tables 2.

A motion to remove the tetracycline footnotes from Tables 1, keep in Tables 2, and add a comment to the Tables 1 introduction to refer to Tables 2 was made and seconded. Vote: 12 for, 1 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:

- Tables 2 information is helpful for people outside of the US.

SC DISCUSSION (MAIN POINTS)

- Need to add minocycline to *S. pneumoniae* in the new Tables 2 comment.
- Need to change that of *Acinetobacter*? Yes, that has been crossed out.
- Concern that there should be a comment highlighting that the drug was not directly tested, and that susceptibility was predicted based on tetracycline.

A motion to accept the Tables 2 tetracycline comment, "Isolates that test susceptible to tetracycline may be reported as susceptible to doxycycline or minocycline. Isolates that test intermediate or resistant to tetracycline should be tested against doxycycline or minocycline if those results are needed for reporting." for Enterobacterales, *Salmonella* and *Shigella* spp., *Staphylococcus* spp., *Acinetobacter* spp., other Non-Enterobacterales, and *Enterococcus* spp. was made and seconded. Vote: 11 for, 1 against, 0 abstain, 2 absent (Pass)

Against Vote Reasoning:

- Does not know if this fits into surrogacy or equivalency.

SC DISCUSSION (MAIN POINTS)

- The minocycline package insert says to use tetracycline to predict minocycline. Need to add minocycline to the first sentence, but not the second sentence.
- What are the expectations to labs to report if tetracycline is I or R?
- Suggestion to exclude minocycline.
- Is this surrogacy or equivalent?
    - Some think this is not a surrogate or equivalent. Equivalent would imply identical susceptibility patterns between drugs.
- Concerns about how broadly this comment should be applied.
- What about urine only tetracycline breakpoints?

A motion to accept the Tables 2 tetracycline comment, "Isolates that test susceptible to tetracycline may be reported as susceptible to doxycycline. Isolates that test intermediate or resistant to tetracycline should be tested against doxycycline if those results are needed for reporting." for *Streptococcus pneumoniae* was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

SC DISCUSSION (MAIN POINTS)

- Preference to report a comment that the organism tested susceptible to tetracycline and can be assumed to be susceptible to doxycycline and minocycline.
- For ß-hemolytic *Streptococcus*, it currently says tetracycline predicts doxycycline and minocycline.
- Suggestion to change the word "reported" to "considered".
- AST manufacturers would build a rule to report the additional drugs.

A motion to accept the Tables 2 tetracycline comment, "Isolates that test susceptible to tetracycline may be reported as susceptible to doxycycline and minocycline." for *Haemophilus influenzae* and *Haemophilus parainfluenzae*, *Neisseria gonorrhoeae*, *Streptococcus* spp. ß-hemolytic group, and *Streptococcus* spp. viridans group was made and seconded. Vote: 5 for, 5 against, 0 abstain, 1 absent (Fail)

Against Vote Reasoning:
- The new wording is not current practice.
- Would like to keep the wording as “considered” rather than “report”.

SC DISCUSSION (MAIN POINTS)
- The CDC would like the original *Neisseria gonorrhoeae* comment to stay the same because it has public health implications.
- Change the word “reported” to “considered”.

A motion to accept the Tables 2 tetracycline comment, “Isolates that test susceptible to tetracycline may be considered as susceptible to doxycycline and minocycline.” for *Haemophilus influenzae* and *Haemophilus parainfluenzae*, *Neisseria gonorrhoeae*, *Streptococcus* spp. ß-hemolytic group, and *Streptococcus* spp. viridans group was made and seconded. Vote: 12 for, 1 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:
- Wording is confusing.

A motion to change the voted on Tables 2 tetracycline comments to state “considered” instead of “reported” was made and seconded. Vote: 12 for, 0 against, 1 abstain, 1 absent (Pass)

TETRACYCLINE PREDICTION COMMENTS

Organism Group	Breakpoints		Table 1 (footnote)	Table 2 (comment)	Organism included in FDA Label
	CLSI	FDA			
Staphylococcus spp.	LZD TZD (*S. aureus*)	LZD TZD (*S. aureus*)	None	*S. aureus* that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that test resistant to linezolid may be susceptible to tedizolid.	LZD TZD (*S. aureus)*
Enterococcus spp.	LZD TZD (*E. faecalis*)	LZD TZD (*E. faecalis*)		*E. faecalis* that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that are intermediate or resistant to linezolid may be susceptible to tedizolid.	LZD TZD (*E. faecalis*)
Streptococcus spp. β-Hemolytic Group	LZD TZD (GAS/GBS)	LZD, TZD		*S. agalactiae* and *S. pyogenes* that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that are nonsusceptible to linezolid may be susceptible to tedizolid.	LZD TZD (GAS/GBS)
Streptococcus spp. Viridans Group	LZD TZD (*S. anginosus* group)	LZD, TZD		*S. anginosus* group that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that are nonsusceptible to linezolid may be susceptible to tedizolid.	LZD TZD (*S. anginosus* group)
Streptococcus pneumoniae	LZD	LZD		None	LZD

LZD – linezolid; TZD – tedizolid

Organism Group	Breakpoints		Table 1 (footnote)	Table 2 NEW Comment Template
	CLSI	FDA		
Staphylococcus spp.	LZD TZD (*S. aureus*)	LZD TZD (*S. aureus*)	None	**New template proposed:** Isolates that test susceptible to linezolid by MIC may be reported as susceptible to tedizolid. Isolates that test [intermediate/resistant/nonsusceptible] to linezolid should be tested against tedizolid if that result is needed for reporting.
Enterococcus spp.	LZD TZD (*E. faecalis*)	LZD TZD (*E. faecalis*)		
Streptococcus spp. β-Hemolytic Group	LZD TZD (GAS/GBS)	LZD, TZD		
Streptococcus spp. Viridans Group	LZD TZD (*S. anginosus* group)	LZD, TZD		
Streptococcus pneumoniae	LZD	LZD		None

- TTWG Recommendation: To update oxazolidinone comments for these groups with new template. TTWG in agreement.

SC DISCUSSION (MAIN POINTS)

- Change the word “reported” to “considered”.

A motion to accept the Tables 2 oxazolidinone comment, “Isolates that test susceptible to linezolid may be considered as susceptible to tedizolid. Isolates that test intermediate/resistant/nonsusceptible to linezolid should be tested against tedizolid if that result is needed for reporting.” for *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp. ß-hemolytic group, and *Streptococcus* spp. viridans group was made and seconded. Vote: 12 for, 0 against, 1 abstain, 1 absent (Pass)

TETRACYCLINE PREDICTION COMMENTS *STREPTOCOCCUS* SPP. VIRIDANS GROUP

Organism Group	Breakpoints		Table 1 (footnote)	Table 2 (comment)	Organism included in FDA Label
	CLSI	FDA			
Streptococcus spp. Viridans Group	TE		N/A	Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, resistance to doxycycline cannot be inferred from tetracycline resistance.	

- TTWG Recommendation: To remove current comment.
- For viridans groups, no breakpoints in CLSI or FDA, not included in FDA label, and no breakpoint or prediction comment for tetracycline, doxycycline, or minocycline in EUCAST.

SC DISCUSSION (MAIN POINTS)

- Consensus of the Subcommittee is to keep the current Tables 2 *Streptococcus* spp. viridans group comment. No vote taken.

OTHER M100 COMMENTS

- *Streptococcus* spp. β-Hemolytic Group

Table 1H-1 footnote (d) – no update

Penicillin and ampicillin are drugs of choice for **treating** β-hemolytic streptococcal infections. Susceptibility testing of penicillins and other β-lactams approved by the US Food and Drug Administration for treatment of β-hemolytic streptococcal infections does not need to be performed routinely, because nonsusceptible isolates (ie, penicillin MICs > 0.12 and ampicillin MICs > 0.25 µg/mL) are extremely rare in any β-hemolytic **streptococci** and have not been reported for *S. pyogenes*. If testing is performed, any β-hemolytic streptococcal isolate found to be nonsusceptible should be re-identified, retested, and if confirmed, submitted to a public health laboratory (see Appendix A for additional instructions).

Table 2H-1 comment (5) – update to harmonize with Table 1H-1 (d)

Penicillin and ampicillin are drugs of choice for **treating** ~~treatment of~~ β-hemolytic streptococcal infections. Susceptibility testing of penicillins and other β-lactams approved by the US Food and Drug Administration for treatment of β-hemolytic streptococcal infections does not need to be performed routinely, because nonsusceptible isolates (ie, penicillin MICs > 0.12 and ampicillin MICs > 0.25 µg/mL) are extremely rare in any β-hemolytic **streptococci** ~~streptococcus~~ and have not been reported for *S. pyogenes*. If testing is performed, any β-hemolytic streptococcal isolate found to be nonsusceptible should be re-identified, retested, and, if confirmed, submitted to a public health laboratory~~.~~ **(s**ee Appendix A for additional instructions**)**.

- Reference to Glossary I

Table 1C footnote (b) – update to refer to Glossary I and harmonize with Table 2C (8)

Penicillin-susceptible staphylococci are also susceptible to other β-lactam agents with established clinical efficacy for staphylococcal infections **(including both penicillinase-labile and penicillinase-stable agents; see Glossary I)**. Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins **(see Glossary I).** Methicillin (oxacillin)-resistant staphylococci are resistant to all currently available β-lactam antimicrobial agents, with the exception of ceftaroline. Thus, susceptibility or resistance to a wide array of β-lactam antimicrobial agents may be determined from testing only penicillin and either cefoxitin or oxacillin. Routine testing of other β-lactam agents, except ceftaroline, is not advised.

Table 2C comment (8) – no change

Penicillin-susceptible staphylococci are susceptible to other β-lactam agents with established clinical efficacy for staphylococcal infections **(including both penicillinase-labile and penicillinase-stable agents; see Glossary I).** Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins.

- Reference to cell-wall agent

Table 2D comment (5) – update to align with Table 3L

Synergy between a **cell wall-active agent (eg,** ampicillin, penicillin, or vancomycin**)** and an aminoglycoside can be predicted for enterococci by using a high-level aminoglycoside (gentamicin and streptomycin) test (see Table 3K).

Table 3L Tests for Detecting High-Level Aminoglycoside Resistance in Enterococci – no update

Table 3L. (Continued)

Test	Gentamicin HLAR			Streptomycin HLAR		
Additional testing and reporting	Resistant: is not synergistic with cell wall-active agent (eg, ampicillin, penicillin, and vancomycin). Susceptible: is synergistic with cell wall-active agent (eg, ampicillin, penicillin, and vancomycin) that is also susceptible. If disk diffusion result is inconclusive: perform an agar dilution or broth dilution MIC test to confirm. Strains of enterococci with ampicillin and penicillin MICs ≥ 16 µg/mL are categorized as resistant. However, enterococci with penicillin MICs ≤ 64 µg/mL or ampicillin MICs ≤ 32 µg/mL may be susceptible to synergistic killing by these penicillins in combination with gentamicin or streptomycin (in the absence of high-level resistance to gentamicin or streptomycin, see Subchapter 3.12.2.3 in M07[1]) if high doses of penicillin or ampicillin are used. Enterococci possessing higher levels of penicillin (MICs ≥ 128 µg/mL) or ampicillin (MICs ≥ 64 µg/mL) resistance may not be susceptible to the synergistic effect.[2,3] Physicians' requests to determine the actual MIC of penicillin or ampicillin for blood and CSF isolates of enterococci should be considered.					
QC recommendations - routine[c]	*E. faecalis* ATCC®[d] 29212: 16-23 mm	*E. faecalis* ATCC® 29212 - susceptible	*E. faecalis* ATCC® 29212 - susceptible	*E. faecalis* ATCC® 29212: 14-20 mm	*E. faecalis* ATCC® 29212 - susceptible	*E. faecalis* ATCC® 29212 - susceptible
QC recommendations - lot/shipment[e]		*E. faecalis* ATCC® 51299 - resistant	*E. faecalis* ATCC® 51299 - resistant		*E. faecalis* ATCC® 51299 - resistant	*E. faecalis* ATCC® 51299 - resistant

	***SALMONELLA/SHIGELLA* TABLE 2A-2**
	• During discussion of comment harmonization between Table 1A-2 and new Table 2A-2, a comment about how to address drugs that were not brought over into Table 2A-2.
	• For example:
	  ○ Question was raised about piperacillin-tazobactam from a customer
	  ○ Manufacturer commented that updates were made to expert rules to suppress reporting of all drugs not represented in Table 2A-2 for *Salmonella/Shigella* isolates. Previously, they would be reported as they were included in the old Table 2A when these organisms were combined with Enterobacterales. Some examples below:
	  ▪ aztreonam
	  ▪ ß-lactamase inhibitor combinations (including piperacillin-tazobactam)
	  ▪ colistin, polymyxin B
	  ▪ doripenem
	  ▪ folate pathway antagonists
	  ▪ etc.
	
	SC DISCUSSION (MAIN POINTS)
	• Action item: CLSI needs to review this table because some drugs were removed when the table was split out from the Enterobacterales.
	• Do not think it is a typo.

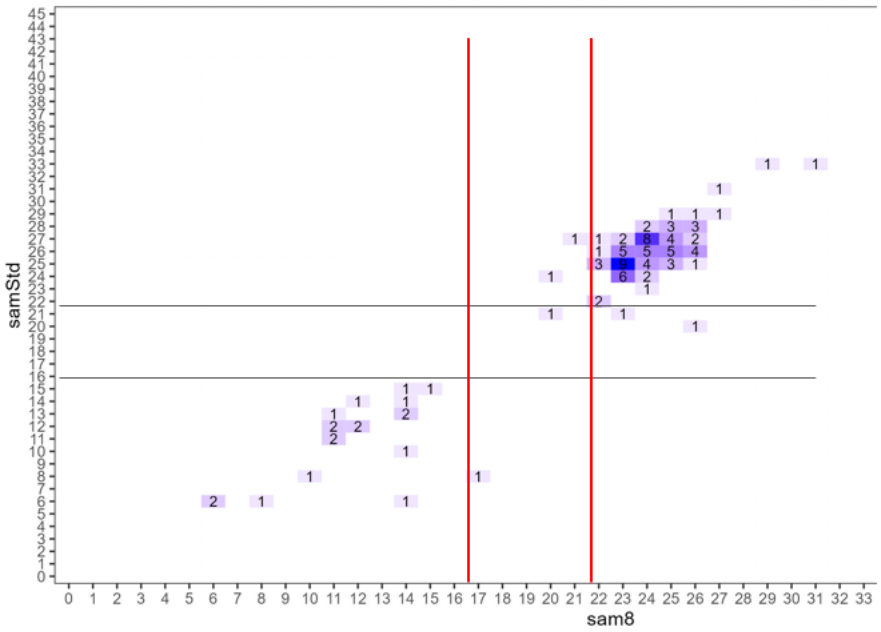
4. **METHODS DEVELOPMENT AND STANDARDIZATION (T. DINGLE)**

DIRECT BLOOD CULTURE AST AD HOC WORKING GROUP REPORT

- Update
  - Reviewed performance of direct disk for piperacillin-tazobactam with old breakpoints
  - Propose reviewing performance with updated breakpoints for Enterobacterales and *P. aeruginosa*
- Ampicillin-sulbactam and *Acinetobacter* Direct Disk Diffusion Breakpoints
  - Set 16-18 hr direct breakpoints (already in M100 34th edition)
    - Aligned with standard (34th ed.) breakpoints
  - Direct DD ad hoc WG December 2023
    - 8-10 hr low categorical agreement with standard (34th ed.) breakpoints
    - Voted and agreed on proposed breakpoints for 8-10 hr
    - The same proposed breakpoints were approved by vote for 16-18 hr to align with 8-10 hr
  - Methods Development WG Jan 2024
    - Reviewed the data on ampicillin-sulbactam for *Acinetobacter* direct
    - Did not vote on any breakpoints due to knowledge of upcoming potential changes to the breakpoints
    - Was not brought to the AST SC
  - Ampicillin-sulbactam 8-10h vs. Std DD *Acinetobacter*

	Std DD			
8-10 hr	S	I	R	Grand Total
S	82	2		84
I	2	1	1	4
R			19	19
Grand Total	84	3	20	107

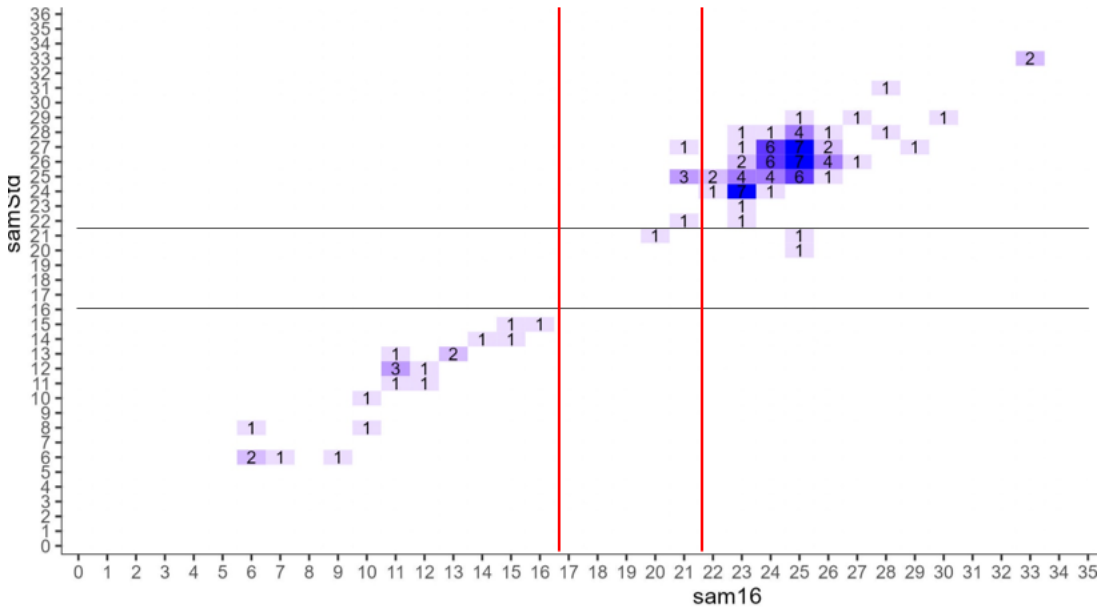
CA	102/107	95.3%
VME	0/20	0
ME	0/84	0
mE	5/107	4.7%



- Ampicillin-sulbactam 16-18h vs. Std DD *Acinetobacter*

	Std DD			
16-18 hr	S	I	R	Grand Total
S	79	2		81
I	5	1		6
R			20	20
Grand Total	84	3	20	107

CA	100/107	93.5%
VME	0/20	0
ME	0/84	0
mE	7/107	6.5%



- Proposed new ampicillin-sulbactam 8-10 h and 16-18h read disk diffusion breakpoints

NEW breakpoints (mm)		
S	I	R
≥22	17-21	≤16

- Who should handle updates to breakpoints/disk correlate changes going forward?

SC DISCUSSION (MAIN POINTS)

- For future direct from blood disk diffusion updates to match new MIC breakpoints, the consensus of the Subcommittee is for the Direct Blood Culture AST AHWG to have the responsibility.

A motion to accept the ampicillin-sulbactam direct blood disk breakpoints for *Acinetobacter* (S≥22, 17-21 I, R≤16 mm) for an 8-10h and 16-18h reading time was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

CEFIDEROCOL AD HOC WORKING GROUP REPORT

- Objective: Reproducible means of testing cefiderocol by broth microdilution or disk diffusion for Enterobacterales, *P. aeruginosa*, *Acinetobacter*, *S. maltophilia*, and appropriate quality control to ensure testing method is accurate
- Goals

Topic	Status
Source of cefiderocol and form	☑
Reading guidance, especially with Acinetobacter (skips, trailing, pinpoint colonies)	☑
Mueller-Hinton Broth…even with iron chelation at 6 hours, media other than BBL and Difco may not give same MICs. There seems to be something else going on.	No
Is MHA really iron-depleted?	?
Quality control to know media is correct	No

- Revised reading guidelines in M100 Appendix H and M07 Quick Guide.
- Next Steps: To focus on disk variability and addressing the question of whether MHA is iron-depleted enough.

SC DISCUSSION (MAIN POINTS)

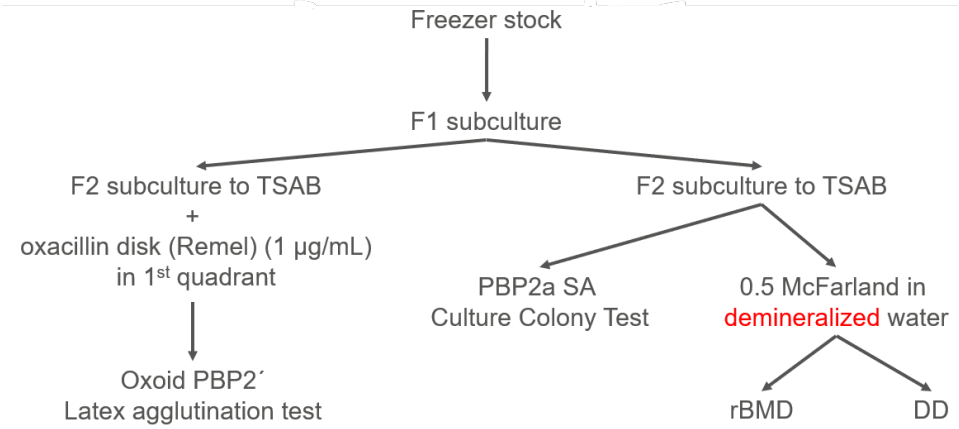
- Suggestion to update the solvents for cefiderocol. The patient care product must be resuspended in a different solvent than the AST method.

COAGULASE NEGATIVE *STAPHYLOCOCCUS* AD HOC WORKING GROUP REPORT

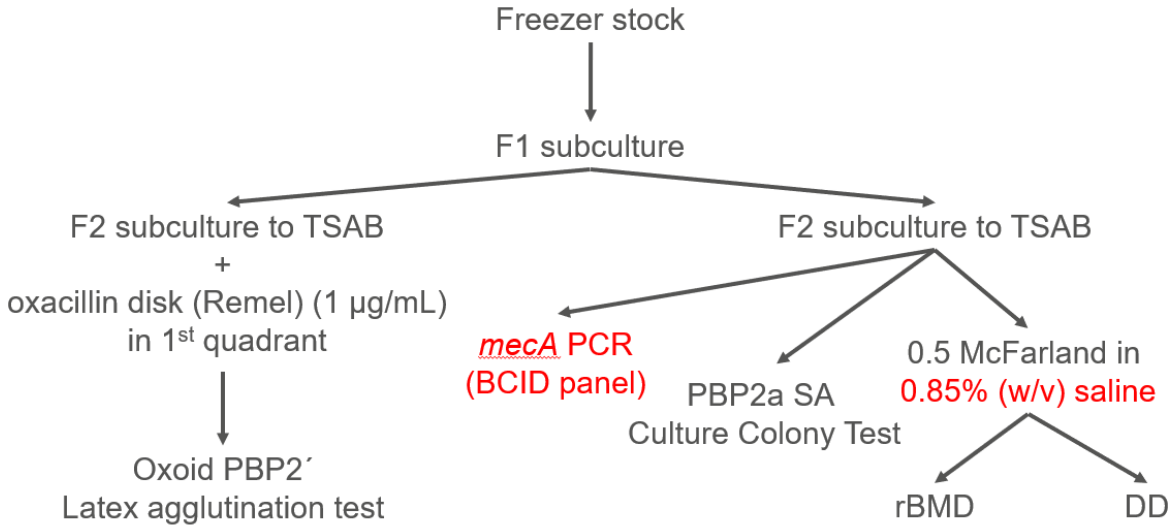
- Goal: Systematically evaluate the performance of AST methods and penicillin-binding protein 2a (PBP2a) immunoassays to detect *mecA/C*-mediated β-lactam resistance in staphylococci other than *Staphylococcus aureus* (SoSA)
- Overview of CLSI updates to staphylococcal testing recommendations to predict the presence of *mecA*

Year	Species	Recommendation	Reference
1986	All staphylococci	Publication of methicillin, nafcillin, and oxacillin MIC and DD susceptibility criteria in M100, first informational supplement	NCCLS, 1986
1999	SoSA	Establishment of oxacillin MIC and DD breakpoints in M100 that are different than those for *S. aureus*	Tenover *et al.*, 1999 *J Clin Microbiol*
1999	All staphylococci	Deletion of methicillin MIC and DD susceptibility criteria - recommendation to test oxacillin alone	Tenover *et al.*, 1999 *J Clin Microbiol*
2004	*S. aureus*/SoSA	Introduction of the cefoxitin disk diffusion test to predict oxacillin resistance	Swenson *et al.*, 2005 *J Clin Microbiol*
2005	*S. lugdunensis*	Inclusion of *S. lugdunensis* with *S. aureus* oxacillin and cefoxitin breakpoints	Swenson *et al.*, 2005 *J Clin Microbiol*
2006	*S. lugdunensis*	Warning that cefoxitin and not oxacillin should be used for disk diffusion or *S. lugdunensis*	Swenson *et al.*, 2005 *J Clin Microbiol*
2012	*S. aureus*	Deletion of oxacillin disk breakpoints	Swenson *et al.*, 2005 *J Clin Microbiol*
2012	SoSA	Recommendation to perform cefoxitin disk, PBP2a, or mecA test if oxacillin MIC of 0.5-2.0 µg/mL for species other than *S. epidermidis*	Swenson *et al.*, 2005 *J Clin Microbiol*
2014	*S. pseudintermedius*	Publication of oxacillin MIC and disk breakpoints; warning against use of cefoxitin tests for this species	Wu *et al.*, 2016 *J Clin Microbiol*
2015	*S. schleiferi*	Publication of oxacillin MIC and disk breakpoints; warning against use of cefoxitin tests for this species	Huse *et al.*, 2017 *J Clin Microbiol*
2018	*S. epidermidis*	Addition of oxacillin disk test for *S. epidermidis*, confirmation of MIC breakpoint	Naccache *et al.*, 2019 *J Clin Microbiol*
2021	SoSA	Oxacillin breakpoint updated	Humphries *et al.*, 2021 *J Clin Microbiol*

- Testing workflow for *Staphylococcus saprophyticus* study (presented in January 2024)



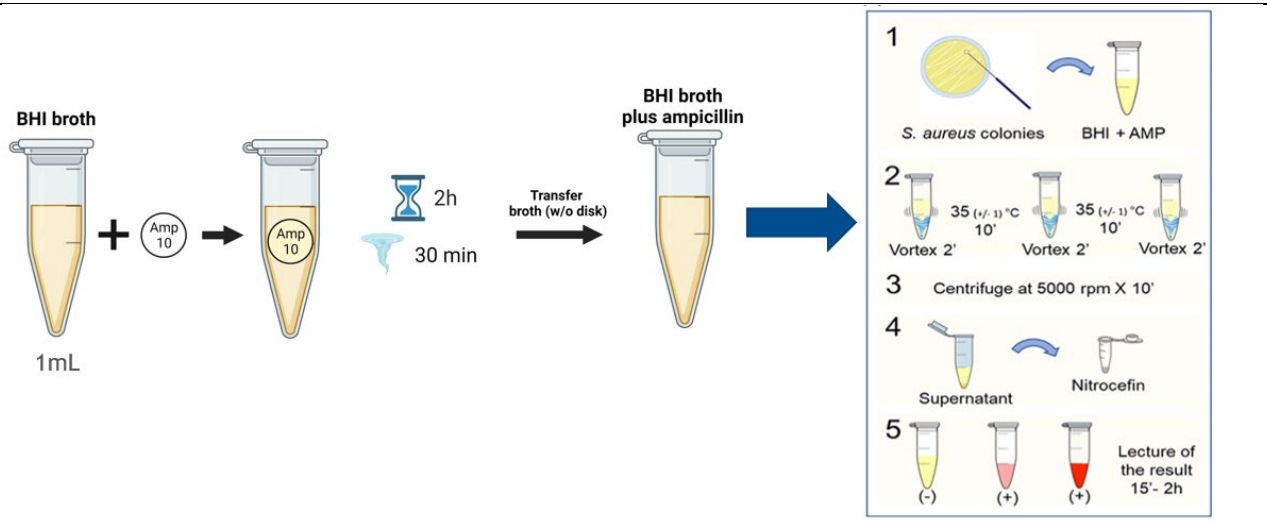
- Testing workflow (revised after counsel from CLSI in January 2024)



- Data analysis in progress. Data for *Staphylococcus saprophyticus* to be presented at January 2025 meeting.

CEFAZOLIN INOCULUM EFFECT AD HOC WORKING GROUP REPORT

- Objectives
  - PHASE 1: Assess the prevalence of CzIE phenotype in MSSA isolates in contemporary US strains. -> Done
  - PHASE 2: Evaluate the revised rapid CzIE assay. Assess suitability for multi-center evaluation. -> Done
  - PHASE 3: Perform multi-center evaluation of the revised rapid CzIE assay.
- Rapin CzlE Nitrocefin Test
  - CLSI January 2023: Performs well for BlaZ Type A in BHI broths from different manufacturers but only performs well for BlaZ Type C in 1/3 BHI broths from different manufacturers.



Slide courtesy of Sara Gomez-Villegas

- Multi-center Evaluation
  - Study protocol confirmed – Rapid CzIE Nitrocefin Method
  - Study sites confirmed – LAC, CHLA, UAH, DEA, CAB
  - Isolates selected
  - Required supplies need to be defined, acquired and distributed

Material/Reagent	Manufacturer	Cost/Donation
Sterile BHI broth	Oxoid	
	Manufacturer 2?	
Ampicillin disks	Oxoid	
	BD BBL	
DMSO	Thermo	
Nitrocefin	Thermo	
PBS	Thermo	
Eppendorf Tubes	-	Supplied by study site
0.2mL Tubes	-	Supplied by study site
1uL calibrated sterile loops	-	Supplied by study site

- Clinical Data
  - Miller, W.R., et al. 2018. OFID.
    - 77 patients in Argentina with SAB, from 2011 to 2014
    - 42 (54.5%) patients had CzIE positive isolates
    - Increase in 30-day all-cause mortality 39.5% vs 15.2%, p=0.034

- In multivariate analysis, the CzlE remained associated with mortality: Risk ratio [RR] 2.65; 95% confidence interval [CI] 1.1-6.42
- McNeil, J.C., et al. 2020. AAC.
  - 250 MSSA acute hematogenous osteomyelitis (AHO) cases in children
  - 36 (14.4%) patients had CzlE positive isolates
  - A higher rate of chronic osteomyelitis was observed with CzlE isolates regardless of definitive antibiotic choice (13.8% vs 3.7%, p=0.03)
- Lee, S., et al. 2018. CMI.
  - Patients in Korea with SAB, 2013-2015
  - 24/110 (21.8%) of patients had CzlE positive isolates
  - Treatment failure rates were not significantly different between CzlE positive and CzlE negative groups
  - Among patients who received cefazolin, treatment failure was higher and rate of 1-month mortality was higher in CzlE positive group
- Bourreau, A. et al. 2023. Infectious Diseases Now, 53(1).
  - 51 patients with MSSA infective endocarditis (17.6% positive for CzlE)
  - CzlE NOT associated with higher rate of persistent bacteremia nor with clinical failure in patients treated with cefazolin.
- Lo, Calvin Ka-Fung. et al. 2024. JAC Antimicrob Resist.
  - n=23 observational studies
  - CzlE prevalence: 0-55%
  - Question: In patients with serious MSSA infection treated with cefazolin, does infection due to CzlE-positive MSSA isolates result in worse clinical outcomes than infection due to CzlE-negative MSSA isolates?
  - No significant difference in mortality in 2 studies comparing MSSA infections with and without the CzlE
  - One study (out of 4) showed a significant increase in treatment failure for CzlE positive isolates, but there was no adjustment for confounders
  - "Our findings do not support CzlE testing in clinical practice currently."

- MDSWG Discussion and Recommendation
  - Difficult to provide testing guidance until more clinical data supporting the utility/impact of CzlE is published and guidance is developed on what to do with a positive result
  - Recommended holding on the multicenter study until more clinical data available
  - Work to find a QC strain (BlaZ type C) that could work to QC BHI broth

TABLE 6A SOLVENTS

- Updates to Table 6A have primarily been done for new agents. For all other drugs, some changes may be warranted.
- For example: Water is the solvent and diluent for both ciprofloxacin and doxycycline. Ciprofloxacin hydrochloride and doxycycline hydrochloride should be used for easier solubility in water and the use of these powders recommended/added to Table 6A as a footnote.
- For all makers of AST broth and/or agar plates, please provide any suggested edits to Table 6A to the Methods Working group, attention Laura Koeth (lkoeth@labspec.org). The goal is to present the review and edits at the January 2025 AST meeting.

SC DISCUSSION (MAIN POINTS)

- There is a UK paper that details this topic.

- The Clinical Microbiology Procedures Handbook recently updated this information.
- CDC added compound name example: avibactam-sodium instead of just avibactam.

"EARLY GROWTH" AST

- Concept: AST turn-around time (TAT) can be by reducing the time that cultures are incubated before setting up AST

CLSI Document	Current Subculture Incubation Guidance PRIOR to AST
CLSI M02 (2024) - DD	18-24 hours
CLSI M07 (2024) - Agar Dilution and BMD	18-24 hours

- MDSWG Discussion and Recommendation
  - Support the formation of an AHWG to review early growth AST (consensus from WG, no vote due to no quorum)
  - Many labs may already be doing this, so data may be available
  - Extend review to BMD? AD?

SC DISCUSSION (MAIN POINTS)

- The consensus of the Subcommittee supports the formation of an early read AST AHWG. No vote taken.
- AST TAT can be reduced.
- This does not work well for anaerobes.
- Would this early read time be considered a reference method, a standard method, or equivalent method?
- Think about what can go wrong and how.
- Do not want *S. pneumoniae* from >24hrs. Concern for isolate purity. Need to think about what species this will work for.

5.	**OUTREACH WORKING GROUP (A. SCHUETZ)**
	
	WORKING GROUP GOALS
	• Educate practicing clinical microbiologists and health care professionals about AST practices and recommendations.
	• Provide resources to facilitate individuals in their understanding and implementation of CLSI AST recommendations.
	• Solicit suggestions from members of other CLSI Working Groups for educational activities; encourage AST SC volunteers to engage in these educational activities.
	
	PRODUCTS OF ORWG
	• Education Workshops
	• News updates
	• Webinars
	  ○ CLSI-CAP
	  ○ CLSI-ACCP-SIDP
	  ○ Other
	• Programs at other meetings (eg, ASM, IDWeek)
	• Other educational products
	  ○ M100 Educational Program (2024 updates in progress)
	  ○ 2023 Breakpoint Implementation Toolkit (BIT) and accompanying materials
	• Other publications
	  ○ Annual mini-review of new M100
	  ▪ M100 32nd Edition and 33rd Edition in press (JCM)
	  ▪ M100 34th Edition in progress (JCM)
	  ○ Other
	
	WEBINARS/PRESENTATIONS
	• CLSI Annual Update (21st)
	  ○ What's New in the 2024 CLSI Standards for Antimicrobial Susceptibility Testing (AST)?
	  ○ https://clsi.org/standards/products/microbiology/education/astupdate24wr/
	  ○ April 17, 2024
	  ○ Speakers: April Bobenchik and Romney Humphries
	  ○ Moderator: Janet Hindler
	  ○ 989 registered
	  ○ 450 joined the live webinar
	  ○ At the time of the webinar, 70% of registrants attended live webinar
	  ○ 741 views of the on-demand recording (on-demand was posted 4/19)
	• CLSI-SIDP-ACCP Annual Webinar
	  ○ Breaking Bad Bacteria: Mastering the 2024 CLSI Antimicrobial Susceptibility Testing Updates
	  ○ August 7, 2024

- Speakers: Virginia Pierce and Navaneeth Narayanan
- Learning objectives:
  - Summarize highlights from the 2024 CLSI M100 standards for antimicrobial susceptibility testing and reporting
  - Examine nuances that may be encountered when performing antimicrobial susceptibility testing (AST) for carbapenem-resistant bacteria.
  - Evaluate species-specific guidance for AST of *Staphylococcus* spp. and ceftriaxone dosing guidance for methicillin-susceptible *Staphylococcus aureus*
  - Discuss the revised breakpoints, reporting, and treatment recommendations for *Stenotrophomonas maltophilia*

- CLSI-CAP Annual Webinar
  - *Staphylococcus* spp. Other than *S. aureus* Identification and AST (tentative)
  - October 2024
  - Speakers: Jennifer Dien Bard and Lars Westblade
  - Proposed content:
    - Species ID – when species ID is needed vs. descriptive ID
    - When to report AST
    - Oxazolidinone testing
    - *mecA* updates
    - Ceftriaxone dosing
- CLSI Webinar
  - Troubleshooting Unusual AST Results
  - October/November 2024
  - Case-based webinar:
    - Material built off examples in M100 Appendix A (confirming AST results and organism ID), Appendix B (intrinsic resistance), Appendix G (molecular assays for resistance detection)
    - Situational examples of unusual AST results
    - Troubleshooting
    - Intrinsic resistance

ASM MICROBE 2024

- CLSI's New Guidance on Antifungal Intrinsic Resistance
  - Track Hub
  - Saturday, June 15, 2024
  - Antifungal Intrinsic Resistance
  - Speaker: Tanis Dingle
  - Moderator: Audrey Schuetz

ASM MICROBE 2025

- β-lactam combination agents
  - Proposed content:

        - Newer available antimicrobials
        - When to test and report
        - Role of these agents in assessing carbapenem resistance mechanisms
        - Streamlined Q
    - Practical; in-depth symposium vs. track hub vs. other

ATTENDEE ORIENTATION

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

2023 BREAKPOINT IMPLEMENTATION TOOLKIT (BIT)

- Launched June 2023
- BIT Webinar
    - Get Current! Using the 2023 Breakpoint Implementation Toolkit to Update and Document AST Breakpoints
    - October 26, 2023
    - Speakers: April Abbott, Felicia Rice, Tsigereda Tekle
    - Moderator: Romney Humphries
    - https://clsi.org/meetings/ast/breakpoints-in-use-toolkit/
    - Free, posted June 2023
    - Created by the Breakpoint Implementation Ad Hoc Working Group
    - 1,945 webinar registrants across 48 countries
    - 628 live attendees – 44% of registered attendees watched the webinar live. Usually industry standard is 30-40%.
    - 1,143 on-demand views (this number is not unique)
- Additional resources in development:
    - CLSI M68 Validation of Commercial Antimicrobial Susceptibility Testing Breakpoints
    - Addressing questions received

M100 EDUCATIONAL PROGRAM

- M100 34th edition in progress
- No fee
- Enhance user ease of access
- Hope to release M100 34th edition summer 2024
- New look!
- Sections:
    - Using M100
    - Exercises
    - Remove setting disk breakpoints to separate section
- Need beta testers!!!

ORWG NEWS UPDATE

- Previous news updates released with January and June AST SC meetings
- Change publication dates
  - September
  - March
- September 2024
  - Feature: *Stenotrophomonas maltophilia*
  - Case: Reporting cefepime for carbapenemase producers
  - Practical tips: Linezolid/tedizolid
  - Hot topic: New antifungal rezafungin

AST SC MEETING EDUCATION SESSIONS

- June 2024
  - Exploring Beta-lactam Combination Agents: Opportunities, Gaps, and Challenges
  - June 22, 2024
  - Speakers: Greg Moeck, Romney Humphries, Andrew Fratoni
  - Will be available for on-demand viewing and PACE credit!
- January 2025
  - AST, Antifungal and Veterinary Subcommittees
  - LDTs
  - FDA (CDER and CDRH), lab, public health perspectives
- Past Education Sessions

Date	Title
June 2015	Antibacterial Therapy – New Drugs and Approaches
January 2016	Emerging Molecular and Novel Methods to Detect Antimicrobial Resistance
June 2016	"Unusual Suspects" – Resistance Concerns and Susceptibility Testing Among Less Common, but Noteworthy Bacteria
January 2017	One Health – One Medicine: Linking Human, Animal, and Environmental Health
June 2017	Rapid Diagnostics, Algorithms, Interpretive Comments, and Antimicrobial Stewardship
June 2018	Implementation of the 21st Century Cures Act for Breakpoints and Interpretive Categories
January 2019	Recent Advances in PK/PD and Its Uses in Setting Breakpoints
June 2019	To MIC or Not to MIC; That Is the Question. Molecular Characterization of Antimicrobial Resistance for Healthcare in 2019.
January 2020	Beyond SIR: Enhancing Laboratory Communication with Reporting Comments
June 2022	Updating Breakpoints – Challenges and Solutions for Various Stakeholders
June 2023	Standard Reference Methods for AST: Perspectives From Various Stakeholders
January 2024	Addressing the gaps in defining, detecting and reporting MDRO in clinical, veterinary, and public health laboratories
June 2024	Exploring Beta-lactam Combination Agents: Opportunities, Gaps, and Challenges

PUBLICATIONS

- Schuetz, A, A Farrell, 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 Farrell, J Hindler, A Schuetz. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100 34th Edition. Planning.

ORWG SUMMARY 2024-2025

- AST SC Meeting Workshop
- Webinars
    - CLSI-SIDP-ACCP Annual Webinar
    - CLSI-CAP Annual Webinar
    - Annual M100 (2025) Update
    - CLSI Fall Webinar
- Updated M100 Educational program for 2024
- Mini-review of M100 34th ed for JCM
- News Update September 2024; March 2025
- Other programs

	○ Submit ASM Microbe Proposal 2025 • Explore possibility of podcasts vs. Micro-learning • Other applicable items that result from AST SC June 2025 discussions **VOLUNTEER OPPORTUNITIES** • News Update ○ Provide feedback on content, delivery, and structure ○ Suggest content ○ Partner with others to write articles (case studies and more) • Other Publications ○ Assorted topics • Webinars / Workshops / Lectures/Podcasts ○ Suggest content ○ Speakers • Other Projects **SC DISCUSSION (MAIN POINTS)** • Suggestion to have podcasts/videos in Spanish.

6. **ADDITIONAL ITEMS**

TESTS FOR CARBAPENEM DETECTION TABLE

- Proposed Plenary Revisions by Virginia Pierce
- Recall that we already decided in January to add a comment to the eCIM reporting that says: If both a serine carbapenemase and a metallo-ß-lactamase are co-produced by one organism, differentiation between enzymes will not be possible and false-negative eCIM results may occur.

	Tests for Carbapenemase Detection			
	CarbaNP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Organisms	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales that are positive by mCIM	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by CarbaNP 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
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 type of carbapenemase.	Requires overnight incubation. Does not determine type of carbapenemase.	Requires overnight incubation. Does not determine 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: eCIM, EDTA-modified carbapenem inactivation method; mCIM, modified carbapenem inactivation method.

A motion to accept the proposed revisions to the Carbapenemase Detection Table was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

7. **ADJOURNMENT**

Dr. Lewis thanked the participants for their attention. The meeting was adjourned at 12:00 PM Central Standard (US) time.

PLENARY ATTENDEES

Plenary 1	Plenary 2	Plenary 3
Abdelraouf Kamilia	Abdelraouf Kamilia	Abdelraouf Kamilia
Adams Jennifer K.	Adams Jennifer K.	Adams Jennifer K.
Alby Kevin	Alby Kevin	Alby Kevin
Andermann Tessa	Andermann Tessa	AliuYamah Musa
Arbefeville Sophie	Arbefeville Sophie	Andermann Tessa
Asempa Tomefa	Asempa Tomefa	Arbefeville Sophie
Atkinson Dunn Robyn	Atkinson Dunn Robyn	Asempa Tomefa
Bala Shukal	Bala Shukal	Atkinson Dunn Robyn
Balbuena Rocio	Balbuena Rocio	Bala Shukal
Barber Meagan	Barber Meagan	Balbuena Rocio
Barman Lipika	Barman Lipika	Barber Meagan
Belanger Myriam	Belanger Myriam	Barman Lipika
Belley Adam	Belley Adam	Barnett Katie
Bennett Jill	Bennett Jill	Belanger Myriam
Bensman Timothy J.	Bensman Timothy J.	Belley Adam
Berger Jane	Bhatnagar Amelia	Bennett Jill
Bhatnagar Amelia	Bidkorpeh Elma Kamari	Bensman Timothy J.
Bidkorpeh Elma Kamari	Bittencourt Cassiana	Bhatnagar Amelia
Bittencourt Cassiana	Bixby Morgan	Bidkorpeh Elma Kamari
Bixby Morgan	Blosser Sara	Bittencourt Cassiana
Blosser Sara	Bobenchik April M.	Bixby Morgan
Bobenchik April M.	Boswell Malcolm	Blosser Sara
Boswell Malcolm	Bowden Robert	Bobenchik April M.
Bowden Robert	Bradford Patricia	Boswell Malcolm
Bradford Patricia	Brasso William B.	Bowden Robert
Brasso William B.	Breton John	Bradford Patricia
Breton John	Brown Carrine	Brasso William B.
Brown Carrine	Bryan, MD, PhD Andrew	Breton John
Bryan, MD, PhD Andrew	Bryant Kendall	Bryan, MD, PhD Andrew
Bryant Kendall	Bryowsky Jason	Bryant Kendall
Bryowsky Jason	Bryson Alexandra Lynn	Bryowsky Jason
Bryson Alexandra Lynn	Buccat Ryan	Bryson Alexandra Lynn
Buccat Ryan	Bulman Zackery P.	Buccat Ryan
Bulman Zackery P.	Burgess David S	Bulman Zackery P.
Burgess David S	Burgos-Garay Maria	Burgess David S
Burgos-Garay Maria	Burnham Carey-Ann	Burgos-Garay Maria
Burnham Carey-Ann	Bush Karen	Burnham Carey-Ann
Bush Karen	Butler Deborah	Bush Karen
Butler Deborah	Caidi Hayat	Butler Deborah
Caidi Hayat	Campbell Davina	Campbell Davina

Campbell Davina
Campeau Shelley
Campodonico Victoria
Canton Rafael
Capraro Gerald A.
Carpenter Darcie E.
Carvalhaes Cecilia
Castanheira Mariana
Castillo-Martinez Nydia
Chaintoutis Serafeim
Chandler Courtney
Chandrasekaran Sukantha
Chantell Christina
CHEN YAMIN
Cicala Katherine
Cintron Cotto Melvili
Cole Nicolynn
Conville Patricia S.
Cooper Elizabeth
Creager Hannah
Cudiamat Ruben
Cullen Sharon K.
Danielsen Zhixia
Datta Pradip
Debabov Dmitri
DeDonder Keith
DeJonge Boudewijn
DeStefano Ian
Dhara Animesh
Dingle Tanis
Donohue Lindsay
Dressel Dana C.
Dumm Rebekah
Duncan Elaine
Edelstein Paul
Elanany Mervat
Esparza German
Farley John
Fedorenko Marianna
fernandez erica
Ferrell Andrea L.
Feßler Andrea T.
Fisher Mark A.
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Flemming Laurie
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Galas Marcelo F.

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Fratoni Andrew
Galas Marcelo F.
Gancarz Barb
Gandhi Mukesh
García Cañete Patricia
Garcia-Effron Guillermo
Garg Rahul
Garrett Elizabeth
Gitman Melissa
Glasgow Heather
Goldstein Beth P.
Gomez Emily J.
Grande Roche Kerian K.
Gray Alice
Gray Kamisha
Greninger Alex
Griffin Natasha
Gutierrez Carlos
Hackel Meredith
Hernandez Esther
Herrera Elide
Hindler Janet A.
Hirsch Elizabeth
Hoffard Rita
Holliday Nicole
Hope Katie
Howe Zachary
Huband Michael D.
Humphries Romney M
Huse Holly
Iarikov Dmitri
Iguchi Mitsutaka
Jean Sophonie
Jimenez Antonieta
Johnson Brian
Johnson Jasmin
Johnson Kristie
Jorgensen James H.
Joshi Abhay
JOSHI PRASHANT
Kamau Edwin
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Kasapidis Cara
Kersh Ellen N.
Khalid Haziq
Kim Peter
Kirn Thomas J.

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Khalid Haziq
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Kim Peter
Kirn Thomas J.
Klavins Anna
Koeth Laura M.
Koralur Munegowda
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Lam Christine M.
LaVoie Stephen
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Lozano Sergio
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Nigg Benjamin
North Michael
Ohkusu Kiyofumi
Onishi Motoyasu
Ordonez Smith de Danies Margaret
O'Rourke Susan
Otima Evans
Otterson Linda G.
Oyarzun Sebastian Cifuentes
Palavecino Elizabeth
Patel Jean B.
Paukner Susanne
Pearson Jeffrey
Perez Omar
Pham Cau Dinh
Pierce Virginia M.
Pillar Chris
Pischel Kelsey
Ramos Karl Anthony
Ransom Eric
Razaki Hamid
Re David
Redell Mark A
Rice Felicia
Richter Sandra S.
Rossi Flavia
Salinas Catalina
Sanchez Susan
Satlin Michael
SAUVONNET Veronique
Scangarella-Oman Nicole
Schuermeyer Linda
Schuetz Audrey N.
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Ransom Eric
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Sanchez Susan
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Schuermeyer Linda
Schuetz Audrey N.
Scott-Pittman Arianne
Selby Ashley
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Sharp Susan
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Ordonez Smith de Danies Margaret
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Otima Evans
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Oyarzun Sebastian Cifuentes
Palavecino Elizabeth
Patel Jean B.
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Sanchez Susan
Satlin Michael
SAUVONNET Veronique
Scangarella-Oman Nicole
Schuermeyer Linda
Schuetz Audrey N.
Scott-Pittman Arianne
Selby Ashley
Seyedmousavi Amir
Sharp Susan
Shawar Ribhi M.
Shi Wanliang
Shier Kileen
Shurland Simone M
Simner Patricia J.
Slaughter Jennifer
Smart Jennifer
Smith Kaylee
Snippes Vagnone Paula M.

Selby Ashley
Seyedmousavi Amir
Sharp Susan
Shawar Ribhi M.
Shi Wanliang
Shier Kileen
Shurland Simone M
Simner Patricia J.
Slaughter Jennifer
Smart Jennifer
Smith Kaylee
Snippes Vagnone Paula M.
Staats Dylan
Steenbergen Judith
Stewart Laura
Stone Gregory G.
Takemura Miki
Tamma Pranita D.
Tarlton Nicole
Tekle Tsigereda
Tenllado Jolyn
Thomson Susan
Thrupp Lauri D.
Torumkuney Didem
Trabold Peter
Trauner Andrej
Trebosc Vincent
Turng Ben
Uprety Priyanka
vaidya suyog
Van Tam T.
Viel Alexis
Wehr Collette
Weingarten Rebecca
Weinstein Melvin P.
Wenzler Eric
Wikler Matthew A.
Winkler Marisa
Won Christina
won sarah
Wong Frederick TS
Wungwattana Minkey
Yamashiro Hidenori
Shawar Ribhi M.
Shi Wanliang
Shier Kileen
Shurland Simone M
Simner Patricia J.
Slaughter Jennifer
Smart Jennifer
Smith Kaylee
Snippes Vagnone Paula M.
Staats Dylan
Steenbergen Judith
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Wong Frederick TS
Wungwattana Minkey
Yamano Yoshinori
Yamashiro Hidenori
Yang Christine
Zimmer Barbara L.
Staats Dylan
Steenbergen Judith
Stewart Laura
Stone Gregory G.
Takemura Miki
Tamma Pranita D.
Tarlton Nicole
Tekle Tsigereda
Tenllado Jolyn
Thomson Susan
Thrupp Lauri D.
Torumkuney Didem
Trabold Peter
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Uprety Priyanka
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Yang Christine
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