Meeting Title:	**Subcommittee on Antimicrobial Susceptibility Testing (AST)**	**Contact:**	egomez@clsi.org
Meeting Location:	Tempe, Arizona, USA		
Meeting Dates and Times: All times are Mountain Standard (US) time.	**Plenary 1:** Monday, 22 January 2024, 7:30 AM – 12:00 PM **Plenary 2:** Monday, 22 January 2024, 1:00 – 5:30 PM **Plenary 3:** Tuesday, 23 January 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
Tanaya Bhowmick, MD	Rutgers Robert Wood Johnson Medical School
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
Christian G. Giske, MD, PhD	Karolinska University Hospital
Howard Gold, MD, FIDSA	Beth Israel Deaconess Medical Center

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
Maria Machado	Centers for Disease Control and Prevention
Linda A. Miller, PhD	CMID Pharma Consulting LLC
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
Eric Wenzler, PharmD, BCPS, AAHIVP	Roche
Barbara L. Zimmer, PhD	Beckman Coulter
Reviewers and Guests (Non-SC-roster attendees): see Plenary Attendee List below	
Staff:	
Jennifer Adams, MT(ASCP), MSHA	CLSI
Kathy Castagna, MS, MT(ASCP)CT, MB	CLSI
Emily Gomez, MS, MLS(ASCP)MB	CLSI
Barb Jones, PhD	CLSI
Christine Lam, MT(ASCP)	CLSI

Plenary Agendas

PLENARY AGENDA: Session 1 Monday, 22 January 2024 (In-person) 7:30 AM – 12:00 PM Mountain Standard (US) Time			
Time	**Item**	**Presenter**	**Page**
7:30 AM – 7:40 AM (10 min)	Opening Remarks	J. Lewis	7
7:40 AM – 7:50 AM (10 min)	CLSI Welcome and Update	J. Adams	7
7:50 AM – 8:00 AM (10 min)	CLSI Awards	B. Jones	7
8:00 AM – 8:10 AM (10 min)	Expert Panel on Microbiology Update	A. Schuetz	8
8:10 AM – 8:20 AM (10 min)	Antifungal Subcommittee Update	T. Dingle	10
8:20 AM – 8:30 AM (10 min)	EUCAST Update	C. Giske	13
8:30 AM – 9:00 AM (30 min)	Outreach WG	J. Hindler A. Schuetz	15
9:00 AM – 9:20 AM (20 min)	Break		
9:20 AM – 11:00 AM (1 hr 30 min)	Quality Control WG	S. Cullen C. Pillar	19
11:00 AM – 12:00 PM (1 hr)	Breakpoints WG: Part 1	N. Narayanan M. Satlin	40

PLENARY AGENDA: Session 2 Monday, 22 January 2024 (In-person) 1:00 PM – 5:30 PM Mountain Standard (US) Time			
Time	**Item**	**Presenter**	**Page**
1:00 PM – 3:00 PM (2 hr)	Breakpoints WG: Part 2	N. Narayanan M. Satlin	40
3:00 PM – 3:20 PM (20 min)	Break		

3:20 PM – 5:30 PM (2 hr)	Methods Application and Interpretation WG	T. Kirn K. Johnson	62
PLENARY AGENDA: Session 3 Tuesday, 23 January 2024 (In-person) 7:30 AM – 12:00 PM Mountain Standard (US) Time			
Time	**Item**	**Presenter**	**Page**
7:30 AM – 8:00 AM (30 min)	Joint CLSI-EUCAST WG	J. Hindler E. Matuschek	82
8:00 AM – 10:30 AM (2 hour 30 min)	Methods Development and Standardization WG	T. Dingle B. Zimmer	85
10:30 AM – 10:50 AM (20 min)	Break		
10:50 AM – 11:30 AM (40 min)	Text and Tables WG	A. Bobenchik S. Campeau	108
11:30 AM – 12:00 PM (30 min)	M45 Update	T. Simner R. Humphries	113
12:00 PM	Closing Remarks	J. Lewis	121

Summary of Voting Decisions and Action Items

Summary of Passing Votes			
#	**Motion Made and Seconded**	**Results[a]**	**Page[b]**
1.	To accept the meropenem-ANT3310 QC ranges for *A. baumannii* NTCC 13304 (0.12/8 - 1/8 µg/mL), *E. coli* ATCC 25922 (0.008/8 – 0.03/8 µg/mL), *K. pneumoniae* BAA-2814 (0.06/8 – 0.25/8 µg/mL), and *P. aeruginosa* ATCC 27853 (0.12/8 – 0.5/8 µg/mL). For meropenem/ANT3310, the recommended routine QC strains are *A. baumannii* NCTC 13304 and *K. pneumoniae* ATCC BAA-2814.	14-0-0-0	21
2.	To accept the BWC0977 QC ranges for *E. faecalis* ATCC 29212 (0.03 - 0.12 µg/mL), *E. coli* ATCC 25922 (0.03 – 0.25 µg/mL), *H. influenzae* ATCC 49247 (0.002 - 0.016 µg/mL), *P. aeruginosa* ATCC 27853 (0.12 – 1 µg/mL), *S. aureus* ATCC 29213 (0.004 - 0.03 µg/mL), and *S. pneumoniae* ATCC 49619 (0.004 - 0.016 µg/mL). Add footnote to Table 5A and 5B stating, "MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available." Add footnote to Table 5B stating, "MIC ranges were established using HTM and CAMHB with LHB (2.5% to 5% v/v). Performance with MH-F broth was not evaluated."	14-0-0-0	24
3.	To accept the ceftibuten-xeruborbactam QC ranges for *K. pneumoniae* ATCC 700603 (0.016/4 - 0.12/4 µg/mL), *K. pneumoniae* ATCC BAA-1705 (0.03/4 – 0.25/4 µg/mL), and *K. pneumoniae* ATCC BAA-2814 (0.12/4 - 0.5/4 µg/mL). For ceftibuten-xeruborbactam, the recommended routine QC strain is *K. pneumoniae* ATCC BAA-2814. Add footnote to Table 5A-2 stating, "MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available."	14-0-0-0	26
4.	To accept the Debio 1452 QC ranges for *S. aureus* ATCC 25923 (20 - 27 mm).	14-0-0-0	28
5.	To accept the ceftibuten-avibactam QC ranges for *E.coli* ATCC 25922 (28 – 36 mm), *E.coli* NCTC 13353 (28 - 34 mm), *K. pneumoniae* ATCC 700603 (24– 30 mm), *K. pneumoniae* ATCC BAA-1705 (24 – 30 mm), and *K. pneumoniae* ATCC BAA-2814 (22 – 28 mm). For ceftibuten-avibactam, the recommended routine QC strain is *E. coli* NCTC 13353. Add footnote to Table 5A-2 stating, "MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available."	14-0-0-0	31
6.	To approve the M45 penicillin MIC breakpoints for *Abiotrophia/Granulicatella, Lactococcus,* and *Micrococcus* (S≤2, I 4, R≥8 µg/mL).	11-3-0-0	43
7.	To approve the M45 penicillin MIC breakpoints for *Lactobacillus* and *Pediococcus* (S≤2 µg/mL).	14-0-0-0	44
8.	To approve the M45 penicillin MIC breakpoint for *Leuconostoc* (S≤4 µg/mL).	14-0-0-0	45
9.	To approve the M45 tetracycline MIC breakpoints for *Aerococcus* (S≤0.5, I 1, R≥2 µg/mL), *Campylobacter* (S≤2, I 4, R≥8 µg/mL), *Corynebacterium* (S≤2, I 4, R≥8 µg/mL), HACEK (S≤1, I 2, R≥4 µg/mL), *Lactococcus (*S≤1, I 2, R≥4 µg/mL), and *Vibrio (*S≤1, I 2, R≥4 µg/mL), the doxycycline MIC breakpoints for *Campylobacter (*S≤1, I 2, R≥4 µg/mL), *Corynebacterium (*S≤1, I 2, R≥4 µg/mL), and *Vibrio* (S≤1, I 2, R≥4 µg/mL), and the minocycline MIC breakpoints for *Leuconostoc* (S≤1, I 2, R≥4 µg/mL).	14-0-0-0	50
10.	To remove "fluoroquinolones" from the CSF warning box in the Introduction to Tables 1.	13-0-0-1	54

Summary of Passing Votes			
11.	To add the comment stating, "In institutions that serve patients at high risk for metallo-ß-lactamase producing Enterobacterales, aztreonam may be considered a Tier 3 agent following cascade reporting rules established at the institution." to aztreonam in Tier 4 in Tables 1A.	8-0-0-6	59
12.	To recategorize disk diffusion from a reference method to a standard method.	14-0-0-0	64
13.	To accept the proposed eCIM revisions with the comment stating, "False-negative results are likely to occur for isolates co-producing a serine carbapenemase and a metallo-ß-lactamase." in the Introduction to Tables 3B and 3C.	14-0-0-0	68
14.	To remove the *Burkholderia cepacia* complex breakpoints in M100.	10-4-0-0	79
15.	To approve the M23S Subchapter 2.5 chapter additions.	12-0-0-2	83
16.	To approve the M23S proposed comment for when a sponsor elects not to interact with the Joint WG for disk content selection.	12-0-0-2	83
17.	To support the agar dilution + PIH method for rifabutin testing of *Acinetobacter baumannii* as the reference method for Tier 1 and Tier 2 QC studies with approval of this specific method contingent on CLSI receiving further information that satisfies the Subcommittee concerns.	12-2-0-0	87
18.	To accept the CAMHB + 20% heat inactivated horse serum read at 100% inhibition method for zosurabalpin (RG6006) broth MIC testing.	13-0-1-0	95
19.	To accept the cefepime direct blood disk breakpoints for Enterobacterales (S≥23, I 19-22, R≤18 mm) for an 8-10h and 16-18h reading time.	13-0-1-0	103
20.	To accept the ceftazidime direct blood disk breakpoints for *Acinetobacter* (S≥17, I 15-16, R≤14 mm) for an 8-10h reading time.	13-0-1-0	103
21.	To accept the piperacillin-tazobactam direct blood disk breakpoints for *Acinetobacter* (S≥19, I 17-18, R≤16 mm) for an 8-10h and 16-18h reading time.	13-0-1-0	104
22.	To accept the ceftazidime direct blood disk breakpoints for *P. aeruginosa* (S≥18, 15-17 I, R≤14 mm) for an 8-10h reading time with a footnote that the I zone size would need to be reincubated and read at 16-18h.	13-0-1-0	106
23.	To accept the current CLSI QC ranges for *E. coli* 25922 ampicillin (15-22 mm), *E. coli* 35218 ampicillin-sulbactam (13-19 mm), *P. aeruginosa* 27853 ertapenem (13-21 mm), and *E. coli* 25922 trimethoprim-sulfamethoxazole (23-29 mm) for direct blood disk early reads.	14-0-0-0	107
24.	To accept the proposed direct blood disk early read QC table edits with wordsmithing the "daily or weekly QC".	14-0-0-0	107

[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.

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

2024 JANUARY AST SUBCOMMITTEE MEETING SUMMARY MINUTES PLENARY 1: Monday, 22 January 2024 (In-person) 7:30 AM – 12:00 PM Mountain Standard (US) Time	
#	**Description**
1.	**OPENING REMARKS (J. LEWIS)** Dr. Lewis opened the meeting at 7:30 AM Mountain Standard (US) time by welcoming the participants to the CLSI meeting in Tempe, Arizona
2.	**CLSI WELCOME AND UPDATE (J. ADAMS)** Ms. Adams provided an update on CLSI activities. The topics included: • Products and publications for fiscal year 2023 • Product ideas and proposals • Projects in progress • Estimated fiscal year 2024 publications • Volunteer engagement increase (20% increase per project) • Standards development organization • Document use • New international document pricing
3.	**CLSI AWARDS (B. JONES)** Dr. Jones presented CLSI awards to: • John V. Bergen Excellence Award – Romney Humphries • Excellence in Standards Development Award – Audrey Schuetz • Russell J. Eilers Memorial Award – Melvin Weinstein

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

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

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

Document Number	Document Name	Publication Year of Prior Version
M35-ED3	Abbreviated Identification of Bacteria and Yeasts	2008
M52-ED2	Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems	2015
M56-ED2	Principles and Procedures for Detection of Anaerobes in Clinical Specimens	2014
M63-ED1	Principles and Procedures for the Gram Stain	N/A
M64-ED1	Implementation of Taxonomy Changes	N/A
M66-ED1	Methods for Active Surveillance of Multidrug-Resistant Organisms	N/A
M67-ED1	Verification of Laboratory Automation in Microbiology	N/A
M68-ED1	Validation of Commercial Antimicrobial Susceptibility Test (AST) Breakpoints	N/A
M69-ED1	Principles of Serologic (Antibody and Antigen) Testing for Infectious Diseases	N/A

- Upcoming Revisions to Microbiology Documents

Document Number	Document Name	Publication Year of Prior Version
M29-ED5	Protection of Laboratory Workers From Occupationally Acquired Infections	2014
M48-ED2	Laboratory Detection and Identification of Mycobacteria	2008
M58-ED2	Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry	2017

- Upcoming New Microbiology Documents
  - Laboratory Testing for Lyme Borreliosis (M70-ED1)
  - Diagnostic Stewardship in Clinical Microbiology
  - Quality Assurance for AST
- How can you help?
  - Reach out to Microbiology Expert Panel members or advisors with ideas for documents
  - Respond to requests for volunteers for document development committees (DDCs)

5. **ANTIFUNGAL SUBCOMMITTEE UPDATE (T. DINGLE)**

Dr. Dingle provided an update on the activities of the CLSI Antifungal Subcommittee. The main points included:

- Antifungal Subcommittee Working Groups
  - Reporting Working Group
    - Intrinsic Resistance Working Group
    - Body Site Reporting Working Group
  - Breakpoint Working Group
    - Rezafungin Ad Hoc Working Group
    - Azole/*Aspergillus fumigatus* Ad Hoc Working Group
  - ECV Working Group
  - Document Review Working Group
    - M27 Review Working Group
    - M38 Review Working Group
  - MIC Reading Working Group
- Antifungal Documents
  - Procedural documents: M27, M38, M44, M57, M51 (archived)
  - Supplements: M27M44S, M38M51S, M57S
- Documents Approved for Revision
  - Procedural documents:
    - M27, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, DDC formed; work to begin March 2024
    - M38, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi, DDC formed; work to begin April 2024
    - M44, Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts, limited revision approved at January 2024 meeting
  - Supplements (Approved for revision at January 2024 meeting)
    - M27M44S, Performance Standards for Antifungal Susceptibility Testing of Yeasts
    - M38M51S, Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi
    - M57S, Epidemiological Cutoff Values for Antifungal Susceptibility Testing
- Breakpoint Working Group Update
  - *Aspergillus fumigatus* and Azoles
    - Voriconazole: Breakpoint approved in June 2020. A draft rationale document was reviewed by FDA; a formal rationale to be sent to FDA to review.
    - Isavuconazole: Breakpoint approved in January 2023. Rationale document to be submitted to FDA for review.
    - Posaconazole: Data presented in November 2022, but breakpoint not approved yet.
  - Rezafungin: Breakpoints passed in January 2024.

Species	CLSI Breakpoint (Susceptible)	FDA Breakpoint (Susceptible)	Dilution Difference
C. albicans	≤ 0.25	≤ 0.12	1
C. auris	≤ 0.5	---	---
C. dubliniensis	≤ 0.12	---	---
C. glabrata	≤ 0.5	≤ 0.12	2
C. krusei	≤ 0.25	---	---
C. parapsilosis	≤ 2	≤ 2	0
C. tropicalis	≤ 0.25	≤ 0.12	1

- ECV Working Group Update
  - New revisions made to M57S Table 6 in 2022 for rare yeast species and *Scedosporium*/*Lomentospora* species.
  - Ongoing reviews of *Aspergillus* species and *Sporothrix* species.
  - Approved flucytosine ECVs for *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, and *Candida krusei* in January 2024.

Species	Antifungal	MICs (labs)	Proposed ECV (µg/mL)
C. albicans	Flucytosine	3668 (10)	TR-L
C. glabrata	Flucytosine	2191 (10)	TR-L
C. tropicalis	Flucytosine	827 (10)	TR-L or 1
C. krusei	Flucytosine	299 (6)*	32

- Antifungal Reporting Working Group
  - Updating definition for intrinsic resistance
    - When an organism/antifungal combination does not meet the CLSI intrinsic resistance definition, but the MICs of most isolates to the antifungal agent are high (ie, the MICs of more than half of isolates are higher than the midpoint of the concentration range that CLSI recommends for in vitro testing), the criteria for "reduced susceptibility" are met.
    - ECVs for these organism/antifungal combinations are high, or the MIC distributions are truncated on the high end of the recommended testing range. MIC distributions for agents with reduced susceptibility will be shifted to the right.
    - Reduced susceptibility indicates potential limited activity of the antifungal agent. A "reduced susceptibility" designation is strictly based on *in vitro* MIC distributions and does not necessarily predict clinical response. Clinicians should be cautious about using these agents and should refer to treatment guidance.
- How is "Reduced Susceptibility" determined?
  - Organism/antifungal combinations with suspected intrinsic resistance are reviewed by the Intrinsic Resistance Working Group.
  - If IR criteria are not met, a "reduced susceptibility" category will be considered.
  - MIC distributions (preferable those distributions prepared by the ECV working group) will be critically reviewed in this process.

- Clinical data will be considered when available.
- Any organism/antifungal combination designated as having “reduced susceptibility” by the IRWG has final approval and vote by the CLSI Antifungal Susceptibility Testing Subcommittee.
- Organism/antifungal combinations with reduced susceptibility will be designated with a footnote or be listed directly with the ECV in the CLSI M57 document.

- How may labs report or proceed with “reduced susceptibility” category?
  - Susceptibility testing for these combinations is advised as some isolates may test with low MICs within the therapeutic range of the antifungal agent
  - A reduced susceptibility comment should be added to wild-type isolates. Comment to be drafted.
  - Important to determine if or how we want clinicians to treat these differently.
  - Initial discussions have occurred with bacterial IRWG about this new category.
- Reviewed Fungal Documents for Quality Assurance (QA) and Quality Control (QC) for Opportunities for Improvement
  - QC Mode (in addition to expected range)
    - M100 Table 5As. Not included. Future improvement.
    - Fungal document already includes mode. No action needed.
  - QC Frequency
    - M100 Table 5F: In process of adding guidelines to define routine user QC using IQCP. Potentially streamline frequency and/or number of strains (eg, up to 8)
    - Fungal: Fewer QC strains so cost is not as big of an issue. No action needed.
  - QC Strain characteristics
    - M100 Table 5A-2, Appendix C: Includes strain characteristics.
    - Fungal: Potential improvement
  - Troubleshooting Guide
    - M100 Table 5G
    - Fungal: Potential improvement.
  - Suggestions for Confirming AST
    - M100 Appendix A
    - Fungal: Potential improvement

SC DISCUSSION (MAIN POINTS)

- Was there any discussion about loosening the definition of intrinsic resistance rather than creating a new category “reduced susceptibility”?
  - No, that was not truly discussed, but perhaps that is where the group is headed.
- Why is voriconazole more than 20 years old and we do not have breakpoints?
  - There is still a lack of clinical evidence.

6. **EUCAST UPDATE (C. GISKE)**

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

- Dr. Christian Giske will be stepping down as EUCAST Chair in 2024. The new Chair will be Sören Gatermann.
- Consultations and New Breakpoints during 2023
  - New changes to the anaerobic bacteria, *Clostrioides difficile*, *Bacillus anthracis*, and *Brucella melitensis* breakpoint tables.
  - Fosfomycin breakpoint revision for *E.coli*.
  - Removal of the PK-PD tab for PK-PD (non-species related breakpoints).
  - New EUCAST guidance on "When there are no breakpoints in breakpoint table?".
- There is an ongoing assessment of PCG vs *S. pneumoniae*.

A simulation was carried out in Pmetrics. The following assumptions were made:
-1 compartment model
-CL 24L/h, V 26L, 25% CV on each parameter, fraction unbound 55% (fixed) (based on Visser et al. 1993)

Dosage	0.6 g x 4	1.2 g x 4	1.2 g x 6	2.4 g x 6	3 g x 3	3 g x 4	3 g x 6
f%T>MIC	40	40	40	40	40	40	40
0.06	97.1	98.3	100	100	99.3	99.1	100
0.125	93.3	97.1	100	100	98.3	98.8	100
0.25	81.9	93.0	100	100	97.0	97.6	100
0.5	61.0	81.9	99.8	100	93.7	94.5	100
1	26.8	61.0	99.5	99.8	84.9	86.1	99.9
2	6.6	26.8	95.0	99.5	65.6	68.4	99.8
4	0.9	6.6	49.6	95.0	32.8	38.8	97.9
8	0.1	0.1	8.1	49.6	8.6	10.5	72.0

Effectively PTA is achieved at 1 dilution higher with the higher protein binding figure

- S ≤ 0.06mg/L, with 0.6-1.2g x 4 as the lowest dose assuming 40% fT>MIC
- R > 1mg/L if 1.2g x 6 is considered

- Upcoming consultations
  - Viridans group streptococci – breakpoints and MIC vs zone
  - Overlook of the breakpoint tables to adapt to requirements in endocarditis
  - *Nocardia* spp. – AST methodology and breakpoints
  - EUCAST dosing tab adapted to pediatric use
- EUCAST Development Lab
  - Investigation of alternative media for disk diffusion of fastidious organisms
  - Development of a disk diffusion method for *N. gonorrhoeae*
  - Investigation of alternative disks for determining the benzylpenicillin susceptibility in *Streptococcus pneumoniae* (to avoid the frequent need for MIC testing)
  - Evaluation of AST methods for cefiderocol (reference BMD, disk diffusion, and commercially available)

SC DISCUSSION (MAIN POINTS)

- The lack of fosfomycin breakpoints is a challenge for the international community because they do not have many drugs for *Pseudomonas* or carbapenemase producing Enterobacterales. What suggestions does EUCAST have for people in Latin America, who apply older breakpoints in the absence of current breakpoints?
    - There are ECOFFS for *Klebsiella* (128 μg/mL) and *Pseudomonas* (256 μg/mL), which are not encouraging. You should not consider fosfomycin as monotherapy. One manufacturing company in Europe is using agar dilution to look at antibiotic combinations with fosfomycin, and they are not seeing a real signal between wild type and non-wild type populations. They are struggling to identify what will be effective for combination therapy. EUCAST does think fosfomycin is important to consider for treatment guidelines, but it is not clear if an ECOFF of 128 μg/mL or 256 μg/mL should be used to encourage or discourage fosfomycin use in combination with other drugs. Fosfomycin may also be important for staphylococcal infections.
- Since EUCAST is looking at *Streptococcus pneumoniae* and penicillin susceptibility testing, is EUCAST also looking into ceftriaxone?
    - EUCAST has not revisited ceftriaxone. They have some guidance on predictions based benzylpenicillin or oxacillin, and EUCAST would be happy to share that data. There might be more to look at based on how different tweaks of different disks play out. The main issue right now is that EUCAST is not in full agreement on where the breakpoint should be. Another issue is that there are different dosing regimens between Europe and the United States. The EUCAST susceptible breakpoint cannot be as high as in the United States because the lowest dose in US is much higher than in Europe.
- The off scale 0.001 susceptible increased exposure was implemented several years ago. Has EUCAST studied the impact of that change?
    - This unfortunately coincided with the pandemic, so EUCAST is still understanding the impact. There have been lots of misunderstandings particularly around wildtype populations of *Pseudomonas* and "I" on the antibiogram; however, this seems to be improving. It seems that people have accepted it as a new concept and this concept is now being taught in medical schools.
- There are several pharmaceutical sponsors on the verge of initiating registrational studies for oral β-lactam and β-lactamase inhibitor compounds in the next few months to years. Traditionally EUCAST has been good at publishing rationale documents; however, those have been lagging for the oral cephalosporins. When does EUCAST expect those documents to be available? Companies are interested in the parent β-lactam component.
    - EUCAST has started looking into that; however, it is not a priority, and there are numerous cephalosporins out there. Ceftibuten is one that has been under the radar, but it is hard to find good data. It has probably always been underdosed. EUCAST is trying to make more progress on this topic, and this is a good reminder to EUCAST that this is something companies are looking for.

7. **OUTREACH WORKING GROUP (J. HINDLER)**

WORKING GROUP GOALS

- Educate practicing clinical microbiologists and health care professionals about AST practices and recommendations.
- Provide resources to facilitate individuals in their understanding and implementation of CLSI AST recommendations.
- Solicit suggestions from members of other CLSI Working Groups for educational activities; encourage AST SC volunteers to engage in these educational activities.

PRODUCTS OF ORWG

- AST SC Meeting Workshops
- Newsletters
- Webinars
    - Annual Update
    - CLSI-CAP
    - CLSI-ACCP-SIDP
    - Other (recent include 2023 BIT)
- New attendee orientation
- Programs at other meetings (eg, ASM)
- Other educational products
    - M100 Educational Program (will update for 2024)
    - 2023 BIT
- Other publications
    - Annual mini-review of new M100
    - M100 32nd ed and 33rd Edition in press

WEBINARS/PRESENTATIONS

- CLSI-SIDP-ACCP Annual Webinar
    - Partnering Laboratory with Stewardship to Navigate Breakpoint Updates and Other New CLSI Recommendations to Enhance Clinical Practice
    - August 31, 2023
    - Speakers: April Bobenchik and Natasha N. Pettit
    - >700 registered; 449 participated on the day of the webinar
    - 1133 on demand participants
- 2023 BIT and BIT Webinar
    - 2023 BIT
        - https://clsi.org/meetings/ast/breakpoints-in-use-toolkit/
        - Free, posted June 2023
        - Created by the Breakpoint Implementation Ad Hoc Working Group
        - 16,158 Toolkit downloads
        - 22,146 Toolkit website views

        - 6,206 YouTube video views
    - Webinar
        - Get Current! Using the 2023 Breakpoint Implementation Toolkit to Update and Document AST Breakpoints
        - https://clsi.org/standards/products/webinars/education/bit-webinar/
        - October 26, 2023
        - Speakers: April Abbott, Felicia Rice, Tsigereda Tekle
        - Moderator: Romney Humphries
        - 1,550 webinar registrants across 48 countries
        - 628 live attendees – 44% of registered attendees watched the webinar live. Usually industry standard is 30-40%.
        - 1133 on-demand views (this number is not unique)
- CLSI Annual Update (21st)
    - What's New in the 2024 CLSI Standards for Antimicrobial Susceptibility Testing (AST)?
    - April 2024
    - Speakers: April Bobenchik and Romney Humphries
    - Moderator: Janet Hindler
- CLSI-CAP Annual Webinar - To be determined
- CLSI-SIDP-ACCP Annual Webinar – To be determined

ASM MICROBE 2024

- CLSI's New Guidance on Antifungal Intrinsic Resistance and Reporting by Body Site
    - Track Hub
    - Saturday, June 15, 2024 10:45-11:30 AM EST
    - Intrinsic Resistance of Yeasts and Molds Against Antifungals
    - Speaker: Tanis Dingle
    - Moderator: Audrey Schuetz

ATTENDEE ORIENTATION

- ORWG will assist CLSI to update for June 2024
- On demand via YouTube as CLSI New Member Orientation

ORWG NEWS UPDATE

- January 2024 Edition
    - https://clsi.org/ast-news-updates-january-2024/
    - Feature: Acinetobacter
    - Case: Fungal Body Site Reporting
    - Practice Tips: M100 Table 1
    - Hot Topic: Sulbactam-durlobactam
    - In Memoriam: Clyde Thornsberry, PhD

AST SC MEETING EDUCATION SESSIONS

- January 2024
  - Addressing the Gaps in Defining, Detecting and Reporting MDRO in Clinical, Veterinary, and Public Health Laboratories
  - https://clsi.org/standards/products/webinars/education/astedujan24wr/
  - Speakers: April Bobenchik, Paula Snippes Vagnone, Kelli Maddock, and Allison Brown
  - Moderator: Stella Antonara
  - Will be available for on-demand viewing and CE credit
- June 2024
  - Topic suggestion: Beta-lactam Combination Agents

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. In Press.
- Pierce, V, T Bhowmick, P Simner. 2023. Guiding antimicrobial stewardship through thoughtful antimicrobial susceptibility testing and reporting strategies: an updated approach in 2023. 2023 Nov 21;61(11):e0007422. doi: 10.1128/jcm.00074-22.
- Patel, J, K Alby, R Humphries, M. Weinstein, J Lutgring, S Naccache, P. Simner. 2023. Updating breakpoints in the United States: A Summary from the ASM Clinical Microbiology Open 2022. JCM. October 2023 Volume 61 Issue 10 e01154-22. doi.org/10.1128/jcm.01154-22
- Will be working on JCM mini-review for M100-34th Edition.

ORWG PROJECTS

- Summary Projected for 2024
  - Webinars
    - Annual M100 Update
    - CLSI-SIDP-ACCP Annual Webinar
    - CLSI-CAP Annual Webinar
  - June 2024 AST SC Education Session
  - Update M100 Educational Program for 2024
  - Mini-review of M100 34th Edition for JCM
  - News Update Second Half of 2024
  - ASM Microbe 2024

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 **SC DISCUSSION (MAIN POINTS)** • Consider putting together a podcast to go along with the CLSI rationale documents or other key topics CLSI wants to communicate to a broader audience. • Is the working group collaborating with IDSA or SHEA? CLSI does a good job reaching the clinical microbiology community but should consider expanding the target audience. ○ The working group could do more to connect with IDSA and SHEA. ○ Trish Simner is on the ID Week Committee, so she could be a good resource to help connect the working group to a wider audience.

8. **QUALITY CONTROL WORKING GROUP (S. CULLEN)**

TIER 2 QC

MEROPENEM-ANT3310

- Background

Drug: Meropenem-ANT3310 (fixed 8 µg/mL	**Abbreviation (Glossary II & III)**: pending compound name	**Previous ID**: N/A
Solvent (Table 6A): MEM = water ANT3310 DMSO	**Diluent (Table 6A):** MEM = water, ANT3310 = water	**Preparation(Table 6C combination agents):** See following slide for example
Route of administration (Glossary II): IV	**Class (Glossary I & II):** β-lactam combination agents	**Subclass (Glossary I & II):** N/A
Study Report by: IHMA Inc.	**Pharma Co**: Antabio	**Control Drugs**: Meropenem, Ceftazidime-avibactam, Sulbactam-durlobactam, and ANT3310

Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, cations, zinc, surfactants, etc):
	  • Yes, study completed March 2021. Meropenem-ANT3310 only affected by high inoculum conc.
	• **Equivalency of agar dilution to broth dilution**:
	  • Completed for QC strains, October 2023.
	• **ISO/TS 16782 assessment of Tier 2 study materials**:
	  • Confirmed.

Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):** N/A
Discussion	• 4 dilution QC range approved for A. baumannii NCTC 13304 (58.8% shoulder. 3/8 labs with mode at top of the range

- Meropenem-ANT3310 Preparation (fixed 8 µg/mL)

***Preparation**

Prepare 10x starting concentration of meropenem at twice the concentration needed and dilute as usual using serial 2-fold dilutions.

Prepare 1600 ug/mL starting concentration of ANT3310 in DMSO then dilute 1:10 in water to a working concentration of 160 ug/mL.

Add an equal volume of ANT3310 160 ug/mL to each of the diluted meropenem tubes.

Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.

Example:

For a starting concentration of 64/8 ug/mL in the panel, prepare a 10x stock concentration of meropenem at 1280 ug/mL and dilute using serial 2-fold increments down to the desired final concentration needed in the panel. Prepare 1600 ug/mL starting concentration of ANT3310 in DMSO then dilute 1:10 in water to a working concentration of 160 ug/mL. Add an equal volume of ANT3310 160 ug/mL solution to each diluted tube of meropenem. For example, 5 mL of 1280 ug/mL meropenem + 5 mL of 160 ug/mL ANT3310 =10 mL of 640/80 ug/mL meropenem/ANT3310. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.

- Proposed QC Ranges

Drug Name:	Meropenem-ANT3310 (fixed 8 µg/mL)	Votes:	14/0/1/1 (For, Against, Absent, Abstain)

QC Strain	Range	% In	Mode	Dil	Shoulder	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
A. baumannii **NCTC 13304**	0.12/8 1/8	100	0.25/8	4	58.8% @ 0.5	0.25/8	0.25/8 (5), 0.5/8 (3)	0.12/8-0.5/8, (3) 95.8%	0.12/8-1/8, (4) 100%	Lab Variability, mode for 3 of 8 labs at top of range, 58.8% shoulder Routine QC
E. coli **ATCC 25922**	0.008/8-0.03/8	99.6	0.016/8	3	<5% @ 0.03	0.016/8 (3)	0.016/8 (8)	0.008/8-0.03/8, 99.6%	0.016/8, 98.3%	
K. pneumoniae **ATCC BAA-2814**	0.06/8-0.25/8	100	0.12/8	3	5% @ 0.12	0.016/8 (3)	0.12/8 (8)	0.06/8-0.25/8, 100%	0.12/8-0.25/8, 98.8%	Routine QC
P. aeruginosa **ATCC 27853**	0.12/8-0.5/8	98.3	0.25/8	3	25% @ 0.12	0.25/8 (3)	0.25/8 (7), 0.5/8 (1)	0.12/8-0.5/8, 98.3%	0.12/8-0.5/8, 98.3%	
ANT3310 only	Information only. Not for publication.									
A. baumannii **NCTC 13304**	64-256	100	128	3	7% @ 256	128 (3)	128 (8)	64-256, 100%	64-256, 100%	
E. coli **ATCC 25922**	32-128	99.6	64	3	<5% @ 128	64 (3)	64 (8)	32-128, 100%	64, 100%	
K. pneumoniae **ATCC BAA-2814**	32-128	97.9	256	3	44% @ 128	64 (2), 128 (1)	64 (5), 128 (3)	32-128, 97.9%	32-128, 97.9%	
P. aeruginosa **ATCC 27853**	128-512	100	256	3	NA	256 (3)	256 (8)	128-512, 100%	256, 100%	

A motion to accept the meropenem-ANT3310 QC ranges for *A. baumannii* NTCC 13304 (0.12/8 - 1/8 µg/mL), *E. coli* ATCC 25922 (0.008/8 - 0.03/8 µg/mL), *K. pneumoniae* BAA-2814 (0.06/8 - 0.25/8 µg/mL), and *P. aeruginosa* ATCC 27853 (0.12/8 - 0.5/8 µg/mL) was made and seconded. For meropenem/ANT3310, the recommended routine QC strains are *A. baumannii* NCTC 13304 and *K. pneumoniae* ATCC BAA-2814. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

BWC0977

- Background

Drug: BWC0977	**Abbreviation (Glossary II & III)**: pending compound name	**Previous ID**: N/A
Solvent (Table 6A): DMSO	**Diluent (Table 6A):** water	**Preparation(Table 6C combination agents):** N/A
Route of administration (Glossary II): IV and PO	**Class (Glossary I & II):** Quinolones	**Subclass (Glossary I & II):**
Study Report by: Element Iowa City (JMI Laboratories)	**Pharma Co**: Bugworks Research Inc.	**Control Drugs**: levofloxacin, cefepime

Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, cations, zinc, surfactants, etc):
	  • Not completed
	• **Equivalency of agar dilution to broth dilution**:
	  • Not established. Add footnote.
	• **ISO/TS 16782 assessment of Tier 2 study materials**: Confirmed.

Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):** NA • Add footnote to Table 5A and 5B "MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available." • Add footnote to Table 5B: MIC ranges were established using HTM and CAMHB with LHB (2.5% to 5% v/v). Performance with MH-F broth was not evaluated.
Discussion	• HTM media used for H. influenzae and LHB for S. pneumoniae. Add footnotes to Tables 5A and 5B. Request for sponsor to provide data to also support use of Mueller-Hinton F media.

- Proposed QC Ranges

Drug Name:	BWC0977				Votes:		14/0/1/1 (For, Against, Absent, Abstain) See previous slide for Table footnotes			
QC Strain	**Range**	**% In**	**Mode**	**Dil**	**Shoulder**	**Media Mode**	**Lab Mode**	**M23 Range**	**Range Finder**	**Comments**
***E. faecalis* ATCC 29212**	0.03-0.12	100%	0.06	3	31.0% @ 0.03	0.06 (3)	0.03 (2), 0.06 (6)	0.03-0.12, 100%	0.03-0.12, 100%	Some lab variability
***E. coli* ATCC 25922**	0.03-0.25	100%	0.12	4	83.1% @ 0.06	0.06 (2), 0.12 (1)	0.03 (1), 0.06 (4), 0.12 (3)	0.03-0.25, 100%	0.03-0.25, 100%	Media variability, lab variability, large shoulder.
***H. influenzae* ATCC 49247**	0.002-0.016	98.8%	0.004	4	72.1% @ 0.008	0.004 (2), 0.008 (1)	0.002 (2), 0.004 (4), 0.008 (3)	0.002-0.016, 98.8%	0.002-0.016, 98.8%	Media variability, lab variability, bimodal MIC values (lab F), large shoulder
***P. aeruginosa* ATCC 27853**	0.12-1	100%	0.5	4	94.2% @ 0.25	0.25 (2), 0.5 (1)	0.25 (5), 0.5 (3)	0.12-1, 100%	0.12-1, 100%	Media variability, lab variability, large shoulder
***S. aureus* ATCC 29213**	0.004-0.03	100%	0.008	4	86.4% @ 0.016	0.008 (2), 0.016 (1)	0.004 (1), 0.008 (4), 0.016 (3)	0.004-0.03, 100%	0.004-0.03, 100%	Media variability, lab variability, large shoulder
***S. pneumoniae* ATCC 49619**	0.004-0.016	95.9%	0.008	3	35.5% @ 0.004	0.008 (3)	0.004 (3), 0.008 (5)	0.004-0.016, 95.9%	0.004-0.016, 95.9%	Lab variability

SC DISCUSSION (MAIN POINTS)

- BWC0977 has impressively low concentrations of drug. Does that have anything to do variability?
  - No, this is one lot of panels so it would be unlikely to be related to the antibiotic concentration. It is not practical to do lot-to-lot studies for MICs; however, the working group will see data/evidence in future tier 3 studies if variation exists.
- Strictly speaking, this is not a fluroquinolone so the class designation of "fluroquinolone" should be revisited.
  - This drug is classified as a novel bacterial topoisomerase inhibitor (NBTI). It targets different residues that fluroquinolones, so it is not cross resistant to fluroquinolone resistant strains.
  - Action item for the QCWG: Follow-up on what the correct classification should be for BWC0977 before publishing the QC ranges.
- Emphasize to the drug sponsor that it is important to test both CLSI media approved for *H. influenzae*. Right now, the Table 2 says labs can use either HTM or fastidious media for *H. influenzae* and here the sponsor has only generated data using one media type.

- It might be worth investigating a quality control organism with an MIC at the higher end of the scale to make this easier for manufacturers to keep the drug on scale. Labs that make their own broth microdilution panel may have a hard time accurately achieving this low of a drug concentration.
    - Maybe consider if there should be a different strain used for validation than for QC. Perhaps the working group can consider how much data is needed for a validation vs. a routine QC organism.

A motion to accept the BWC0977 QC ranges for *E. faecalis* ATCC 29212 (0.03 - 0.12 µg/mL), *E. coli* ATCC 25922 (0.03 – 0.25 µg/mL), *H. influenzae* ATCC 49247 (0.002 - 0.016 µg/mL), *P. aeruginosa* ATCC 27853 (0.12 – 1 µg/mL), *S. aureus* ATCC 29213 (0.004 - 0.03 µg/mL), and *S. pneumoniae* ATCC 49619 (0.004 - 0.016 µg/mL) was made and seconded. Add footnote to Table 5A and 5B stating, "MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available." Add footnote to Table 5B stating, "MIC ranges were established using HTM and CAMHB with LHB (2.5% to 5% v/v). Performance with MH-F broth was not evaluated." Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

CEFTIBUTEN-XERUBORBACTAM

- Background

Drug: Ceftibuten-xeruborbactam (fixed 4 µg/mL)	**Abbreviation (Glossary II & III)**: TBD	**Previous ID**: ceftibuten-QPX7728 (fixed 4 µg/mL)
Solvent (Table 6A): ceftibuten – phosphate buffer pH 8.0 (0.1M), xeruborbactam – water	**Diluent (Table 6A):** ceftibuten – phosphate buffer pH 8.0 (0.1M), xeruborbactam – water	**Preparation(Table 6C combination agents):** same as aztreonam-avibactam
Route of administration (Glossary II): PO	**Class (Glossary I & II):** β-lactam combination agents	**Subclass (Glossary I & II):** NA
Study Report by: Element Iowa City (JMI Laboratories)	**Pharma Co**: Shionogi	**Control Drugs**: Ceftibuten and ceftazidime-avibactam

Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, cations, zinc, surfactants, etc):
	  • Has not been conducted yet.
	• **Equivalency of agar dilution to broth dilution**:
	  • Equivalence testing has not been performed yet. Add footnote.
	• **ISO/TS 16782 assessment of Tier 2 study materials**:
	  • Confirmed

Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):** NA • Add footnote to Table 5A-2 "MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available."
Discussion	• *K. pneumoniae* ATCC BAA-2814 is recommended for routine QC as there is no overlap with ranges with ceftibuten alone.

QCWG January 2024

- Proposed QC Ranges

Drug Name:	Ceftibuten-xeruborbactam (fixed 4 µg/mL)	Votes:	13/0/1/2 (For, Against, Absent, Abstain)

QC Strain	Range	% In	Mode	Dil	Shoulder	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
K. pneumoniae **ATCC 700603**	0.016/4-0.12/4	97.1%	0.03/4	4	92.6% @ 0.06/4	0.016/4 (1), 0.03/4 (1), 0.06/4 (1)	0.016/4 (2), 0.03/4 (4), 0.06/4 (3)	0.016/4-0.12/4, 97.1%	0.008/4-0.12/4, 5 dilutions, 99.6%	Media and lab variability. Laboratory C bimodal 0.12/4, 0.06/4. Large shoulder
K. pneumoniae **ATCC BAA-1705**	0.03/4-0.25/4	100%	0.06/4 0.12/4	4	Bimodal 0.06/4-0.12/4	0.06/4 (1), 0.12/4 (2)	0.06/4 (3), 0.12/4 (5)	0.03/4-0.25/4, 100%	0.03/4-0.25/4, 100%	Media and lab variability. Bimodal MIC distribution.
K. pneumoniae **ATCC BAA-2814**	0.12/4-0.5/4	100%	0.25/4	3	9.8% @ 0.12/4	0.25/4 (3)	0.25/4 (8)	0.12/4-0.5/4, 100%	0.12/4-0.5/4, 100%	Routine QC

A motion to accept the ceftibuten-xeruborbactam QC ranges for *K. pneumoniae* ATCC 700603 (0.016/4 - 0.12/4 µg/mL), *K. pneumoniae* ATCC BAA-1705 (0.03/4 - 0.25/4 µg/mL), and *K. pneumoniae* ATCC BAA-2814 (0.12/4 - 0.5/4 µg/mL) was made and seconded. For ceftibuten-xeruborbactam, the recommended routine QC strain is *K. pneumoniae* ATCC BAA-2814. Add footnote to Table 5A-2 stating, "MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available." Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

DEBIO 1452

- Background

Drug: Debio 1452	**Abbreviation (Glossary II & III)**: FAB	**Previous ID**: ANF-1252
Solvent (Table 6A): DMSO	**Diluent (Table 6A):** DMSO	**Preparation(Table 6C combination agents):** See Table 8B preparing dilutions of water-insoluble antimicrobial agents
Route of administration (Glossary II): Oral, IV	**Class (Glossary I & II):** FabI inhibitor	**Subclass (Glossary I & II):** NA
Study Report by: IHMA	**Pharma Co**: Debiopharm	**Control Drug**: Rifampin

Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, cations, zinc, surfactants, etc): • NA • **Equivalency of agar dilution to broth dilution**: NA • **ISO/TS 16782 assessment of Tier 2 study materials**: Confirmed
Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):** TBD
Discussion	2 mm difference in zone diameters between disk manufacturers with lots in the Tier 2 Study. Approved a narrower range and requesting additional information from sponsor to reassess at future meeting. (see next slide).

- Proposed QC Ranges

Drug Name:	Debio 1452 (0.1 µg disks)	**Votes:**	14/0/0/1 (For, Against, Absent, Abstain)

QC Strain	Range	% In	Median	mm	Media	Disk	Labs	Gavan	Range Finder	Comments
S. aureus **ATCC 25923**	~~20-28~~ 20-27	~~98.1~~ 97.1	24	~~9~~ 8	24 (2), 23 (1)	25 23	22 (1), 23 (2), 24 (2), 25 (3), 26 (1)	20-28, 98.1%, 9 mm	20-28, 98.1%, 9 mm	Disk variability 2mm, Media variability 1mm. Lab variability 5mm

- QCWG Discussion and Recommendation
  - Proposal approved for 20-27 mm (dropped 1mm from upper limit for a slightly narrower range).
  - Follow up to reassess range due to concerns with 2 mm difference in disk median between the disk manufacturers.

- Potency of manufacturer disks (eg, bioassay).
- Suggest exchanging disks between manufacturers to confirm potency.
- Request manufacturer internal data for multiple disk lots (eg, additional data, evaluation of lot to lot reproducibility)
- Combine Tier 2 disk data with QC data from disk content study (eg, evaluate data with expanded sample size)
- Ask for manufacturers for information if there are any other potential sources of variability.
- Present/propose range for *S. aureus* ATCC 29213 data as potential supplemental QC strain that is easier to read (fuzzy zones observed with *S. aureus* ATCC 25923) to potentially help with troubleshooting.

SC DISCUSSION (MAIN POINTS)

- The disk diffusion QC range for the QC organism (*S. aureus* ATCC 25923) are quite large, is this OK?
  - QC ranges with 9 mm are on the larger size, so it was narrowed to be 8 mm for *S. aureus* ATCC 2592. There may be an opportunity to decrease it to 7 mm in the future. CLSI has approved 9 mm QC ranges before.
  - There was a 2 mm difference in QC range based on the disk manufacturer, so the QCWG is asking the manufacturers to follow-up on that discrepancy.

A motion to accept the Debio 1452 QC ranges for *S. aureus* ATCC 25923 (20 - 27 mm) was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

CEFTIBUTEN-AVIBACTAM

- Background

Drug: ceftibuten-avibactam 10/4 µg disks	**Abbreviation (Glossary II & III)**: CBA	**Previous ID**: NA
Solvent (Table 6A): N/A	**Diluent (Table 6A):** N/A	**Preparation(Table 6C combination agents):** N/A
Route of administration (Glossary II): PO	**Class (Glossary I & II):** β-lactam combination agents	**Subclass (Glossary I & II):** N/A
Study Report by: Element Iowa City (JMI Laboratories)	**Pharma Co**: Pfizer Inc.	**Control Drug**: ceftibuten 30 µg, cefepime 30 µg, ceftazidime-avibactam 30/20 µg

Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, cations, zinc, surfactants, etc):
	  • Tier 1 impact studies are completed. Small inoculum effect observed.
	• **Equivalency of agar dilution to broth dilution**:
	  • The equivalency testing has been completed.
	• **ISO/TS 16782 assessment of Tier 2 study materials**:
	  • Confirmed.

Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):** Any need to add comment regarding small inoculum effect?
Discussion	• *E. coli NCTC* 13353 recommended for routine QC. No zone diameter overlap with ceftibuten alone. K. pneumoniae ATCC BAA-2814 MIC ranges overlap slightly. • Labs A, D, G tested more than 60 reps due to other testing reasons. • Need to review ceftibuten ranges in Tables 4A1 and 4A2 and align as appropriate.

- Proposed QC Ranges

Drug Name:	ceftibuten-avibactam 10/4 µg disks	Votes:	11/3/1/1 for ATCC 25922 and 14/0/1/1 for the other QC strains

QC Strain	Range	% In	Median	mm	Media	Disk	Labs	Gavan	Range Finder	Comments
E. coli **ATCC 25922**	28-36	99.6	32	9	32 (1), 33 (2)	32 (2)	29 (1), 32 (3), 33 (3), 35 (1)	29-35, 94.6%, 7 mm	28-36, 99.6%, 9 mm	Alternative 29-36, 97.1%, 8 mm,
E. coli **NCTC 13353**	~~27-35~~ 28-34	~~100~~ 98.9	31	~~9~~ 7	31 (3)	31 (2)	29 (2), 30 (2), 31 (1), 32 (2), 33 (1)	29-33, 87.2%, 5 mm	27-35 100%, 9 mm	Routine QC
K. pneumoniae **ATCC 700603**	24-30	96.8	27	7	27 (1), 28 (2)	27 (1), 28 (1)	26 (1), 27 (3), 28 (1), 29 (2)	24-30, 96.8%, 7 mm	24-31, 99.3%, 8 mm	Slight disk variability.
K. pneumoniae **ATCC BAA-1705**	24-30	97.6	27	7	27 (3)	27 (2)	26 (3), 27 (3), 28 (1), 29 (1)	24-30, 97.6%, 7 mm	24-31, 99.0%, 8 mm	
K. pneumoniae **ATCC BAA-2814**	22-28	99.5	25	7	24 (1), 25 (2)	25 (2)	24 (3), 25 (3), 26 (2), 27 (1)	22-28, 99.5%, 7 mm	22-28, 99.5%, 7 mm	Slightly overlaps range for ceftibuten alone.

SC DISCUSSION (MAIN POINTS)

- Action Item: The QCWG is assigning themselves to harmonize/cleanup what is published for ceftibuten alone in Table 5A1 vs. Table 5A2.
- There was a concern to have *E. coli* ATCC 25922 at the wider 9 mm range and not tighten it like the QCWG tightened the range for *E. coli* NCTC 13353.
    - *E. coli* ATCC 25922 is not the recommended QC, and it will not be listed in green. *E. coli* ATCC 25922 is not the recommended QC for any of the ß-lactam combinations and it is causing some confusion for laboratories. The working group is looking for ways to clean up the presentation of this organism.
- There is a large shoulder for *K. pneumoniae* ATCC 700603, what did the QCWG discuss on this issue?
    - There was not a lot of discussion on this topic, but this organism will not be listed as the routine QC for this drug. It will not be listed in green.
- CLSI has an archived QC table. For these organisms that are approved but not intended to be used for routine QC, perhaps it would be better to place them in the archived table rather than publish them in the routine QC table. That way if people want these ranges for future data, they will have them.

- CLSI is proposing to only have one of these organisms as the routine QC organism for disk diffusion; however, multiple of these organisms are approved as routine QC for MIC test methods. Will that be confusing for laboratories?
    - Action Item: The QCWG should investigate how this data is presented and if any additional explanation is needed to clarify this point for laboratories. The choice for which organism would be the routine QC organism for disk diffusion was based on avoiding overlap in zone sizes for ceftibuten alone to make sure the inhibitor was being evaluated by the QC organism.

A motion to accept the ceftibuten-avibactam QC ranges for *E.coli* ATCC 25922 (28 – 36 mm), *E.coli* NCTC 13353 (28 - 34 mm), *K. pneumoniae* ATCC 700603 (24- 30 mm), *K. pneumoniae* ATCC BAA-1705 (24 – 30 mm), and *K. pneumoniae* ATCC BAA-2814 (22 – 28 mm) was made and seconded. For ceftibuten-avibactam, the recommended routine QC strain is *E. coli* NCTC 13353. Add footnote to Table 5A-2 stating, "MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available." Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

TIER 3 MIC QC

- No votes requested.
- Additional data requested/monitor
    - *K. pneumoniae* BAA-1705 with Imipenem/relebactam,
    - *E. coli* ATCC 25922 with Aztreonam/avibactam
    - *S. pneumoniae* ATCC 49619 with Ceftriaxone and Doxycycline
    - *K. pneumoniae* BAA-1705 with Imipenem
    - *K. pneumoniae* BAA-2814 with Imipenem
- Archive
    - *K. pneumoniae* BAA-1705 with Piperacillin/Tazobactam and Ampicillin/sulbactam
- New additions: None
- Discussion and Decision Requests

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 at this time. Continue to monitor and request additional data.	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%. Available data supports current range.	19-Jan
K. pneumoniae ATCC 700603	Piperacillin/ Tazobactam	8/4-32/4	No change at this time. Recommend archiving.	Report for mode at very low end of range from one lab. Dec 2023: Data from 3 additional labs added, resulting in 6 total labs with Tier 3 data (n=989) + Tier 2 (n=240). Apart from three labs, one which was bimodal at the low end of range and two that had a mode at the low end of the range, overall data had a strong mode at 16/4 and no appreciable shoulders along with only 0.2% out of QC; Data supports current range.	21-Jun
K. pneumoniae ATCC 700603	Ampicillin/ sulbactam	8/4-32/16	No change at this time. Recommend archiving.	Report for mode at very low end of range from one lab and another lab out of QC high. Dec 2023: Data from 2 additional labs added, resulting in 6 total labs with Tier 3 data (n=1835); no Tier 2 available. Apart from two labs with datasets having a mode at the low end of the range, and one lab with data out of QC high, overall data has a strong mode at 16/4 and no appreciable shoulders along with only 1.1% out of QC; Data supports current range.	21-Jun

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recommended	Concern/Analysis	Reported
E. coli ATCC 25922	Aztreonam/ avibactam	0.03/4-0.12/4	Discuss expanding range to include 0.25/4 in June 2024. 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.	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.	22-Nov
S. pneumoniae ATCC 49619	Doxycycline	0.016-0.12	Request additional data	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 Dec 2023: no reference data submitted	23-Jun

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recommended	Concern/Analysis	Reported
K. pneumoniae BAA-1705	Imipenem	4-16	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	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

TIER 3 DISK DIFFUSION QC

- Agreed to archive all *S. aureus* ATCC 25923 with quinolones, *E. coli* ATCC 25922 with Minocycline
- Request additional data/monitor *N. gonorrhoeae* ATCC 49466 with Spectinomycin
- Archive *P. aeruginosa* ATCC 27853 with Cefiderocol. Potentially revisit based on recommendations from MDSWG (discussions in process)
- New additions
    - *E. coli* NCTC 13353 with Ceftibuten (based on performance as a control drug in Tier 2 study)
- Discussion and Decision Requests

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recmd	Concern	Update	Date Reported
S. aureus ATCC 25923	Ciprofloxacin 5 µg Levofloxacin 5 µg Moxifloxacin 5 µg Ofloxacin 5 µg Norfloxacin 10 µg	22-30 25-30 28-35 24-28 17-28	Archive	Fuzzy zone edges results in too small zones (also observed for S. aureus ATCC 29213).	January 2024: No new data. The issue on reading fuzzy zone edges has been referred to the the reading guide group.	May-21
P. aeruginosa ATCC 27853	Cefiderocol 30 µg	22-31	Archive and wait for work on additional QC strains by the cefiderocol ad hoc WG	Major media differences observed in M23 study, which resulted in a 10 mm range. EUCAST QC range is set to 23-29 mm.	January 2024: No new data.	Jan-21
E. coli ATCC 25922	Minocycline 30 µg	19-25	Archive if no new data is submitted before the June meeting	Values at top of range and above range from one lab.	January 2024: No new data.	Jan-21
N. gonorrhoeae ATCC 49226	Spectinomycin	23-29	Continue to monitor until June 2025. Request additional data.	QC study out high	January 2024: No additional data. June 2022: Observations in gentamicin QC study, especially with one lab and media	June-22

AST ROUTINE USER QC IMPROVEMENTS

- Current State and Issues
  - Can we reduce routine user QC and increase focus on detecting common issues?
    - AST QC costs are high and contributes to staffing challenges.
    - This is especially true in the following situations:
      - Low numbers of ASTs performed (eg, physician office labs (POL), small hospitals, reference labs doing limited AST)
      - Small numbers of one type of AST (eg, anaerobes, enterococci)
      - Multiple backup methods for select organisms/AST methods
      - Testing newer beta lactam combinations: Up to 8 QC strains if all agents are tested
    - Some bug/drug QC provides little or no value
      - Off scale MIC results (eg, *E. coli* ATCC 25922)
      - Does not detect common issues
- 2023 AST Routine User QC Survey – Highlights
  - Respondents
    - 69 respondents provided QC data/causes (>350 started the survey but didn't provide data)
    - Good representation of methods: agar dilution, BD Phoenix, Disk Diffusion, Beckman Coulter MicroScan, bioMérieux Vitek 2, In house broth microdilution, Pathnostics, Etest, gradient diffusion, Sensititre
  - Frequency Out of Range: Includes system and random errors
    - Percent out of range is very low 0.1% overall
      - QC strain frequency out of range 0-0.4%

- Meets criteria >95% in range QC
- No trends in increased or decreased frequency between 2021 and 2022.
- QC strains reported
  - Most frequent QC strains tested: *E. coli* 25922, *P. aeruginosa* 27853, *S. aureus* 29213, *E. faecalis* 29212, *E. coli* 35218, *K. pneumoniae* 700603
  - New QC strains/ranges have lower out of QC %

- System Errors – 44
  - Removed causes and follow up actions if clearly random (eg, new QC sub, resolved with repeat). May include a few random errors that could not definitively be excluded.
  - Manufacturing issues were most common causes. A few related to contamination and degradation.
  - New/replacement lot/recall was most common follow up.
- Comment Highlights (from one or more respondents)
  - QC failures rarely due to instrument or panel
  - Automated systems reliable and may require less QC than KB and gradient diffusion
  - QC strains marginally serve their purpose (MIC tests) to avoid erroneous AST reporting
  - Some resources expended for QC would be better served checking patient results
  - Current QC approach too costly/issues with staffing, can be cost prohibitive to implement newer drugs
  - CLSI should provide guidance on main causes of quality problems and how to address them (like CAP?)
  - QC failures more likely with less robust QC strains (not EC 25922, PSA 27853, SA 25923, 29213)
- See additional slides or excel file for details.

- QC Statements in Commercial AST Instructions for Use (IFU)
  - IFU statements don't conflict with proposed CLSI on QC frequency/strains
  - Could consider revisions/improvements for clarification or point to CLSI recommendations
- Additions to Table 5F MIC Reference Guide to QC Frequency and QC Strain Selection
  - New Sections
    - QC Frequency Recommendations for Test Modifications (existing section, new section title)
    - QC and QA Responsibilities for Users and Manufacturers
    - Recommendations for Determining User Routine QC Testing
    - Definitions (Random vs Systemic Failures, Routine vs Supplemental QC)
    - Add link to access IQCP from ASM and CLSI
  - Highlights on approach and content on following slide
  - Refer to mockup Table 5F for additional details
- Table 5F MIC Reference Guide to QC Frequency and QC Strain Selection – Highlights
  - Refer to CLSI M07 and CLSI M02 for user and manufacturer responsibilities
  - Describe process for using Individualized Quality Control Plan (IQCP) and CLSI EP23-A to determine the individual lab's appropriate Quality Assurance (QA) and Quality Control (QC)
  - Assess risks and review historic QC data to determine if a lab qualifies for a revised QC plan
    - ≥ 3 consecutive lots, ≥ 3 shipments, ≥ 3 consecutive seasons (for seasonal shipping variance)
    - Acceptable: ≥ 95% in range

- Identify common causes of QC failures (random vs system) from historic data
- Also use Troubleshooting Guide for key indicators of issues based on user responsibilities
  - eg, *P. aeruginosa* ATCC 27853 with carbapenems, *K. pneumoniae* ATCC 700603 with clavulanate to detect drug deterioration due to storage/shipping issues.
- Determine appropriate QA (training, proficiency, handling testing materials/QC strains, procedures, Appendix A – Confirming AST Results)
- Determine appropriate routine QC Plan (refer to user responsibilities)
- QC Plans might include revisions to one or more of the following
  - QC strain selection (eg, critical indicator), QC frequency (eg, lot/shipment, monthly, twice a month, weekly, daily) or combination (eg, rotate strains through the month, key indicators for deterioration more frequent than other QC strains)

- QC: Table 2 Routine QC box
  - Table 2s Routine QC box recommendations
    - Additional recommendations are not clear/consistent between individual organism Table 2s (see back up slides, testing *P. aeruginosa* 27853 not on all tables)
    - Many QC ranges are off-scale, minimal value (eg, *E. coli* ATCC 25922)
    - Propose removing details and refer to applicable QC tables for acceptable ranges. Add reference to Table 4D/5F to develop QC plan for routine QC testing.
- Statistical Options to Evaluate Outliers for Disk and Media Lots
  - John Turnidge demonstrated potential revisions to RangeFinder Tool to evaluate differences in media and disk lots
    - Testing more lots of disks and media would be ideal (best evaluation of lot and manufacturing differences) but is not practical.
    - Lowered central tendency criteria in RangeFinder as option to identify lots with different performance (eg, 50%)
    - Will not “exclude” data since sample size significantly reduced but could use to refine proposed expected ranges and/or request supplemental studies (eg, test multiple replicates with additional disk or media lot in single lab if lab variability was minimal)
  - Next steps
    - Refine RangeFinder changes: John Turnidge
    - Identify previous presentations with observations of disk or media differences to pilot revised RangeFinder tool: QCWG
    - Review results of pilot and decide path forward (eg, reject, refine, finalize RangeFinder changes)
- Next Steps
  - AST Routine User QC testing recommendations
    - Refine/Finalize revisions to Table 5F (MIC) and 4D (disk diffusion) with recommendations to develop QC Plan (IQCP)
    - Update Troubleshooting Guide: update from surveys, enhance critical indicator information
    - Refine/Finalize IQCP Example
    - Share with CMS/CAP for feedback and request support for inspector guidance
    - Pursue White Paper to explain rationale and process.
    - Timing not dependent on M100 publication. Address CLSI and commercial methods.
    - Propose education session topic to Outreach Working Group
  - Refine/Finalize revisions to Table 2s Routine QC Recommendation box
  - Review RangeFinder pilot and refine/finalize proposal to identify media and disk differences
  - Future topics
    - Table 4A-2 and 5A-2 (QC for Beta lactam combination agents)

- Options for single agents in the table (eg, remove ranges, remove from table/archive on CLSI website, revise comments)

SC DISCUSSION (MAIN POINTS)

- Did the QCWG get data on commercial reporting range vs. QC ranges? There are several reporting ranges that are dilutions outside QC ranges, so the user would have to fail significantly to notice issues.
  - No, but the manufacturer should have thoroughly tested that issue. This effort to streamline QC is more about checking for degradation of materials.
- TTWG is concerned that CLSI already has 13 tables for QC and adding in a new table could easily get lost. Has the QCWG considered moving QC out of the M100?
  - There is debate among the QCWG as to where the QC information belongs, and the group can consider moving it outside of the M100.
- The goal of the streamlined QC is to confirm what lab directors know, which is that QC is being overdone. The IQCP is not new. Labs can decide to do QC less frequently but have been hesitant to implement because CLSI recommends 1 week.
  - Action Item: Check if CAP states that QC must be performed weekly.
  - The QCWG needs to work with CAP to help align and educate inspectors on any new CLSI recommendations around IQCP.
- Will this section include information about media as separate components such as lyophilized panels from one company and media from another company?
  - That specific example has not been discussed. The QCWG needs to work on what examples will be published.
- For the Table 2 Routine QC Box, labs struggle with supplemental QC vs. routine QC and depend on this table to help them figure it out. There is concern about removing details from this table. If information is removed, CLSI needs to be clear about how labs find this information.
  - The QCWG needs to consider how footnotes referring to the manufacturer instructions appear. As an example, consider what happens to 5-A2 for β-lactam/β-lactamase inhibitor combinations.
  - If CLSI is going to move this table, the voting board needs to look at mockups first in June 2024.
  - Action Item: The QCWG to mockup what this table for discussion at the June 2024 meeting. TTWG is willing to help make example tables.
- For the statistical options to evaluate outliers for disk and media lots, TTWG is available to help look at how/where this information can be included in the CLSI documents.

9. **ADJOURNMENT**

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

2024 JANUARY AST SUBCOMMITTEE MEETING SUMMARY MINUTES PLENARY 2: Monday, 22 January 2024 (In-person) 1:00 PM – 5:30 PM Mountain Standard (US) Time	
#	**Description**
1.	**OPENING** Dr. Lewis opened the meeting at 1:00 PM Mountain Standard (US) time.

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

M45 PENICILLIN BREAKPOINTS

- Should M45 breakpoints for Gram-positive bacteria be revised to reflect/align with the extrapolated PK/PD cutoff and a tentative ECV?
- Challenges with M45 Organisms
  - Breakpoints for penicillin "borrowed" from a variety of microorganisms in M100 (*Staphylococcus*, *Streptococcus*, *Enterococcus*)
  - In some cases, susceptible breakpoint is above the tentative ECV
  - Two schools of thought:
    - Keep as-is, with different MIC breakpoints for each organism
    - Attempt to extrapolate available PK-PD data, which may lead to higher breakpoints
- ECV and Breakpoints

	ECV	CLSI	EUCAST	USCAST	Notes
S. aureus	0.125	0.12/0.25*	0.12/0.25	0.12/0.25	Breakpoint needs to stay narrow for penicillinases
Beta-Streptococci	0.03	0.12/-	≤0.25/≥0.5 (0.125/0.25)	0.25/0.5	Resistance extremely rare
S. pneumoniae (meningitis)	0.06	2/4/8 (0.06/0.12)	0.06/0.125-2/4 (0.06/0.12)	0.06/4 (0.06/0.12)	1. IV PCN doses of ≥2 MU (1.2g) Q4h (12 MU TDD) ok for non meningitis with MICs ≤2 mg/L 2. 18-24 MU TDD (2 MU Q4h/4 MU Q6h-4 MU Q4h) for MIC ≤4 mg/L 3. Only dosing listed in App E 4. S breakpoint low to help detect PBP mods and ensure treated with higher dosing
Viridans Streptococci	0.06	≤0.12/0.25-2/≥4	≤0.25/0.5-2/≥4	≤0.12/≥4	
Enterococcus	8	8/16**	--	--	1. Breakpoint based on amp dose of 2g Q4-6h (appr. 1.2g or 2 MU Q4-6h (12 MU TDD) of PCN but not PCN dosing listed) 2. Combo therapy for serious infections
PK-PD	-	--	≤0.25/0.5-2/≥4***	--	

*based on ability to detect *blaZ*

**Rx: Combination therapy with high-dosage, parenteral ampicillin, amoxicillin, penicillin or vancomycin plus an aminoglycoside, is usually indicated for serious enterococcal infections.

***Note wide susceptible, increased exposure (ie, SDD) range based on dosing recs

- Questions
  - Should we consider updating PCN (and AMP) breakpoints for M45 Gram positive bacteria to:
    - ≤2 µg/mL (S), 4 µg/mL (I) and ≥8 µg/mL (R) based on PK-PD
    - Add comment re: dose & combination therapy for endocarditis?
    - Is there concern about disconnect with M100 organisms?
  - Should we change PCN (and AMP) breakpoints for M45 Gram positive bacteria only when ECV is above breakpoint?
    - Should this be ≤2 µg/mL

M45 *ABIOTROPHIA/GRANULICATELLA, LACTOCOCCUS,* AND *MICROCOCCUS*

- Proposed Breakpoints

	Current M45 Breakpoint					
Organism	S	I	R	Source of PCN BP	PCN ECV (tentative)	PK/PD cutoff
Abiotrophia/ Granulicatella	**≤0.12**	0.25-2	≥4	Viridans Strep	**2**	**2-4**
Aerococcus	≤0.12	0.25-2	≥4	Viridans Strep	0.12	
Corynebacterium	≤0.12	0.25-2	≥4	Viridans Strep	≥0.5	
Erysipelothrix	≤0.12	-	-	Viridans Strep	≤0.12	
Gemella	≤0.12	0.25-2	≥4	Viridans Strep	0.12	
Lactococcus	**≤1**	2	≥4	MIC distribution	**2-4**	
Bacillus	≤0.12	-	≥0.25	Staphylococcus	0.12	
Micrococcus	**≤0.12**	-	≥0.25	Staphylococcus	**0.5**	
Lactobacillus	≤8	-	-	Enterococcus	0.5-2	
Leuconostoc	≤8	-	-	Enterococcus	4	
Pediococcus	≤8	-	-	Enterococcus	1	
Listeria	≤2	-	-	M100 S15 (2005)	~1	
Rothia mucilaginosa	≤0.12	0.25-2	≥4	MIC distributions	0.125	

Some BPs are below ECV (red)

- BPWG Discussion and Recommendation
  - Concerns about limited PK/PD data to make more the breakpoints more aggressive (ie, increasing the breakpoints)

- No more data to dig up in the literature – what we have here is all we got
- Issue = the current breakpoints and set below the tentative ECV
- Should M45 be a supplement in M100?
- Note of the work to finish a draft of updated M45 (aim for Feb 2024) to move to next stages (eg, public comment)
- M45 = guideline; M100 = standard: difference is M100 has substantial evidence for breakpoint decisions
- Motion to increase the breakpoint for *Abiotrophia/Granulicatella*, *Lactococcus*, and *Micrococcus* from 0.12/0.25-2/4 to 2/4/8 µg/mL (S/I/R). WG Vote: 9-1-1-1.
- Discussion if *Abiotrophia* breakpoint should be removed because inaccuracy of AST.
- Concern with increasing the breakpoint of *Micrococcus* to 2 when the ECV is 0.5. It was noted that the *BlaZ* is not found in those isolates between 0.5 and 2.

SC DISCUSSION (MAIN POINTS)

- Should M45 breakpoints for Gram-positive bacteria be revised to reflect/align with the extrapolated PK/PD cutoff and a tentative ECV?
- It is interesting that EUCAST removed PK/PD breakpoints because it is species specific. The PK/PD data presented here is being pulled from other organisms.
  - There is an overall lack of data. The M45 is already borrowing PK/PD, so the goal is to try to match the ECV with the best guess PK/PD. We do know that testing these bacteria are hard, and it is even harder when the breakpoint bisects the wildtype.
- For *Abiotrophia/Granulicatella,* is the working group thinking of including comments or different breakpoints for different disease states/treatments (eg, endocarditis)? The variation of the test is important.
  - There will be one comment stating what the maximum dose was based from: "Breakpoint is based on a dosage of 24 million units/day (4 million units every 4 hours)." This dosing falls confidently at an MIC of 2 maybe even 4 µg/mL. For the existing borrowed breakpoints, the intermediate range goes up to 2 µg/mL and there is no comment about dosing.
- While the PK/PD are species specific, similar organisms have similar PK/PD, so might be reasonable to go by related organism groups. With respect to pneumonia, EUCAST is probably going to see the dosing regimen 1.2 time 6 be more compatible with a breakpoint of 1 µg/mL due to the fact that they prefer the 40% target. EUCAST can continue to discuss with CLSI.
- There is anxiety around *Abiotrophia/Granulicatella* susceptibility testing. Some clinical microbiology labs do not test because of the variability. Many of these cases are in serious endocarditis, and the treatment guidelines say, "Do not perform susceptibly testing". There is concern about having breakpoints at all. Is the variability in susceptibility results because they are in the middle of the wildtype?
  - Dr. Humphries' lab looked at test methods a while ago and found Etest was not reliable. Broth microdilution was reproducible under a caveat that a certain brand of media needs to be used. The M45 cannot comment on specific media brands, but companion manuscripts can be published to help provide this information.
  - Physicians and pharmacists are putting too much weight on the MICs and do not understand the testing challenges/limitations. It is best to use the maximum dose.
  - The M45 Working Group is working to make significant edits to the M45 Forward to help providers better understand the limitations of M45 breakpoints.
- In *Micrococcus*, there was a concern for the *BlaZ* gene. The BPWG tested 150 *Micrococcus* isolates for *BlaZ* and looked though all published genomes and did not find any evidence of *BlaZ*.
- There is concern that the treatment guidelines say "do not test these organisms" so should CLSI even have breakpoints? However, not everyone agreed that testing should not be performed. The "do not test" guideline is specific to one endocarditis guideline. Providers will still ask their laboratories to

perform susceptibility testing. The BPWG received data from the major reference laboratories, which demonstrated that reference labs are regularly receiving requests to perform *Abiotrophia/Granulicatella* susceptibility testing.

- For the *Micrococcus* penicillin MIC distribution, the MICs start at 0.03 μg/mL, peak at 0.12 μg/mL and come down at 0.5 μg/mL. The ECV sits at 0.5 μg/mL. Even though the ECV sits at 0.5 μg/mL, it is on the high side, so it could be pushing 1 μg/mL. The current breakpoint is 0.12 μg/mL, so it bisects the wildtype distribution.

A motion to approve the M45 penicillin MIC breakpoints for *Abiotrophia/Granulicatella*, *Lactococcus*, and *Micrococcus* (S≤2, I 4, R≥8 μg/mL) was made and seconded. Vote: 11 for, 3 against, 0 abstain, 0 absent (Pass)

Against Vote Reasoning:

- Do not want to increase the breakpoint this high for *Micrococcus* because the ECV is 0.5 μg/mL.

M45 *LACTOBACILLUS* AND *PEDIOCOCCUS*

- Proposed Breakpoints

	Current M45 Breakpoint					
Organism	S	I	R	Source of PCN BP	PCN ECV (tentative)	PK/PD cutoff
Abiotrophia/ Granulicatella	≤0.12	0.25-2	≥4	Viridans Strep	**2**	**2-4**
Aerococcus	≤0.12	0.25-2	≥4	Viridans Strep	0.12	
Corynebacterium	≤0.12	0.25-2	≥4	Viridans Strep	≥0.5	
Erysipelothrix	≤0.12	-	-	Viridans Strep	≤0.12	
Gemella	≤0.12	0.25-2	≥4	Viridans Strep	0.12	
Lactococcus	≤1	2	≥4	MIC distribution	**2-4**	
Bacillus	≤0.12	-	≥0.25	Staphylococcus	0.12	
Micrococcus	≤0.12	-	≥0.25	Staphylococcus	**0.5**	
Lactobacillus	**≤8**	-	-	Enterococcus	**0.5-2**	
Leuconostoc	≤8	-	-	Enterococcus	4	
Pediococcus	**≤8**	-	-	Enterococcus	**1**	
Listeria	≤2	-	-	M100 S15 (2005)	~1	
Rothia mucilaginosa	≤0.12	0.25-2	≥4	MIC distributions	0.125	

	• BPWG Discussion and Recommendation
	  ○ Motion to reduce the breakpoint for *Lactobacillus* and *Pediococcus* from ≤8 µg/mL to ≤2 µg/mL (S only). WG Vote: 10-0-1-1.
	  ○ More conservative breakpoint to align with tentative ECV and PK/PD cutoff extrapolated from *Pneumococcus* versus adopting *Enterococcus* breakpoints.
	
	SC DISCUSSION (MAIN POINTS)
	• The current penicillin breakpoint is not achievable, so want to reduce the breakpoint to be ≤2 µg/mL (S only).
	  ○ The original breakpoint was based on *Enterococcus*.
	• Are there MIC distributions?
	  ○ No, the MIC distributions were not included in the slide set, but the ECV for these organism does not match the *Enterococcus* ECV. Therefore, the current breakpoint is wrong. The *Enterococcus* ECV is higher than *Lactobacillus* and *Pediococcus*.

	A motion to approve the M45 penicillin MIC breakpoint for *Lactobacillus* and *Pediococcus* (S≤2 µg/mL) was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)
	M45 LEUCONOSTOC • Proposed Breakpoints

	Current M45 Breakpoint					
Organism	S	I	R	Source of PCN BP	PCN ECV (tentative)	PK/PD cutoff
Abiotrophia/ Granulicatella	≤0.12	0.25-2	≥4	Viridans Strep	2	2-4
Aerococcus	≤0.12	0.25-2	≥4	Viridans Strep	0.12	
Corynebacterium	≤0.12	0.25-2	≥4	Viridans Strep	≥0.5	
Erysipelothrix	≤0.12	-	-	Viridans Strep	≤0.12	
Gemella	≤0.12	0.25-2	≥4	Viridans Strep	0.12	
Lactococcus	≤1	2	≥4	MIC distribution	2-4	
Bacillus	≤0.12	-	≥0.25	Staphylococcus	0.12	
Micrococcus	≤0.12	-	≥0.25	Staphylococcus	0.5	
Lactobacillus	≤8	-	-	Enterococcus	0.5-2	
Leuconostoc	**≤8**	-	-	Enterococcus	**4**	
Pediococcus	≤8	-	-	Enterococcus	1	
Listeria	≤2	-	-	M100 S15 (2005)	~1	
Rothia mucilaginosa	≤0.12	0.25-2	≥4	MIC distributions	0.125	

Some BPs are below ECV (red)

- BPWG Discussion and Recommendation
  - Motion to reduce the breakpoint for *Leuconostoc* from ≤8 µg/mL to ≤4 µg/mL (S only). WG Vote: 10-0-1-1.
  - More conservative breakpoint and the 1 log2 dilution is not trivial. Aligns with PK/PD cutoff (maximal achievable MIC with max dosing).

SC DISCUSSION (MAIN POINTS)

- Was there any discussion about harmonizing the breakpoints between penicillin and ampicillin?
  - The BPWG did not specifically discuss this. The general plan is to try to align penicillin and ampicillin where we can. This is step one in improving the breakpoints for *Leuconostoc*. The BPWG will not leave the ampicillin and penicillin breakpoints in a way where there are split results between the two drugs. They will plan match in interpretation between the two drugs.

A motion to approve the M45 penicillin MIC breakpoint for *Leuconostoc* (S≤4 µg/mL) was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

M100 PENICILLIN DOSING COMMENT

- TTWG needs to revise the penicillin breakpoint dosing comment to use standard language.
- The comment to edit is: “Breakpoint is based on a dosage of 24 million units/day (4 million units every 4 hours).”

Table 2G. *Streptococcus pneumoniae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
(7) For nonmeningitis isolates, a penicillin MIC of ≤0.06 µg/mL (or oxacillin zone ≥20 mm) can predict susceptibility to the following β-lactams: ampicillin (oral or parenteral), ampicillin-sulbactam, amoxicillin, amoxicillin-clavulanate, cefaclor, cefdinir, cefditoren, cefepime, cefotaxime, cefpodoxime, cefprozil, ceftaroline, ceftizoxime, ceftriaxone, cefuroxime, doripenem, ertapenem, imipenem, loracarbef, meropenem. See general comment (5).								
Penicillin	1 µg oxacillin	≥20	-	-	-	-	-	(8) Isolates of pneumococci with oxacillin zone sizes ≥20 mm are susceptible (MIC ≤0.06 µg/mL) to penicillin. Penicillin and cefotaxime, ceftriaxone, or meropenem MICs should be determined for isolates with oxacillin zone diameters ≤19 mm, because zones ≤19 mm occur with penicillin-resistant, -intermediate, or certain -susceptible strains. For isolates with oxacillin zones ≤19 mm, do not report penicillin as resistant without performing a penicillin MIC test.
Penicillin parenteral (nonmeningitis)	-	-	-	-	≤2	4	≥8	(9) *Rx:* Doses of intravenous penicillin of at least 2 million units every 4 hours in adults with normal renal function (12 million units per day) can be used to treat nonmeningeal pneumococcal infections due to strains with penicillin MICs ≤2 µg/mL. Strains with an intermediate MIC of 4 µg/mL may necessitate penicillin doses of 18-24 million units per day. (10) For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.

M45 TETRACYCLINE BREAKPOINTS

- Current Breakpoints for Doxycycline and Tetracycline

	CLSI S33	USCAST 2023	EUCAST v13.1	FDA
Enterobacterales	≤4/≥16; ≤4/≥16	--; ≤4/≥16	--; --	CLSI;CLSI
S. aureus	≤4/≥16; ≤4/≥16	≤1/≥4; ≤1/≥4	≤1/≥2; ≤1/≥2	--;CLSI
Enterococcus	≤4/≥16; ≤4/≥16	--;--	--;--	--;CLSI
Beta-Strep	--; ≤2/≥8	≤1/≥4; ≤1/≥4	≤1/≥2; ≤1/≥2	--;CLSI
S. pneumoniae	≤0.25/≥1; ≤1/≥4	≤0.25/≥1; ≤1/≥4	≤1/≥2; ≤1/≥2	CLSI;CLSI
Viridans Strep	--; ≤2/≥8	-- ; ≤2/≥8	--; --	--;--
H. influenzae	--; ≤2/≥8*	-- ; ≤2/≥8	≤1/≥2; ≤2/≥4	--;CLSI
N. gonorrhoeae	--; ≤0.25/≥2	--; 0.25/2	--; ≤0.5/≥0.5	--;CLSI
N. meningitidis	--;--	≤2/-; ≤2/-	--; ≤2/≥4	--;--

EUCAST BP based on PK data, micro data, clinical experience (from 2009)
"Enterobacterales, Enterococcus, Strep species other than beta-and S. pneumoniae are considered poor targets for tetracycline= no BP"
No PK-PD breakpoints from EUCAST. EUCAST Tetracycline BP based on 25-500 mg x 4 / day
*related to change to HTM??

- Current M45 Tetracycline Breakpoints

Organism	Tentative ECV	S	I	R	Notes
Aerococcus	≤0.5	2	4	8	Tet only
Aeromonas	ND	**4**	**8**	**16**	**Tet only**
Bacillus	ND	**4**	**8**	**16**	**Tet only**
Campylobacter	1-2	**4**	**8**	**16**	Doxy 2/4/8
Corynebacterium	~2	**4**	**8**	**16**	**Doxy 4/8/16**
HACEK	<1	2	4	8	Tet only
Lactococcus	~1	2	4	8	Tet only
Leuconostoc	~1-2 (old data)	**4**	**8**	**16**	**Mino only**
M catarrhalis	ND	2	4	8	Tet only
Pasteurella	ND	1	-	-	Doxy, 0.5
Vibrio	~0.5-1	**4**	**8**	**16**	**Doxy same**
BT agents	N/A	varies			

- Should we keep these "high" tetracycline breakpoint?
- Should the susceptible breakpoint be lowered to ≤2 µg/mL if ECV is ≤2 µg/mL, with a stasis endpoint?
- BPWG Discussion and Recommendation
    - Tetracycline PK is challenging.
    - M45 extrapolation for tetracyclines is derived from Enterobacterales.
    - Should M45 breakpoints harmonize with EUCAST?
    - Motion to lower the breakpoint for *Aeromonas*, *Bacillus*, *Campylobacter*, *Corynebacterium*, *Leuconostoc*, and *Vibrio* from 4/8/16 to 2/4/8 µg/mL (S/I/R)). WG Vote: 9-1-1-1.
    - Attempts to align with estimated PK/PD cutoff (conservative approach) versus copying Enterobacterales breakpoints
    - Concern there is not enough Pk/PD data.
- New Breakpoints Set at the ECV

Organism		NEW			OLD		
	T (ECV)	S	I	R	S	I	R
Aerococcus	≤0.5	0.5	1	2	2	4	8
Aeromonas	TBD	TBD, based on ECV			4	8	16
Bacillus	ND	Evaluate old data for ECV, TBD			4	8	16
Campylobacter	~1-2	2	4	8	4	8	16
Campylobacter, doxycycline	~0.5-1	1	2	4	2	4	8
Corynebacterium	~2	2	4	8	4	8	16
Corynebactrium, doxycycline	~1	1	2	4	4	8	16
HACEK	~1	1	2	4	2	4	8
Lactococcus	~1	1	2	4	2	4	8
Leuconostoc, Minocycline	~1-2	1	2	4	4	8	16
M catarrhalis	2	2	4	8	2	4	8
Pasteurella	~1	1	-	-	1	-	-
Pasteurella	~1	0.5	-	-	0.5	-	-
Vibrio Tet & Doxy	~0.5-1	1	2	4	4	8	16

SC DISCUSSION (MAIN POINTS)

- If the breakpoints are lowered in the M45, is there a plan to lower the breakpoints in the M100 for the Enterobacterales?
  - Action Item: BPWG will review the tetracycline breakpoints for Enterobacterales.
- Need to be cautious with old doxycycline studies because they did not characterize protein binding. Many of those targets are total drug targets. They are not necessarily free drug targets, which is why higher targets are seen compared the more contemporary data with *Acinetobacter* and *Stenotrophomonas*. It is interesting to see targets of 25 and 50 for stasis in one log kill but lower targets for *Acinetobacter* and *Stenotrophomonas*. Based on the Monte Carlo simulations with those lower targets, it was required for the breakpoint to be down at 0.5 μg/mL and 1 μg/mL for *Stenotrophomonas*. That is lower than shown here, so it not consistent with some of the contemporary data.
- Is the PK/PD target based on stasis? Answer: Yes.

- Can the breakpoints be lowered, or will that cut into the ECV?
  - Yes, the breakpoints could go lower. EUCAST went lower to the ECV with *Vibrio*. Looking at ECVs 1/2/4 μg/mL looks like reasonable breakpoints for a lot of these organisms.
- Do clinicians need tetracycline breakpoints for all these organisms?
  - Providers are often out of options on *Corynebacterium*, so they need the tetracyclines for *Aeromonas*, *Vibrio*, and *Corynebacterium*.
  - *Corynebacterium* are for limited therapies. It is less important to have tetracycline breakpoints for the other organisms.
- Did one of the working group members vote against these breakpoints because the breakpoints are "wrong" or is it "a little better"?
  - There was not enough data to make a change and CLSI should look at the M100 organisms too.
  - The current breakpoints are not great data, the current proposal is a conservative move to lower the breakpoints.
- The ECVs are below 2 μg/mL and it did not look like any PK/PD indicates 2 μg/mL. Why is 2μg/mL proposed instead of the ECV?
  - There is not a lot of data, so a best guess ECV and quasi-reasonable PK/PD is being used. If the breakpoint is above the quasi-reasonable PK/PD then we should look at changing it. Baby steps are being taken to lowering the breakpoint a little bit and not going all the way to 1μg/mL.
  - Note that the M45 is only updated every ~5 years, so whatever is decided here will last a while.
- CLSI is looking into ways to update the M45 more frequently.
- Bring the susceptible breakpoint to ECV if the ECV is below or equal to 2 μg/mL. Then I is one dilution above, then R is one dilution above that. This is only for organisms on the slide, which are in M45.
- The veterinary world sees large differences among ECVs between different *Corynebacterium* species.
- How many organisms are the ECVs based on?
  - It varies from a couple hundred to thousands.
- What do we know about precision about tetracycline susceptibility testing?
  - Not much, which is true for all the organisms in the M45.
- For future reference, the M45 working group will work with the CDC to update tetracycline breakpoints for bioterrorism agents in the future.
- Tetracycline in rarely used in clinical practice. Doxycycline is used more commonly. Minocycline has slightly broader spectrum of activity. Would it be possible to move to testing doxycycline instead of tetracycline?
  - The M45 working group agreed in general; however, there is no doxycycline data at this time.

A motion to approve the M45 tetracycline MIC breakpoints for *Aerococcus* (S≤0.5, I 1, R≥2 μg/mL), *Campylobacter* (S≤2, I 4, R≥8 μg/mL), *Corynebacterium* (S≤2, I 4, R≥8 μg/mL), HACEK (S≤1, I 2, R≥4 μg/mL), *Lactococcus* (S≤1, I 2, R≥4 μg/mL), and *Vibrio* (S≤1, I 2, R≥4 μg/mL), the doxycycline MIC breakpoints for *Campylobacter* (S≤1, I 2, R≥4 μg/mL), *Corynebacterium* (S≤1, I 2, R≥4 μg/mL), and *Vibrio* (S≤1, I 2, R≥4 μg/mL), and the minocycline MIC breakpoints for *Leuconostoc* (S≤1, I 2, R≥4 μg/mL) was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

REPORTING OF ANTIMICROBIAL AGENTS FOR BACTERIA ISOLATED FROM CSF

- Current CSF Warning Box in Introduction to Tables 1

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

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

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

- Should fluoroquinolones be reported by the clinical microbiology lab for bacteria from CSF specimens?
- The question is not whether these agents are first-line or preferred or superior agents for treatment of central nervous system infections. To be determined by treating clinician.
- Primer to CSF Warning
  - Reflects drug/drug class only
  - Not for specified drug/bug combinations but notes for 'bacteria included in Table 2A through Table 2J'
- Levofloxacin, Moxifloxacin, and Ciprofloxacin Data
  - Organisms/Organism Group

	Ciprofloxacin	Levofloxacin	Moxifloxacin
Enterobacterales	X	X	
Pseudomonas aeruginosa	X	X	
Acinetobacter spp.	X	X	
Burkholderia cepacia complex		X	
Stenotrophomonas maltophilia		X	
Other non-Enterobacterales	X	X	
Staphylococcus spp.	X	X	X
Enterococcus spp.			
Haemophilus influenzae and *Haemophilus parainfluenzae*	X	X	X
Streptococcus pneumoniae		X	X
Beta-hemolytic *Streptococcus* spp.		X	
Streptococcus spp. Viridans Group		X	
Neisseria meningitidis	X	X	
Anaerobes			X

- Summary

Ciprofloxacin	Levofloxacin	Moxifloxacin
Moderate penetration into CSF	High penetration into CSF	High penetration into CSF
Potentially enough penetration to treat some gram-negative bacilli; Not recommended for *Streptococcus pneumoniae* (but no breakpoints and not included in clinical guidelines)	Potentially enough penetration to treat multiple bacteria	Potentially enough penetration to treat multiple bacteria
Case reports/series of clinical use; experimental models	Limited/no clinical literature but trials for TB meningitis; experimental models	Case reports/series of clinical use; experimental models
Recommended as **alternative agent** for bacterial meningitis in clinical guidelines	Recommended as **alternative agent** for bacterial meningitis in tertiary resources	Recommended as **alternative agent** for bacterial meningitis in clinical guidelines

- Cefazolin Organism/Organism Groups
  - Enterobacterales
  - *Staphylococcus* spp.
  - SS-hemolytic *Streptococcus* spp.
- BPWG Discussion and Recommendation
  - Back and forth about the accuracy of the warning and its application to clinical practice.
  - Motion to remove fluroquinolones from CSF warning box. WG Vote: 9-0-1-2.
  - Cefazolin deliberation:
    - No breakpoints for S. *aureus* so nothing to report – inferred from oxacillin
    - Not comfortable removing from list (inoculum effect, etc.)
    - No motion/vote – not moved forward
    - Further discussion on the warning comment as a whole
    - Centered around meningitis but only says "CSF"
    - Is it too clinically prescriptive?
    - Lists drugs like imipenem (because of seizure concern?) but doesn't carve out other drug/bug combinations that are inappropriate for meningitis (eg, pip-tazo/Enterobacterales in CNS)

SC DISCUSSION (MAIN POINTS)

- In the fungal documents, there is a body site table to highlight drug penetration, which could be good to use as an example for bacteria.
  - Agreement to use the body site table in the fungal documents as an example on how to move forward.
  - The Antifungal Subcommittee looked at body site reporting based on penetration and clinical use.

- The CSF warning box is not easy to find, so CLSI should consider moving it.
- The working group is looking for feedback on what to do with the CSF warning box overall. They are also unsure of how to best handle cefazolin.
- Changing this box would have implications for CAP proficiency testing, so CLSI will need to notify CAP of any changes.
- Comments in Table 2 for *Streptococcus pneumoniae* and *Haemophilus influenzae* need to be reviewed for discrepancies.
- One of the issues with the warning is that it is about the drug class. It is not organism specific.
- Action item: The CSF comment box needs an AHWG and bring it back to BPWG.
- Providers would like to have the MIC for the organisms, and there are no breakpoints for *Staphylococcus*, but this would affect how we report ß-lactam susceptibly.
- The field does not necessarily know what percent of drug level penetration into the CSF is important/clinically relevant. For example, amphotericin B is only ~1%, but that drug is still used. Amphotericin B barely gets into the CSF, but it is still the drug of choice for cryptococcal meningitis. It is also important to consider that inflammation can affect penetration.
- Not all institutions across the world have access to cefazolin.
- This guidance needs to fit all lab sizes. Keep in mind small hospitals get this information too.

A motion to remove "fluoroquinolones" from the CSF warning box in the Introduction to Tables 1 was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

PHENOTYOIC RULES FOR CARBAPENEMASE TESTING FOR CRE

- June 2023 CLSI Meeting Background
  - Data suggesting differences in *in vivo* responses for cefepime and meropenem-vaborbactam depending on the presence and type of carbapenemase
    - Led to comments for meropenem-vaborbactam/Enterobacterales with OXA-48 and for carbapenemase-producing Enterobacterales and cefepime
  - Consensus to develop a phenotypic CRE definition to use for when to recommend carbapenemase testing in Enterobacterales
    - New agents target specific enzymes
    - Testing much easier now with lateral flow and PCR assays
  - Proposed options:
    - Option 1: CDC definition of CRE: resistance to any carbapenem (except *Proteus*/*Providencia*/*Morganella* (*PPM*) and imipenem)
    - Option 2: Meropenem or imipenem intermediate or resistant (except *PPM*/imipenem)
    - Option 3: Meropenem or imipenem resistant (except *PPM*/imipenem)
  - No decision was made. Request to review data on different definitions and correlations with carbapenemases.
- Current wording in M100 Table 2A: "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 H, Table H3 regarding suggestions for reporting when mechanism of resistance-based testing (molecular and phenotypic methods) is discordant with phenotypic AST."
- Data Used to Establish Resistance to Any Carbapenem as CDC's CRE Definition
  - 312 isolates of *K. pneumoniae*, *E. coli*, and *E. cloacae* that tested intermediate or resistant to any carbapenem (including ertapenem) at 6 CDC EIP sites
  - Determination of carbapenemase: Modified Hodge, BMD test with chelators, and PCR

- CDC selected resistance to any carbapenem, including ertapenem, as the preferred phenotypic definition because:
  - Rarely missed carbapenemase-producing strains (high sensitivity)
  - 45% of isolates had a carbapenemase (modest specificity)
  - Simplicity

- Strengths and Weaknesses of Different CRE Definitions for Recommendation for Carbapenemase Testing

Option #	Definition	Strengths	Weaknesses
1	Resistant to any carbapenem, including ertapenem	Good "sensitivity" for carbapenemases (97-99% for KPC and NDM, 95% overall) Aligns with CDC definition	Ertapenem monoresistant isolates rarely produce a carbapenemase (2-12%): poor "specificity"
2	Not susceptible to imipenem or meropenem	Good "sensitivity" for carbapenemases (>95% b/c inclusion of imipenem)	Better "specificity": 50% of isolates in USA and 63% globally have a carbapenemase Not all labs test imipenem
3	Resistant to imipenem or meropenem	Decreased "sensitivity" for carbapenemases (still >95% for KPC and NDM, but down to 90% overall)	Good "specificity": 64-68% of isolates in USA and 72% globally have a carbapenemase Not all labs test imipenem

- Proposed Wording Based on Option #2: "Enterobacterales isolates that are not susceptible to imipenem or meropenem should undergo testing to detect and differentiate the most common carbapenemases, wherever this testing is available. See Appendix H, Table 3 regarding suggestions for reporting mechanism of resistance-based testing is discordant with phenotypic AST."
- BPWG Discussion and Recommendation
  - Concerns about including a definition of imipenem or meropenem resistance (without ertapenem) because many labs do not test imipenem and imipenem not on some automated panels
  - CDC's ARLN may have additional data to review
  - Consideration of a comment about whether *Enterobacter cloacae* that are resistant to ertapenem only need to be tested
  - Desire to align with CDC's CRE definition, although this definition may be re-evaluated
  - Discussion about whether we are recommending tests that detect presence or absence of carbapenemase (eg, mCIM), test that distinguished metallo-ß-lactamase (MBL) from serine (eg, eCIM), or tests that directly detect the carbapenemase type by lateral flow or PCR
  - Suggestion to test isolates with lower meropenem MIC values (eg, outside wild type), but this is not possible with dilutions used by automated systems
  - Motion to recommend carbapenemase testing for Enterobacterales isolates that are resistant to ertapenem, imipenem, or meropenem (except for *Proteus/Providencia/Morganella* that are only resistant to imipenem). WG Vote: 11-0-0-1.

SC DISCUSSION (MAIN POINTS)

- CLSI needs to carve out an exception for *E. cloacae* that are resistant to ertapenem only. These isolates do not necessarily need to be tested for carbapenemases.

- In the data presented which was provided by JMI, the AmpC producers can lead to over testing of isolates for carbapenemases. *Serratia* and *Klebsiella aerogenes* were an issue because they were often ertapenem resistant, but susceptible to all other antibiotics including the cephalosporins.
    - Discussion that *Serratia* should not be excluded.
    - If *E. cloacae* is removed, then the ertapenem specificity issue improves.
- CLSI needs to keep in mind that not all panels have multiple carbapenems.
- EUCAST uses a lower meropenem MIC cutoff to help identify isolates that may need carbapenemase testing. Can CLSI consider looking at a meropenem MIC of 1μg/mL, if only meropenem is tested?
    - The majority of OXA48 isolates are meropenem susceptible with MICs of 1 or 0.5 μg/mL
    - Unfortunately, many panels do not go to 1 or 0.5μg/mL
    - Would using the lower MIC also have a low specificity issue? It would be good to see the data.
- Need to discuss screening for carbapenemases vs. detection and differentiation
- Need to specify if labs need to look for the gene or the enzyme.
- Carbapenemase testing is complicated to understand. It is hard for labs if CLSI has a different definition than state public labs.
- If there is no specification that labs must detect the carbapenemase then commercial labs will use the cheapest methods (like mCIM). So, CLSI needs to state if molecular testing is needed.
- There is a lot of concern about including ertapenem because to the low specificity. It will lead to extra work and expensive testing.
- If the specific organisms with AmpCs are removed, it will increase the ertapenem specificity.
    - Provide the sensitivity of each carbapenem with the AmpC producers excluded from the data set. It would also be good to know how data looks for the AmpC producers alone.
- CDC is not changing the definition of carbapenemase producer.
- There is interest in the methods for detection and CLSI needs specific guidance.
- Any recommendations for which *Pseudomonas aeruginosa* isolates should be tested for carbapenemase production? Answer: Not at this time.
- CLSI could state the preferred option, then have alterative options for labs since this is not practical.
- Prevalence of the different genes matters. The US is mostly KPC at this time.
- This has been discussed before and it was decided to be permissive, not prescriptive. Make a table for labs to understand what the expected phenotypes are for each genotype for the new ß-lactam/ß-lactamase inhibitor drugs. That will help labs understand what this testing means for their labs.
- Is the mCIM testing enough for carbapenemase testing if labs are also testing the new ß-lactam/ß-lactamase inhibitor drugs directly?
- State a recommendation for carbapenemase testing and arm labs with info to decide how sensitive/specific their labs to be. Maybe state carbapenemase testing should be expected for patient care and a table to let labs/hospitals decide the next steps.
- What is true in the US is not true in other parts of the world. So, knowing prevalence thresholds could inform testing strategy; however, is this complicated in recourse limited settings.
- Third party payers are not enthusiastic about paying for molecular epidemiology, CLSI needs to specify this is to direct therapy or costs will not be reimbursed.
- Providers in the room use the specific carbapenemase genes to direct patient care. Starting to see more NDMs.
- Maybe public health needs to move to “send us all your CPOs” which could be good for public health.
    - Minnesota Public Health liked the comment to change surveillance to CPO, not just CP-CRE.
    - The guidelines have already been updated. CPOs are nationally notifiable.
- CLSI needs to specify enzymatic vs. non-enzymatic testing methods and specify what is OK. Labs are still doing modified hodge, so please specify.

- Labs need to know if serine or MBL carbapenemase because new drugs are in the pipeline.
- Should the lower meropenem cutoffs be used because one or two commercial panels do not have lower MICs.
- Missing why labs need to do the testing. Is it for epidemiology or patient care? The why is missing. Need a table to explain why.
- Assume the motion means to report clinically.
- Need to say for "for treatment and enzyme level differentiation" to push commercial labs to do enzyme level testing.
- Need to be careful with the word "level".
- There are concerns about ertapenem for screening due to low specificity. CLSI wants the data on ertapenem level after excluding the key organisms leading to low specificity.
- Action item: The working group needs to review the discussion minutes and address the concerns at the June meeting. One major piece of data needed for June is to look at the sensitivity/specificity of each of the carbapenems with key organisms excluded such as *E. cloacae* and the AmpC producers, as AmpC plus a porin mutation could led to carbapenem resistance.

A motion to recommend carbapenemase testing for Enterobacterales that are resistant to at least one carbapenem ertapenem, imipenem, or meropenem (except for *Proteus*, *Providencia*, and *Morganella*) and add a footnote on *E. cloacae* that is resistant to ertapenem could be resistant by other mechanisms was made and seconded. Vote: 8 for, 6 against, 0 abstain, 0 absent (Fail)

Against Vote Reasoning:
- Need to work out practicality and language. Agree with the direction.
- Missing the points on carbapenem type and clinical implication

AZTREONAM TABLE 1A PLACEMENT
- Introduction and Background
  - Clinical labs and physicians in Latin America are using AST results for aztreonam in the following situations (not just severe ß-lactam allergy)
  - Identifying MBL-producing organisms (that test susceptible to aztreonam)
    - Very important in areas where MBLs are endemic
    - Treating MBL-producing Enterobacterales infections (when susceptible)
    - Decrease the need for testing ceftazidime-avibactam + aztreonam combination testing
    - To have an additional option to detect ESBLs (other than ceftriaxone, cefotaxime, ceftazidime)
  - Aztreonam being removed from certain automated panels and new diagnostic tests
- Reasons Move Aztreonam from Tier 4 to Tier 1 for Enterobacterales in Table 1
  - Tier 4 placement discourages testing
  - Aztreonam an important drug to detect MBLs and ESBLs
  - Can obviate need for aztreonam and ceftazidime-avibactam combination testing
  - Having aztreonam in Tier 2 allows the drug to be tested routinely but reported selectively
- BPWG Discussion and Recommendation
  - Decision previously made to make Table 1 US-centric, but discussion with PAHO to create a Table 1 for Latin America
  - Consideration of Tier 3 given that aztreonam AST may only be needed for areas with MBL-producing Enterobacterales, but would be unclear how to cascade in this Tier

- Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for multi-drug resistant organisms (MDROs) but should only be reported following cascade reporting rules established at each institution
- Motion to change aztreonam from Tier 4 to Tier 2 for Enterobacterales in Table 1A. WG Vote: 9-1-1-1.
- Rejection vote wanted Tier 3.

SC DISCUSSION (MAIN POINTS)

- In the US, it is more a tier 3. Outside of the US is different. One lab cascades aztreonam testing if an MBL is detected by PCR.
  - There is concern that aztreonam should not be used as monotherapy for an MBL producer; however, several ID pharmacists and ID physicians say they consider using aztreonam as monotherapy if it tests susceptible. Often it does not test as susceptible because there are other ß-lactamases present.
  - The guidelines do not say "do not use aztreonam". It is just infrequently susceptible that it is maybe not that useful of a drug.
- Trish Simner thinks tier 3 makes the most sense.
- An ID pharmacist believed the current placement in tier 4 is to account for factors like severe ß-lactam allergy and moving it into tier 3 disregards any other reasons for testing such as the ß-lactam allergy. This could be confusing for laboratories.
- In an institution with a high prevalence of MBLs consider cascading testing off cefotaxime or ceftriaxone along with the carbapenems. Or cascade off a resistant carbapenem.
- In the Instructions for Use, tier 4 includes epidemiology.
- Looking at Pfizer atlas data, ESBLs that are going to be susceptible to aztreonam only are going to be low. In Latin America, it looks like 10% aztreonam susceptible among the MBLs. In Colombia, it looks closer to 30%. There are many MBLs that do not include aztreonam.
- Many antimicrobial susceptibility panels do not include aztreonam and CLSI informs labs to be prepared to routinely report drugs in tiers 1 and 2. Labs might feel pushed to test aztreonam.
- One antimicrobial stewardship team was happy to see aztreonam to be moved back to Tier 4.

A motion to move aztreonam to Tier 2 for Enterobacterales in Table 1A was made and seconded. Vote: 4 for, 10 against, 0 abstain, 0 absent (Fail)

Against Vote Reasoning:

- Leave it where it is in tier 4,and let labs decide when to test it.
- Cascade testing off carbapenem, which belongs in tier 3.
- There are concerns for US centric table.

SC DISCUSSION (MAIN POINTS)

- Since this is a US centric table, add use a footnote indicating in high prevalence regions move this to tier 2 or 3.
- Tier 3 has the middle ground between US vs. and other countries.
- Keep in mind that tier 3 is triggered off something, whereas tier 4 is not. As previously mentioned, labs test aztreonam for many reasons like drug allergies and not just off cascade testing.
- Moving aztreonam to tier 3 will dilute the importance of tier 3.
- Labs take these table very literally/seriously. Need to keep that in mind.

A motion to move aztreonam to Tier 3 for Enterobacterales in Table 1A was made and seconded. Vote: 8 for, 6 against, 0 abstain, 0 absent (Fail)

Against Vote Reasoning:

- Belongs in tier 4.
- Consider developing a non US-centric Table 1.

SC DISCUSSION (MAIN POINTS)

- There is interest in adding a footnote.
- Was there ever consideration that a drug can go in more than one tier?
    - No, but a comment was added that labs can test whatever they want.

AZTREONAM TIER 4 TABLE 1A COMMENT

- Proposed Comment: g. "In institutions that serve patients at high risk for metallo-ß-lactamase producing Enterobacterales, aztreonam may be considered as a Tier 3 agent following cascade reporting rules established at the institution."

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin			
Cefazolin	Cefuroxime		
Cefotaxime or ceftriaxone[b]	Cefepime[c]		
	Ertapenem Imipenem Meropenem	Cefiderocol	
		Ceftazidime-avibactam	
		Imipenem-relebactam	
		Meropenem-vaborbactam	
Amoxicillin-clavulanate Ampicillin-sulbactam			
Piperacillin-tazobactam			
Gentamicin	Tobramycin	Plazomicin	
	Amikacin		
Ciprofloxacin Levofloxacin			
Trimethoprim-sulfamethoxazole			
	Cefotetan Cefoxitin		
	Tetracycline[d]		
			Aztreonam[g]
			Ceftaroline[b]
			Ceftazidime[b]
			Ceftolozane-tazobactam

A motion to add the comment stating, "In institutions that serve patients at high risk for metallo-ß-lactamase producing Enterobacterales, aztreonam may be considered a Tier 3 agent following cascade reporting rules established at the institution." to aztreonam in Tier 4 in Tables 1A was made and seconded. Vote: 8 for, 0 against, 0 abstain, 6 absent (Pass)

ACINETOBACTER AD HOC WORKING GROUP REPORT

AMPICILLIN/SULBACTAM

- Sulbactam for *Acinetobacter baumannii*
  - Penicillanic acid sulfone ß-lactamase inhibitor of some Class A enzymes
  - Intrinsic antibacterial activity against *A. baumannii* due to high affinity for PBP3 (and PBP1)
    - SUL resistance from *Acinetobacter*-derived cephalosporinases, OXA carbapenemases, and mutations in PBP3 and PBP1
  - Only available as a fixed 2:1 combination of ampicillin-sulbactam (AMP-SUL) in U.S. or as the newly approved sulbactam-durlobactam (SUL-DUR)
    - FDA-approved dosages of AMP-SUL: 1.5 (1/0.5) g or 3 (2/1) g (q6h) with a max of 4 g sulbactam/day.
    - FDA-approved dosages of SUL-DUR: 1g/1g over 3 hours every 6 hours (up to every 4 hours with augmented renal clearance)
  - AMP-SUL in combination with ≥1 other agent is recommended as first line for treatment of CRAB by IDSA, ESCMID, SIDP, etc.
    - Recommend 6-9 g of sulbactam/day (18-27 mg of AMP-SUL)
- Current Breakpoints

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

[a]*A. calcoaceticus* only No EUCAST breakpoints for AMP-SUL or SUL for *A. baumannii*

- Summary
  - AMP-SUL recommended first line for treatment of CRAB by IDSA, in combination, at a total daily dose of 6-9 g SUL (18-27 g AMP-SUL) as an extended infusion (eg, 9g Q8h over 4h) for moderate-severe infections
  - ECV at 4 mg/L for SUL against *A. baumannii* based on contemporary MIC distributions
  - PK/PD data variable but suggest extended-infusion regimens are needed for achieving adequate PTA at current breakpoints
    - 3 g AMP-SUL (1g SUL) Q6h over 4h for AMP-SUL MIC ≤8/4 mg/L (S)

- 6-9 g (2-3 g SUL) Q6-8h (6-9 g TDD SUL) over 4h for AMP-SUL MIC 16/8 mg/L (I)
  - 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
- BPWG Discussion and Recommendation
  - Consideration of 3h infusions instead of 4h given SUL-DUR is approved as a 3h infusion
    - Most PK-PD data evaluating prolonged infusions looked at 4h infusions
  - More data coming from Hartford group re: sulbactam and *Acinetobacter*
  - SUL-DUR package insert recommends 1g/1g q4h for patients with augmented renal clearance
  - Notable that PK-PD target (fT>MIC) increases with resistance increases
  - Plan: proposal for vote in June 2024 for AMP-SUL
    - Will also potentially review minocycline breakpoints for *A. baumannii*

SC DISCUSSION (MAIN POINTS)

- Are additional antibiotics going to be address in the future such as minocycline and fluoroquinolones? Answer: Yes
- Disk correlates would be nice to have.
- Need to think about *Acinetobacter* in general vs. carbapenem resistant *Acinetobacter* (CRAB). A lot of CRAB isolates are also resistant at the breakpoint CLSI already has. There is emerging clinical PK/PD data indicating the targets might need to be higher in critically ill patients but might not have safety data for higher doses.
- Joe Kuti will have three papers coming from an FDA funded study to help answer these questions. Foreshadowing that he will be looking for molecular testing as it is the main driver for needing more or less exposure. If it is an OXA23 then no dose of sulbactam alone will help.
- It would be helpful to have a review of criteria of sulbactam-durlobactam decision and what was used for the criteria and cutoffs.

3.

METHODS APPLICATION AND INTERPRETATION WORKING GROUP (T. KIRN)

DISK DIFFUSION REFERNECE VS STANDARD METHOD AD HOC WORKING GROUP REPORT

- Reference Method Definition (Proposed in CLIS M02 14th Edition/CLSI M07 12th Edition)
  - "Reference method: thoroughly investigated method in which exact and clear descriptions of the necessary conditions and procedures are given for the accurate determination of one or more property values, and in which the documented trueness and precision of the method are commensurate with the method's use for assessing the trueness of other methods for measuring the same property values or for assigning reference method values to reference materials."
  - Disk diffusion is a standardized method, but not a reference method
    - Interpretations are correlates to the MIC
    - Several recent issues with disk diffusion requiring "MIC confirmation"
    - May be misleading as reference for new technologies
- Proposal
  - Broth microdilution is the CLSI AST Subcommittee method for assessing the trueness of other AST methods
  - ie, source of "truth" for bacteria is the M07-BMD MIC method for CLSI when:
    - evaluating new methods / tests for publication in M100 or companion documents
    - establishing MIC breakpoints
    - setting disk diffusion correlates
- Outstanding Questions for Recategorizing Disk Diffusion as a "Standard Method"

Question	Answer
Do we have to go back and re-evaluate methods defined by CLSI using disk as the reference?	Group Consensus, "No"
Can disk diffusion be used by clinical laboratories as the comparator method when verifying / validating commercial AST systems?	• Disk diffusion remains a comparator method for verification/validation studies • Akin to other validated methods in a clinical laboratory • Disk diffusion must be verified by laboratories prior to use as it is an FDA-cleared method (CLIA) • Change of disk diffusion to a standardized method will be addressed in M52 and M68 documents (currently in progress)
Are the CLSI M100 "Table 3" tests considered reference methods? If so, how does this impact them?	See next slide please

- CLSI M100 Tables 3 Tests that Involve Disks
  - From CLSI M100 Instructions for Use

- Supplemental tests: test that detects susceptibility or resistance to a drug or drug class by method other than routine disk diffusion or broth or agar dilution MIC and does not need additional tests to confirm susceptibility or resistance
- Some supplemental tests identify a specific resistance mechanism and may be required or optional for reporting specific clinical results
- Screening test: test that provides presumptive results; additional testing typically only needed for a specific result (eg, only if screen is positive)

- No impact with change of disk diffusion to a standardized method

Table	Supplemental Test	Use
3A	ESBL	Optional for ESBL detection
3C	mCIM/eCIM	Optional for carbapenemase detection
3D	Ceftazidime/avibactam +Aztreonam BDE	Optional for Ceftazidime-avibactam + Aztreonam MIC
3E	Colistin BDE	Optional for Colistin MIC
3G	Beta-lactamase	Required for Penicillin-S Staph isolates
3J	Inducible clindamycin resistance	Required for Erythromycin-R and Clindamycin-S *Staphylococcus, S. pneumoniae*, B-hemolytic Streptococcus
3L	HLAR	Screening test for Enterococcus

BDE, broth disk elution

Table	Test	Type
3F	Direct disk from positive blood cultures	For providing early susceptibility results from positive blood cultures, could be considered an optional supplemental
3K	Mupirocin HLR	For detecting high-level mupirocin resistance in *S. aureus*, could be considered an optional supplemental

- Outstanding Questions for Recategorizing Disk Diffusion as a “Standard Method” Continued

Question	Answer
How about other areas of CLSI documents?	• See summary provided January 2023 and again at this meeting. • CLSI documents will be updated as they are revised to include new definition
What about the ISO document under revision for disk diffusion?	• Will be categorized as a technical standard, not necessarily highlighted as a reference method • Draft not yet available for circulation • Members of ISO document WG anticipate ISO will align with decision at CLSI
Would this have any impact on FDA?	CDRH/FDA does not currently foresee any ramifications of referring to disks as a reference vs standardized method
Would this have any impact on the CLSI Fungal or VET Subcommittees?	• Vet – "will bring up in Tempe but chair does not see any issues." • Fungal – "disk diffusion is an adjunct standardized CLSI method" (from SC chair)

- MAIWG Discussion and Recommendation
  - Discussion focused on impact of the change on the use as a comparator method for clinical validations.
  - These issues would be addressed in CLSI M68 and M52.
  - Motion to recategorize disk diffusion as a standard method rather than reference method. WG Vote: 10-0-0-2.

SC DISCUSSION (MAIN POINTS)

- Table 3 includes disk diffusion testing, so the wording in that table needs to be reviewed and standardized with any decision to make disk diffusion a standard method.
- There was a discussion with the Antifungal Subcommittee about the language/definition of a "standard" method.
- One caveat with fungal susceptibility testing is that there are fewer fungal options for disk diffusion. In M68 (or whichever fungal document is appropriate), CLSI should add a statement that disk diffusion in fungi is not as well worked out or studied as bacterial.
  - Yes, the M52 and M68 document development committees will work on that
- Fluconazole for fungi was developed so long ago that the scattergram has not been seen. Do not want people to use fluconazole disk to validate another fluconazole method.

A motion to recategorize disk diffusion from a reference method to a standard method was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

RE-EVALUATION OF eCIM

- Background

- The eCIM (performed in conjunction with mCIM) is a CLSI endorsed method to detect MBL production in Enterobacterales and recommended to guide treatment decisions, infection prevention procedures, or epidemiological investigations.
- Hypothesis: The eCIM method is inaccurate and unreliable when used on organisms that harbor both a MBL and another serine ß-lactamase (KPC, OXA-48 like)

- Data
  - Isolates harboring a MBL and another carbapenemase are more prevalent than previously thought (IHMI data)
  - 110,755 clinical Enterobacterales isolates collected 2018-2022
  - 3217 isolates harbored an MBL
    - Of these, ~ 28% of isolates also harbored another carbapenem-hydrolyzing, serine ß-lactamase
    - 132 isolates harbored MBL + KPC enzymes
    - 767 isolates harbored MBL + OXA-48 like enzymes
- Clinical Impact
  - Many antimicrobials available for MBL-negative isolates
    - Ceftazidime-avibactam, imipenem-relebactam, meropenem-vaborbactam
    - Laboratories that perform testing via eCIM to inform present/absence of an MBL may provide misleading results to clinicians on which antimicrobials to use
  - Testing for these antimicrobials is preferred, but not all labs can test all options

	Ertapenem	Meropenem	Choice
No carbapenemase or not performed	R	R	CZA, I/R, MEV
Not an MBL	R	R	CZA, I/R, MEV
MBL	R	R	CZA+ATM or FDC

- Does the eCIM work if class A/D and class B carbapenemase present?
  - 37 total isolates tested (ß-lactamase genes detected by whole-genome sequencing)
    - 23 isolates harboring MBL + KPC or OXA-48 like enzymes
      - 17 isolates harboring MBL + KPC enzymes
      - 6 isolates harboring MBL + OXA-48 like enzymes
    - 7 isolates harboring MBL
    - 6 isolates harboring KPC alone or OXA-48 like enzyme alone
  - QC strains: *K. pneumoniae* ATCC 1705 (harbors KPC), *K. pneumoniae* ATCC 2146 (harbors NDM), *E. coli* ATCC 35218 (no ß-lactamases)
  - Species: 5 *E. coli*, 11 *E. cloacae*, 15 *K. pneumoniae*, 1 *K. oxytoca*, 3 *K. aerogenes*, 1 *Citrobacter* spp., 1 *Providencia* spp.
  - eCIM and mCIM set up simultaneously as per CLSI M100 guidelines
- Results
  - eCIM yielded false negative results for 15 out of 23 isolates harboring MBL + KPC/ OXA-48 like enzymes
  - Tested all isolates with the CARBA5 assay to confirm if the eCIM positive isolates with MBL and KPC or OXA-48 like genes produced both enzymes.

Genotype	Total	eCIM positive	eCIM negative	Sensitivity %	False negative %
KPC	4	0	4 (mCIM pos)	-	-
OXA-48 like	2	0	2 (mCIM pos)	-	-
MBL	7	7	0	100	0
MBL + KPC	17	5	12	29.4	70.6
MBL + OXA-48 like	6	3	3	50	50
MBL + KPC **or** OXA-48	23	8	15	34.8	65.2

- Summary of Results Post Confirmation of Enzyme Production with CARBA5
  - All eCIM positive MBL + KPC isolates (n=5) were only positive for NDM production by CARBA5 (negative for KPC)
    - All other MBL + KPC isolates were confirmed to produce both enzymes by CARBA5
  - All eCIM positive MBL + OXA-48 isolates harbored OXA-232 (n=3) and were positive for both NDM and OXA-48 like enzymes by CARBA5
    - All other MBL + OXA-48 isolates also harbored OXA-232 and produced both enzymes by CARBA5

Phenotype confirmed	Total	eCIM positive	eCIM negative	Sensitivity %	False negative %
KPC	4	0	4 (mCIM pos)	-	
OXA-48 like	2	0	2 (mCIM pos)	-	
MBL	12	12	0	100	
MBL + KPC	12	0	12	0	100
MBL + OXA-48 like	6	3	3	50	50
MBL + KPC **or** OXA-48	18	3	15	16.7	83.3

- The eCIM method yields excessive false negative results on Entobacterales isolates that have both a MBL and a serine-ß-lactamase
  - 100% false negative rate for isolates with MBL + KPC
  - 50% false negative rate for isolates with MBL + OXA-48 like enzymes
  - Overall sensitivity in presence of class A/D carbapenemase = 16.7%

- Recommendation
  - Remove eCIM as an option from M100 due to:
    - risk of false-negative in presence of class A or D carbapenemase
    - change in epidemiology to an increased prevalence of MBL + class A/D carbapenemase
- MAIWG Discussion and Recommendation
  - Method is not a problem but epidemiology of organisms harboring multiple classes of carbapenemases may make it less useful in certain settings.
  - Concern about taking away a method that could be useful in certain settings.
  - Motion to not remove eCIM from M100, make a note that if eCIM is negative needs some sort of further characterization. WG Vote: 10-0-0-2.
  - May want to state that, if possible, a test that better differentiates the presence of specific carbapenemases should be used.
- eCIM Proposed M100 Revisions
  - To add a comment to Tables 3B and 3C in mCIM with eCIM column that states “False-negative results may occur for isolates co-producing a serine carbapenemase and a metallo-ß-lactamase.”
  - To add text to the Table 3C test interpretation row as shown below:

Test interpretation	eCIM – Interpret only when mCIM test is positive • Metallo-B-lactamase positive: – A ≥ 5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (eg, mCIM = 6 mm; eCIM = 15 mm; zone diameter difference = 9 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figures 3B and 3C). – If the test isolate produces a metallo-B-lactamase, the activity of the carbapenemase will be inhibited in the presence of EDTA such that the meropenem in the disk will not be hydrolyzed as efficiently as in the tube without EDTA. The result is inhibition of the meropenem-susceptible *E. coli* and an increase in the zone diameter for the eCIM zone diameter compared with the mCIM zone diameter. • Metallo-B-lactamase ~~negative~~inconclusive, serine carbapenemase detected: – A ≤ 4-mm increase in zone diameter for the eCIM vs zone diameter of mCIM (eg, mCIM = 6 mm; eCIM = 8 mm; zone diameter difference = 2 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figure 3D). Isolates that co-produce a serine-carbapenemase and a metallo-B-lactamase may give an inconclusive eCIM result. An alternate method should be used rule out the presence of a metallo-B-lactamase – If the test isolate produces a serine carbapenemase, the activity of the carbapenemase will not be affected by the presence of EDTA and there will be no or marginal (≤ 4 mm) increase in zone diameter in the presence of EDTA compared with the mCIM zone diameter.

- To add text to Table 3C reporting row as shown below:

Table 3C. (Continued)

Test	mCIM Only or in Conjunction With eCIM		
Reporting	**mCIM Only**		
	mCIM Result	**eCIM Result**	**Report**
	Negative	Not set up	Carbapenemase not detected
	Positive	Not set up	Carbapenemase detected
	Indeterminate	Not set up	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.*
	mCIM and eCIM Combination Test		
	mCIM Result	**eCIM Result**	**Report**
	Negative	Do not interpret	Carbapenemase not detected
	Positive	Negative	Serine carbapenemase detected; metallo-B-lactamase inconclusive[a]
	Positive	Positive	Metallo-B-lactamase detected
	Inconclusive	Do not interpret	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.[ab]

[a] If both a serine carbapenemase and a metallo-B-lactamase are co-produced by one organism, differentiation between enzymes will not be possible and false-negative eCIM results may occur, resulting in an inconclusive interpretation for metallo-B-lactamase detection.

[b] If **inconclusive** mCIM results are obtained on repeat testing, consider performing a different phenotypic test for carbapenemase detection (ie, CarbaNP), a test for carbapenemase genes or send isolate to a referral laboratory for further testing.

~~If both a serine carbapenemase and a metallo-B-lactamase are co-produced by one organism, differentiation between enzymes will not be possible and false-negative eCIM results may occur.~~

- To add text to Table 3C footnote Note 2 that states "NOTE 2: eCIM: This method demonstrated a sensitivity > 95% and specificity >92% for differentiation of metallo-ß-lactamases (NDM, VIM, IMP) from serine carbapenemases (KPC, OXA, and SME) among Enterobacterales isolates investigated by CLSI. In CLSI studies, one K. pneumoniae co-producing NDM and OXA-181 yielded a false-negative result at 3 of 4 validation sites. Additional studies have demonstrated poor sensitivity for detection of metallo-ß-lactamases (NDM, VIM, and IMP) in isolates co-producing a serine ß-lactamase (KPC or OXA-48), therefore a negative eCIM does not exclude the possibility of a metallo-ß-lactamase."

SC DISCUSSION (MAIN POINTS)

- Any concern about the carbaNP assay?
  - There are some limitations with OXAs with the carbaNP, but only studying the eCIM here.
- Is the comment "may occur" too light, should it state something strong like "will occur"?
  - Would like to keep the wording in "may occur", but could consider changing it to "will occur"
- The title of the table should also say for "clinical testing" to help labs distinguish between clinical vs. epidemiology testing.
- Labs that do not do phenotypic testing on new antibiotics depend on the eCIM, so that is a reason to take a stronger stance.
- The data does not look good for the eCIM. Why not remove the test all together?
  - The test works well if there is only an MBL and a positive test result is helpful. It is an issue if there are two mechanisms.
- If only a KPC is seen, probably not running the eCIM only because it is going to be negative.
- There are situations where labs do not have access to molecular, so this is a half-step. It can be useful in some scenarios.
- There were a few KPCs that tested positive in the data. Can CLSI give guidance on what to do if labs see a lot of MBL or KPCs?
- The initial study of the eCIM excluded the only isolate that had two resistance mechanisms, but now seeing an evolving epidemiology picture with both mechanisms becoming more common. Disk methods are very important for resource limited settings.
- There is support for the verbiage here and cannot take this away for low resource settings. The interim is to go with this verbiage change. This is risk stratification. It comes back to this table of high vs. low risks to help institutions define what the risk is in their institution.
- If there is a carbapenem resistant isolate and a negative eCIM test, it needs confirmation.
- For labs who see a lot of combinations of carbapenemases, the eCIM method fails for those labs. Some are using mCIM plus a lateral flow.
- It's important to understand local epidemiology, so that should be included in the comment.

A motion to accept the proposed eCIM revisions with the comment stating, "False-negative results are likely to occur for isolates co-producing a serine carbapenemase and a metallo-ß-lactamase." in the Introduction to Tables 3B and 3C was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

INTRINSIC RESISTANCE AD HOC WORKING GROUP REPORT

- Intrinsic Resistance and New antibiotics
  - Staphylococci and new drugs: ceftazidime/avibactam, ceftolozane/tazobactam, and cefiderocol
  - *Proteus, Providencia*, and *Morganella* with imipenem and imipenem/relebactam (move out of Appendix B) (same as *S. marcescens* and gentamicin)
  - Non-Enterobacterales – would restructure chart to add new agents as data support
    - *P. aeruginosa* and gentamicin
- Staphylococci Appendix B3
  - If data support adding cefiderocol to either the table, should add – or a comment.

- Support notes for aztreonam/avibactam in appendix B3 and B4.
- The group had a mixed discussion on ceftazidime, ceftazidime/avibactam, and ceftolozane/tazobactam. We are going to look for more references to discuss this in our next meeting.

- Enterobacterales Appendix B.1
  - To remove imipenem/*Proteus, Providencia, Morganella* from Intrinsic Resistance table (Appendix B.1) because as it is written, does NOT meet the intrinsic resistance definition.
  - To remove the corresponding comment for the species above from appendix B.1 (same as removing gentamicin/*S. marcescens*) and move the revised comment including imipenem/relebactam to table 2A.
  - Suggested comment: "*Proteus* spp., *Providencia* spp., and *Morganella* spp. may have elevated minimal inhibitory concentrations to imipenem and imipenem/relebactam by mechanisms other than by production of carbapenemases. Isolates that test as susceptible to imipenem should be reported as susceptible. For these species testing is not recommended for imipenem/relebactam."
- Non-Enterobacterales Appendix B.2
  - Support addition of new agents to non-Enterobacterales appendix B
  - Will work with text and tables for chart
- Proposal to Include Gentamicin as Intrinsically resistant in *P. aeruginosa*
  - Background
    - For 2023, the CLSI M100 33rd edition, included revised breakpoints for aminoglycosides in Enterobacterales and *Pseudomonas aeruginosa*. Those changes were mainly based on new PK/PD data and scarce clinical data.
    - Table 1C included amikacin for urine and tobramycin for other Infection sources. Gentamicin was removed as new evidence demonstrate low target attainment percentages at any dose regimens even lowering the breakpoints.
    - It has been commented in some meetings that gentamicin looks like intrinsically resistant with this data. So a discussion in the IR AHWG would be necessary to propose an action for this drug.
  - From a PK/PD standpoint, it seems that gentamicin is intrinsically resistant in *P. aeruginosa*, as the ECOFF is 3 fold higher (8 ug/mL) that the minimal MIC required for stasis (1 ug/mL)
  - Even lowering the breakpoint, the gentamicin exposure in *P. aeruginosa* would be inappropriate.
  - Despite limited clinical data, gentamicin could cause important nephrotoxicity for isolates at 1 ug/mL
  - We discussed the definition of intrinsic resistance (and it is not met here) and many were not enthused about addition of gentamicin to this table. No action required.
- MAIWG Discussion and Recommendation
  - No vote was taken, and discussion highlighted the need for a better, harmonized "intrinsic resistance" definition and use for the table.
  - Perhaps adding to the Table "clinical resistance"?
  - The ad hoc working group will discuss this with the fungal group and come back with a more information on intrinsic resistance definitions in June.

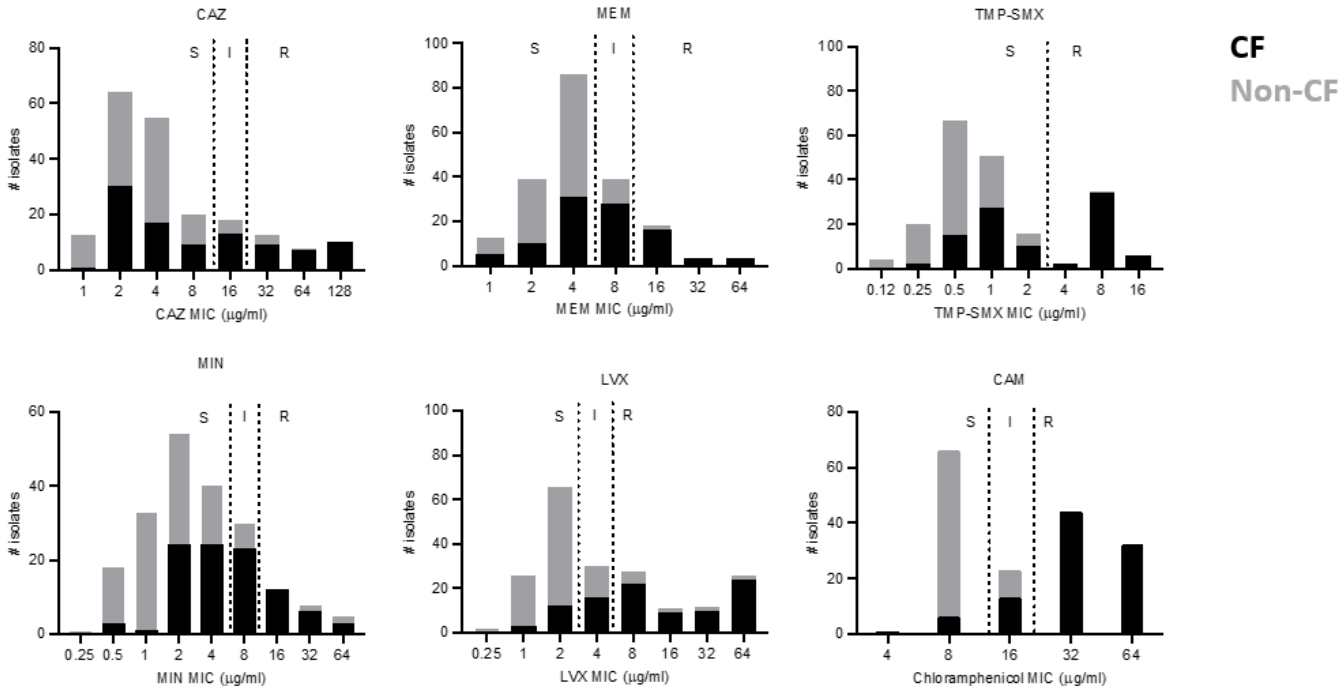
SC DISCUSSION (MAIN POINTS)

- If bacterial killing in a human cannot be achieved, then that is clinical intrinsic resistance. The microbiology definition is very strict, but this is something clinicians look to see what they can and cannot use.

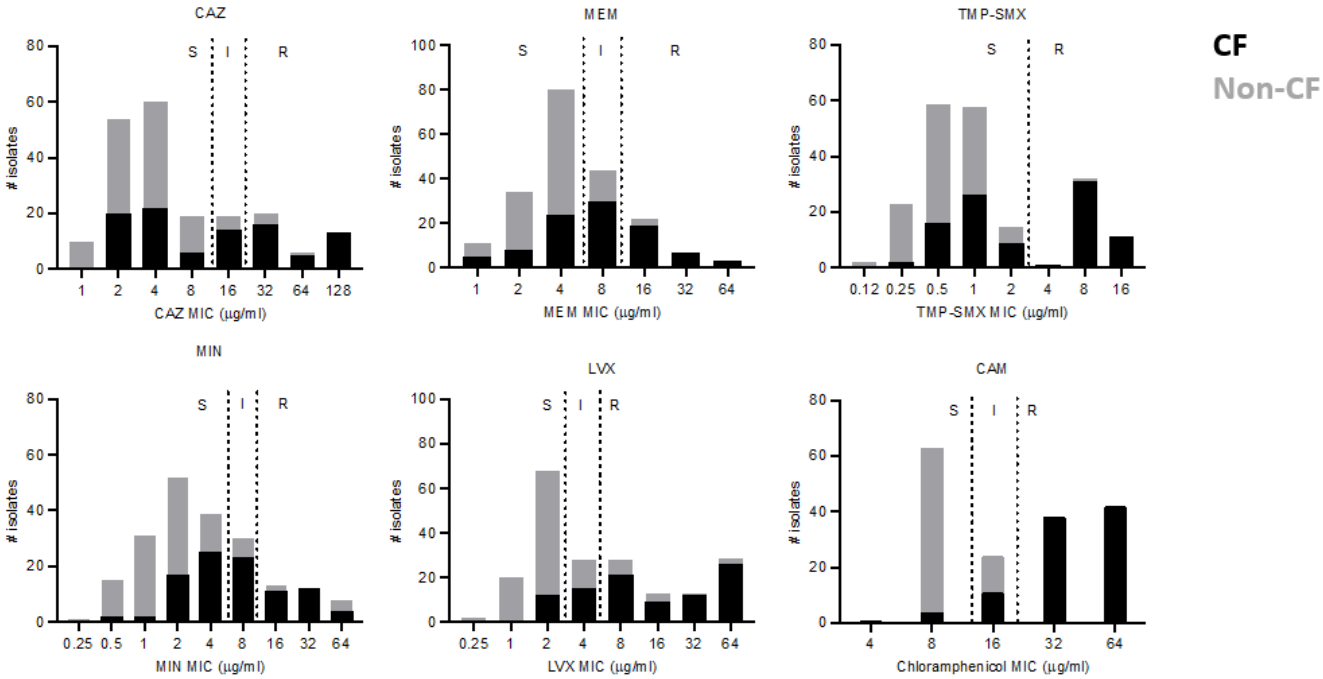
- The microbiology definition of intrinsic resistance might need to be reviewed, particularly the 3 or 4% cutoff for the small percent of isolates. There is a difference between acquired resistance and having a small number of susceptible isolates because most isolates have the acquired resistance vs. when the MICs of the wild-type population are above where the resistant breakpoint is.
- It is easy to get lost in philosophical debate, and EUCAST decided to go with "expected resistance".
- Most pharmacists consider clinical resistance is resistance.
- Gentamicin is used for locks or cement in joints. So, it is important if it is truly intrinsic resistance at any drug level or if it is resistant in the human.
- Should bring in the VET Subcommittee because they are also discussing the definition of intrinsic resistance
- For *Salmonella* and *Shigella,* drugs that appear active *in vitro*, but are not active *in vivo* are listed.
- Action item: Intrinsic Resistance AHWG is charged with updating the table and evaluating gentamicin as intrinsic resistance.
    - The working group needs to clarify, but it might be placing an R in the antibiogram.
- The name of the table is important and need to play with the wording.
- The intrinsic resistance table is helpful to labs because if it is testing susceptible then need to investigate and see if the lab misidentified the organism. There are two separate concepts here.

BURKHOLDERIA CEPACIA COMPLEX AST AD HOC WORKING GROUP REPORT

- June 2023 MAIWG Recommendations
    - AST Subcommittee decision:
        - Remove disk diffusion (DD) breakpoints for the *Burkholderia cepacia* complex (BCC)
        - Recommend testing by a reference dilution method (broth or agar)
    - Can we identify new DD breakpoints for BCC?
        - Determine epidemiological cut-off values (ECVs) for BCC MIC data
        - Compare new ECVs to current BCC CLSI breakpoints (BPs)
        - Evaluate various MIC BPs for determining new DD breakpoints
        - Evaluate performance of agar dilution and ETEST compared to broth microdilution
- Agar Dilution (AD) vs Broth Microdilution (BMD) Studies
    - BCC isolates: n=205
        - 100 from people with cystic fibrosis (CF)
        - 105 from people without CF
        - Isolates sent to Mayo Clinic as freezer stocks
    - Agar dilution performed at Mayo Clinic following CLSI methods
    - MICs read at 20-24h and 24h
    - Data analyses:
        - 4 isolates discarded because they had either two morphologies or contamination during AD
        - Comparator: MIC BMD mode from CMI and VUMC data
            - MICs truncated to last observable value
            - When 2 modes, calculated median and rounded to the next 2-fold dilution
            - When no mode, median was used
    - Agar Dilution MIC Distribution 20h Read



- Agar Dilution MIC Distribution 24h Read



- Summary: AD vs BMD

Antimicrobial	Mayo Clinic	Wootton et al. (EUCAST)	Fehlberg et al.
CAZ	72.6%	52.3%	97.5%
MEM	86.1%	32.9%	95.1%
MIN	87.6%	66.5%	97.5%
LVX	95.5%	Not tested	93.9%
CAM	90.5%	70.3%	89.0%
SXT	67.7%	80.0%	98.8%

- AD vs BMD: Error-Rate Bounded Method

		20 h			24 h		
Antibiotic	**Type**	**VME**	**ME**	**mE**	**VME**	**ME**	**mE**
Ceftazidime	≥I +2	11.1% (4/36)	-	22.2% (8/36)	5.6% (2/36)	-	13.9% (5/36)
	I+1 TO I-1	7.4% (6/81)	1.2% (1/81)	14.8% (12/81)	6.2% (5/81)	1.2% (1/81)	19.8% (16/81)
	≤I-2	-	0% (0/84)	4.8% (4/84)	-	1.2% (1/84)	7.1% (6/84)
Meropenem	≥I +2	14.7% (5/34)	-	29.4% (10/34)	8.8% (3/34)	-	26.5% (9/34)
	I+1 TO I-1	7.3% (11/150)	0% (0/150)	47.3% (71/150)	4.7% (7/150)	0.7% (1/150)	45.3% (68/150)
	≤I-2	-	0% (0/17)	0% (0/17)	-	0% (0/17)	5.9% (1/17)
Minocycline	≥I +2	15.2% (5/33)	-	18.2% (6/33)	9.1% (3/33)	-	12.1% (4/33)
	I+1 TO I-1	4.8% (4/83)	0% (0/83)	50.6% (42/83)	3.6% (3/83)	0% (0/83)	50.6% (42/83)
	≤I-2	-	0% (0/85)	0% (0/85)	-	0% (0/85)	0% (0/85)
TMP-SMX	≥R+1	21.3% (10/47)	-	NA	21.3% (10/47)	-	NA
	R+S	31.6% (24/76)	0% (0/76)	NA	31.6% (24/76)	1.3% (1/76)	NA
	≤S-1	-	0% (0/78)	NA	-	0% (0/78)	NA
Chloramphenicol	≥I +2	4.4% (3/68)	-	7.4% (5/68)	2.9% (2/68)	-	8.8% (6/68)
	I+1 TO I-1	8.3% (11/132)	0% (0/132)	37.9% (50/132)	7.6% (10/132)	0% (0/132)	36.4% (48/132)
	≤I-2	-	0% (0/1)	0% (0/1)	-	0% (0/1)	0% (0/1)
Levofloxacin	≥I +2	0% (0/61)	-	4.9% (3/61)	0% (0/61)	-	3.3% (2/61)
	I+1 TO I-1	3.4% (4/118)	0% (0/118)	28% (33/118)	1.7% (2/118)	0% (0/118)	29.7% (35/118)
	≤I-2	-	0% (0/22)	0% (0/22)	-	0% (0/22)	4.5% (1/22)
Acceptance criteria	≥I +2	<2%	ND	<5%	<2%	ND	<5%
	I+1 TO I-1	<10%	<10%	<40%	<10%	<10%	<40%
	≤I-2	ND	<2%	<5%	ND	<2%	<5%

- DD vs Agar Dilution
  - Total isolates n=199
    - 4 isolates removed for agar dilution
    - 2 isolates removed from DD due to contamination at VUMC
  - Mode for DD performed at VUMC
    - 3 values from 3 different MHA manufacturers
    - Median if no mode
  - AD reference method for DD comparison
  - All data truncated to the last readable values for both methods

		20 h			24 h		
Antibiotic	**Type**	**VME**	**ME**	**mE**	**VME**	**ME**	**mE**
Ceftazidime	≥I +2	22.2% (4/18)	-	5.6% (1/18)	21.1% (4/19)	-	10.5% (2/19)
	I+1 TO I-1	4.1% (2/49)	0% (0/49)	36.7% (18/49)	14.3% (8/56)	0% (0/56)	32.1% (18/56)
	≤I-2	-	1.5% (2/132)	1.5% (2/132)	-	0.8% (1/124)	1.6% (2/124)
Meropenem	≥I +2	0% (0/6)	-	16.7% (1/6)	20% (2/10)	-	10% (1/10)
	I+1 TO I-1	4.3% (6/141)	5.7% (8/141)	27% (38/141)	4.2% (6/144)	3.5% (5/144)	27.8% (40/144)
	≤I-2	-	5.8% (3/52)	3.8% (2/52)	-	6.7% (3/45)	4.4% (2/45)
Minocycline	≥I +2	7.7% (1/13)	-	15.4% (2/13)	15.8% (3/19)	-	26.3% (5/19)
	I+1 TO I-1	3.8% (3/80)	0% (0/80)	36.3% (29/80)	8.6% (7/81)	0% (0/81)	37% (30/81)
	≤I-2	-	1.9% (2/106)	0% (0/106)	-	2% (2/99)	0% (0/99)
TMP-SMX	≥R+1	10.3% (4/39)	-	0% (0/39)	9.8% (4/41)	-	2.4% (1/41)
	R+S	0% (0/18)	61.1% (11/18)	11.1% (2/18)	0% (0/16)	68.8% (11/16)	6.3% (1/16)
	≤S-1	-	3.5% (5/142)	19.7% (28/142)	-	3.5% (5/142)	19.7% (28/142)
Acceptance criteria	≥I +2	<2%	ND	<5%	<2%	ND	<5%
	I+1 TO I-1	<10%	<10%	<40%	<10%	<10%	<40%
	≤I-2	ND	<2%	<5%	ND	<2%	<5%

- Summary: AD vs BMD
  - Essential agreement:
    - > 90% for CAM and LVX
    - > 85% for MEM and MIN
    - < 75% for CAZ and SXT
  - When comparing AD to BMD:
    - LVX meets all acceptance criteria using error-rate bounded method
    - CAZ, MEM, MIN, CAM, and TMP-SMX do not meet acceptance criteria due to high VMEs and mEs
  - When comparing DD to AD:
    - CAZ, MEM, MIN, and TMP-SMX do not met acceptance criteria
  - Limitations:
    - BMD, DD, and AD not performed on the same day using same inoculum
    - AD not performed in triplicate
  - Discussion points
    - Compare CF to non-CF isolates?
    - Remove isolates without a mode MIC?
- Etest Performance
  - Received funding from the CF Foundation: studies in progress
  - Past data, UCLA pilot study:
    - 44 isolates from UCLA (n=4) and the University of Michigan (n=40)
    - ETEST performed on BD MHA following manufacturer's instructions

- Limitations: smaller # isolates, clonal isolates

	ETEST vs. BMD				
ABX	**CA (%)**	**VME (%)**	**ME (%)**	**MiE (%)**	**EA (%)**
CAZ	32/44 (73)	1/11 (9)	0/27 (0)	10/44 (23)	20/39 (51)
MEM	34/44 (77)	0/16 (0)	0/16 (0)	16/44 (36)	30/39 (77)
MIN	27/44 (61)	3/25 (12)	0/14 (0)	16/44 (36)	34/44 (77)
TMP/SMX	40/44 (91)	2/25 (8)	2/19 (11)	0/44 (0)	26/40 (65)
LVX	38/44 (86)	0/21 (0)	0/12 (0)	24/44 (55)	36/37 (97)

- Evaluation of New DD Breakpoints
  - Data analysis: ECVs
    - Antimicrobials:
      - Ceftazidime (CAZ)
      - Levofloxacin (LVX)
      - Meropenem (MEM)
      - Minocycline (MIN)
      - Trimethoprim-sulfamethoxazole (TMP-SMX)
    - MIC dataset:
      - 100 CF isolates tested in triplicate or single replicate at VUMC
      - 105 non-CF isolates tested in triplicate at VUMC
      - Used the mode MIC value when possible; otherwise use single replicate value
      - 267-1106 MIC values for BCC generated at IHMA1
      - # values depend on drug tested
      - Limitation: IHMA data has body site only, does not specify CF-vs non-CF patients
    - Used ECOFFinder to determine ECVs following the user instructions (see raw data slides)
      - https://clsi.org/meetings/susceptibility-testing-subcommittees/ecoffinder/
      - Evaluated data for all isolates (IHMA, CF, and non-CF) and for non-respiratory isolates only
      - Are non-respiratory isolates more “wild type”?
      - ECVs generated using ECOFFinder
        - ECOFF 97.5% is the same for CAZ, LVX, and MEM regardless of body site
        - ECOFF 97.5% is lower for MIN and SXT when analyzing only non-respiratory isolates
        - Current CLSI MIN S BP is 4 µg/ml
        - Current CLSI SXT S BP is 2 µg/ml

All BCC isolates

	CAZ	LVX	MEM	MIN	SXT
ECOFF 95.0%	16	16	16	32	8
ECOFF 97.5%	**16**	**16**	**16**	**32**	**16**
ECOFF 99.0%	16	32	16	64	16
ECOFF 99.5%	32	32	32	128	32
ECOFF 99.9%	32	64	32	256	64

Non-respiratory BCC isolates

	CAZ	LVX	MEM	MIN	SXT
ECOFF 95.0%	16	8	8	4	4
ECOFF 97.5%	**16**	**16**	**16**	**4**	**8**
ECOFF 99.0%	32	16	16	8	8
ECOFF 99.5%	32	32	16	8	16
ECOFF 99.9%	32	32	32	8	32

- Follow-up Studies
  - Consult with Joe Kuti, PharmD on AHWG for BCC AST
  - ECV very high for most drugs tested, not within PK/PD clinically useful range except for MEM and CAZ (16 µg/mL)
  - Recommendations for evaluating new DD BPs using diffusion Breakpoint Estimation Testing Software (dBETS) :
    - Use current CLSI MIC BPs
    - Use PK/PD breakpoints
    - Use new ECV MIC BPs
  - Data input for dBETS:
    - Mode MIC of 100 CF and 105 non-CF isolates tested at CMI and/or VUMC
    - Correlative DD zone of inhibition (ZOI)
      - Mode ZOI (tested on different MHA brands)
  - For analysis 1, use current CLSI BCC MIC breakpoints
  - For analysis 2, use PK/PD breakpoints provided by Joe Kuti
    - PK/PD breakpoints based on maximal doses
    - Limitation: single value only, so no I or R categories
  - For analysis 3, ECV MIC for MEM and CAZ
    - Limitations:
      - ECV MICs were so high, only MEM and CAZ clinically relevant (16 ug/mL)
      - Single value only, so no I or R categories
  - Compare DD to BMD using newly generated DD BPs-error rate bounded method

Analysis 1

	Current CLSI MIC BPs (µg/ml)		
	S	I	R
CAZ	≤ 8	16	≥ 32
MEM	≤ 4	8	≥ 16
MIN	≤ 4	8	≥ 16
TMP/SMX	≤ 2/38	-	≥ 4/76
LVX	≤ 2	4	≥ 8

	Current CLSI Zone Diameter Breakpoints (mm)		
	S	I	R
CAZ	≥ 21	18-20	≤ 17
MEM	≥ 20	16-19	≤ 15
MIN	≥ 19	15-18	≤ 14
TMP/SMX	≥ 16	11-15	≤ 10

	New Zone Diameter Breakpoints Based on Current CLSI MIC Breakpoints (mm)		
	S	I	R
CAZ	≥ 24	21-23	≤ 20
MEM	≥ 24	20-23	≤ 19
MIN	≥ 17	18-22	≤ 23
TMP/SMX	≥ 14	7-13	≤ 6
LVX	≥ 18	16-17	≤ 15

Analysis 2

	PK/PD MIC BPs (µg/ml)		
	S	I	R
CAZ[1]	≤ 8		
MEM[1]	≤ 8		
MIN[2]	≤ 1		
TMP/SMX[2]	≤ 0.25		
LVX[3]	≤ 1		

1. Derived from *P. aeruginosa* data
2. Derived from *S. maltophilia* data
3. Derived from *P. aeruginosa* and *S. maltophilia* data

	Zone Diameter Breakpoints Based on PK/PD Breakpoints (mm)[1]		
	S	I	R
CAZ	≥ 25		
MEM	≥ 20		
MIN	≥ 34		
TMP/SMX	≥ 31		
LVX	≥ 30		

1. When a single MIC breakpoint is entered into dBETS, no I or R breakpoints are generated.

Analysis 3

	ECV MIC BPs (µg/ml)		
	S	I	R
CAZ[1]	≤ 16		
MEM[1]	≤ 16		

	Zone Diameter Breakpoints Based on ECV (mm)[1]		
	S	I	R
CAZ[1]	≥ 23		
MEM[1]	≥ 16		

1. When a single MIC breakpoint is entered into dBETS, no I or R breakpoints are generated.

- Data summary: DD compared to BMD, error-rate bounded method

	Previous study	New DD BPs based on current CLSI MIC BPs[1]	New DD BPs based on PK BPs[1]	New DD BPs based on ECOFF[1]
CAZ	FAIL[2]	PASS	FAIL	FAIL
LVX	FAIL	PASS	FAIL	
MEM	FAIL	FAIL	FAIL	FAIL
MIN	FAIL	FAIL	FAIL	
TMP-SMX	FAIL	FAIL	FAIL	

1. Based on analysis using dBETS
2. Failed due to 1 VME.

	New Zone Diameter Breakpoints Based on Current CLSI MIC Breakpoints (mm)		
	S	I	R
CAZ	≥ 24	21-23	≤ 20
LVX	≥ 18	16-17	≤ 15

- Summary and discussion points
  - DD does not perform well compared to BMD for most drugs tested even when we generate new DD breakpoints using various MIC cut-offs:
    - Using the current CLSI breakpoints, CAZ and LVX meet acceptance criteria
    - All other drugs fail whether using current CLSI BPs, PK/PD BPs with max dosing, or new ECVs
    - Limitation: PK/PD BPs and ECVs do not have I category
  - Discussion:
    - DD BPs for only CAZ and LVX
    - Concerns for overuse of those drugs and not helpful to have DD BPs for these 2 drugs
    - Overall agreement that DD does not work well for this population of organisms
- MAIWG Discussion and Recommendation
  - Motion to remove all *Burkholderia cepacia* complex breakpoints from M100. WG Vote: 5-5-0-2.
  - Discussion of motion: Going back to a Tier 1 study to determine effect of different testing variables? How will this impact clinical decisions on treatment or transplants.
  - Reason for no vote: Wanting to have more information to make the decision, particularly on the impact of removal of breakpoints and methods for BCC AST.

SC DISCUSSION (MAIN POINTS)

- BMD is reproducible within the method (>90% agreement), although the one exception is ceftazidime (89%). The reproducibility of agar dilution was not tested. There is a limitation that that AD and BMD were not tested side by side.
- This is a case of clinical resistance. All these breakpoints are grandfathered in from other organisms. The breakpoint might not be right to start with.
- The breakpoint bisects the wild type population for all the drugs.
- Thank you to Holly Huse and her lab to getting grants from the CF Foundation to do these studies.
- CLSI could send out a questionnaire to transplant ID physicians to ask what they would do if they did not have AST data for *Burkholderia*.

- There is no good justification for giving *Burkholderia* AST results. Need to go all the way back to the groundwork.
- If physicians see susceptible, they use that drug. If it is all resistant, physicians pick what they want anyways and hope for the best at a high dose.
- The recommendation from CF foundation is to use BMD only.
- John LiPuma at the University of Michigan who did a lot of the transplant work is in favor of MICs, and he has a validation set for those interested in performing testing.
- EUCAST removed the *Burkholderia* breakpoints, but think labs are still testing it.
- Consider moving the agents to Tier 4, remove the breakpoint, publish the ECV, and report MIC only.
- It is important to contact the CF Foundation. If CLSI removes the breakpoints and the methods, there is concern that labs will use the outdated information.
- Know the breakpoints are wrong, so need to explore what does this really look like and how to move forward in June.
- IHMA and CF/non-CF isolates were used for the ECV calculation. Using non-respiratory isolates only lowered the ECV a little. There is concern that the ECV is on limited data from mostly from one lab.
- Tested the isolate in triplicate with the same brand of broth and got reasonable agreement, but that mode will not match with agar dilution. It is unlikely that CLSI will get further funding to develop the method.
- BMD appears reproducible, but since AD and BMD were not tested side-by-side, there are concerns around media effect. If a study is done there is a request that BMD and AD are done side-by-side and look at bias on both sides. If it is known that one method is consistently higher or lower, that is actionable and can work with that information.
- EUCAST looked at different media and gave up.
- CLSI should look at moving *Burkholderia* to the M45.
    - The physicians will not understand the difference from M100 vs. M45
    - It would be a tight timeline to get this into the M45 and the M45 is not updated every year. There is concern about moving the breakpoints to the M45 where they might stay incorrect for a while until the document is updated again.
- The ECV sits high, so even if the breakpoint is moved a little bit, it is unlikely to get near the PK/PD aspect. Suggestion to not do more testing.
- Action Item: The BCC Working Group should come back in June with ideas (multiple options) on how to move forward. Would like to see a fact finding mission on damage control if breakpoints are removed and look at an ECV option.

A motion to remove the *Burkholderia cepacia* complex breakpoints in M100 was made and seconded. Vote: 10 for, 4 against, 0 abstain, 6 absent (Pass)

Against Vote Reasoning:
- Would like to talk to transplant centers before making this change.

ANAEROBE AD HOC WORKING GROUP REPORT

- Disk testing – EUCAST efforts
    - Update on study status
        - Protocol drafted – Under revisions – working group to provide feedback
        - MTA in process – MTA finished legal review – will start the signature process by December 8,2023
        - Looking for industry partners for donations of supplies
        - Issue with exporting *Clostridium perfringens* from Wales, all *C. perfringens* will be from Mayo

	• M11 – Revision – Paperwork in process to request revision
	• Antibiogram – Appendix D
	  ○ Received data from several sites, now compiling to update the antibiogram.
	  ○ Review of publications
	  ○ Nothing new to update
	
	NOVEL/EMERGING METHODS AD HOC WORKING GROUP IDEA
	• There are new methods coming out and need to keep up to date with this.
	• All the newer agents are starting to have unique parameters, so we need to think about this.
	• Dr. Tom Kirn may set up an ad hoc working group to address this topic.

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

2024 JANUARY AST SUBCOMMITTEE MEETING SUMMARY MINUTES PLENARY 3: Tuesday, 23 January 2024 (In-person) 7:30 AM – 12:00 PM Mountain Standard (US) Time	
#	**Description**
1.	**OPENING** Dr. Lewis opened the meeting at 7:30 AM Mountain Standard (US) time.

2. **JOINT CLSI EUCAST WORKING GROUP (J. HINDLER)**

WG GOALS

- Describe a method for disk content determination which can be used early in the drug development process to avoid having different disk contents in the CLSI and EUCAST standards. Completed July 2021.
- Discuss differences between CLSI and EUCAST QC criteria, methods for establishing QC criteria and the possibility of harmonizing CLSI and EUCAST QC criteria.

DISK CONTENT SELECTION IN PROGRESS

WG Assigned Study #	Agent	Sponsor	Notes
JWG-2022-3	Imipenem-XNW4107 (Funobactam) (fixed at 8 mg/L)	Evopoint Biosciences [a]	Present at this meeting
JWG-2022-4	~~RG6006~~ Zosurabalpin	Roche	Phase 2 ongoing
JWG-2022-5	Aztreonam-nacubactam (1:1) and Cefepime-nacubactam (1:1)	Meiji	Phase 1 ongoing
JWG-2022-9	Zoliflodacin	GARDP	Phase 2 ongoing
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	Organizing

[a] formerly Sinovent Pharmaceuticals

MHA AGAR EVALUATIONS IN PROGRESS

WG Assigned Study #	Agent	Sponsor	Notes
JWG-2022-6	Debio 1452	Debiopharm	Present at this meeting

M23S PROPOSED REVISIONS

- Add procedure for establishing disk content for combinations of agents
- Add comment for review of data where sponsor did not select disk content in collaboration with Joint WG
- Chapter 2.5 Proposed Revisions
    - Chapter 2.5 Considerations for Selection of the Optimal Disk Content (Potency) for Combinations of Agents
    - Recommendations in current M23S lack detail
    - Consider the standard potency for the active agent (eg, parent compound) and vary the inhibitor component. If there is no agreed disk potency for the parent compound alone, must establish the optimal disk potency of the parent compound.
    - Consider:
        - Does the inhibitor have any secondary antimicrobial activity unrelated to the target inhibition?
        - Do the zone diameters for parent compound plus an inhibitor (without secondary activity) coincide with those of the parent compound alone? If not, there may be an on-disk interaction between parent compound and inhibitor.
    - Selecting isolates:

Phenotype	No. of Isolates	
	Phase 1	Phase 2
WT to the parent compound	≥2	≥10
WT to the combination (NWT to parent)	≥2	≥30
NWT to the combination	≥2	≥30

    - NOTE: For Enterobacterales, a variety of target species should be included and differences in MIC values between species should be taken into consideration when determining the numbers of each species to evaluate.
- What to do when sponsor elects not to interact with the Joint WG for disk content selection?
    - Add proposed comment to M23S: "Although CLSI and EUCAST strongly suggest that pharmaceutical companies approach the joint WG to develop disk content for a new drug or for a drug for which an alternative disk content might be needed, some companies may wish to proceed without collaborating with the joint WG. If the company subsequently seeks approval for disk QC ranges, breakpoints, or ECVs, the company will be asked to provide the data supporting disk content selection to the joint WG. Additional studies may be requested if the data are deemed insufficient to approve the disk content."

A motion to approve the M23S Subchapter 2.5 chapter additions was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)

A motion to approve the M23S proposed comment for when a sponsor elects not to interact with the Joint WG for disk content selection was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)

JOINT WG - WHAT IS NEXT?

- Continue with disk diffusion content selection and MHA (for QC) acceptability studies
- Complete edits on M23S and M23S2
- Pre-QC of CAMBHB
- Colony counts

	• Harmonization of disk diffusion and BMD reading guide

3.	**METHODS DEVELOPMENT AND STANDARIZATION WORKING GROUP (T. DINGLE)**
	
	RIFABUTIN REFERENCE SUSCEPTIBILITY TESTING METHOD AGAINST *ACINETOBACTER BAUMANNII*
	• Objective: The aim of this document is to expose the rationale for using agar dilution in Mueller Hinton medium supplemented with the iron chelator pyridoxal isonicotinoyl hydrazone (PIH) as the reference susceptibility testing method for rifabutin against *A. baumannii*.
	• Mechanism of Action
	  ○ Rifamycins penetrate the bacterial cell wall to inhibit the DNA-dependent RNA polymerase.
	  ▪ Rifamycins have limited clinical use on Gram-negative infections because of their poor ability to penetrate the Gram-negative outer membrane.
	  ○ Rifabutin was identified with potent and specific *in vitro* activity against the Gram-negative pathogen *A. baumannii* when tested in nutrient deprived medium.
	• Agar dilution supplemented with PIH is the most robust method for rifabutin AST
	  ○ In contrast to iron-depletion, iron-chelation was found to be required to achieve the proper iron-limited conditions leading to potent *in vitro* activity of rifabutin.
	  ○ Agar dilution using Mueller Hinton agar supplemented with the non-toxic iron chelator PIH at 0.1 mM showed to be the most robust method for MIC determination, as opposed to microbroth dilution MIC that often leads to skipped wells.
	• Agar dilution MIC are robust across different MHA manufacturers
	  ○ Different MHA (agar dilution) or CAMHB (microbroth dilution) manufacturers were evaluated for MIC determination in the presence of 0.1 mM PIH.
	  ○ Agar dilution MIC were robust across the 3 manufacturers tested (EA ≥ 97 %), while microbroth dilution MIC were not robust.
	• Development of rifabutin MIC testing device in the presence of PIH
	  ○ Rifabutin agar dilution panels supplemented with 0.1 mM PIH, and rifabutin MIC test strips supplemented with PIH are in development at Liofilchem.
	  ○ Preliminary results suggest that PIH is compatible with the development of commercial rifabutin MIC testing devices, such as agar dilution panels and MIC test strips.
	• Summary/Conclusion
	  ○ Rifabutin demonstrates potent and specific *in vitro* activity against the Gram-negative pathogen *A. baumannii* when tested in iron-limited medium.
	  ○ Potent *in vitro* activity in iron-limited medium is due to active uptake of rifabutin by the *A. baumannii* siderophore receptor FhuE.
	  ○ Iron-limited conditions are required to determine MIC that are predictive of rifabutin efficacy *in vivo*.
	  ○ Agar dilution MIC in the presence of the non-toxic iron chelator PIH is required to determine unambiguous rifabutin MIC.
	  ○ Agar dilution MIC in the presence of 0.1 mM PIH is robust across the MHA manufacturers.
	  ○ PIH is compatible with the development of commercial rifabutin MIC testing devices.
	  ○ Proposing that the reference method for rifabutin susceptibility testing against *Acinetobacter baumannii* be an agar dilution MIC method using Mueller Hinton agar medium supplemented with 0.1 mM of the iron chelator.
	• MDSWG Discussion and Recommendation
	  ○ Does this method work with other iron chelators? Yes.
	  ○ Did cefiderocol reference method work? NO – iron depletion alone is not sufficient. Chelation is important.
	  ○ Why not just use RPMI Not standard media. Some strains did not grow in RPMI without serum.

- Does regular reference agar dilution work? Need PIH chelation to separate WT from NWT
- Where else is AD used as the primary reference method?
  - Fosfomycin
  - Anaerobes
  - *Neisseria gonorrhoeae*
- Motion to support the agar dilution + PIH method for rifabutin testing of *Acinetobacter baumannii* as the reference method for Tier 1 and Tier 2 QC studies. WG Vote: 11-0-0-2.

SC DISCUSSION (MAIN POINTS)

- Does PIH inhibit growth of any strains? Does the amount of residual iron need to be quantified before or after chelation?
  - The working group heard that PIH is non-toxic during the sponsor presentation. The working group does not know if the residual iron needs to be quantified after chelation.
  - The QC does not work if there is too much iron.
- Important to give the sponsor concrete advice. Need to know what level of iron is acceptable in the media. It would be good to see the assay work with different media brands.
  - Action Item: MDSWG will go back to the sponsor to clarify the level of residual iron that is acceptable.
- Are there resistant strains and do they correlate with *in vitro*/*in vivo*?
  - The resistant strains do not have the active siderophore.
- The QCWG looked at 3 QC strains but does not remember if the sponsor commented on iron. The sponsor did test multiple brands for media, but it is not clear if they tested multiple brands of PIH.
- It is not great to have two different iron chelation methods in CLSI. Is there a chance that PIH could work for cefiderocol?
  - Shionogi did look a chelator, but found iron depleted media works better; however, they do not remember if Shionogi specifically tried PIH.
- EUCAST strongly discourages media modifications and has heard multiple concerns about multiple methods.
- Maybe this drug can only be tested in reference lab, not a clinical lab.
- If these modifications to the reference method need to be made, need to take this seriously.
- How does chelation vs. depletion matter? CLSI would like to know from the sponsor.
- The sponsor will want to know if they can move forward with tier 1 and 2 studies.
  - That is a sponsor risk question. If the sponsor feels they have enough data that the outcome of the method will not change, then they can more forward concurrently; however, it appears to CLSI that the details are not worked out here. There is potential for modifications.
  - The sponsor should not proceed with tier 1 and 2 studies.
  - Our timeline might not meet the sponsor's timeline. Suggestion to move this off cycle and help review data more.
  - From the perspective of the sponsor, CLSI needs to be as clear on the guidelines as possible. Given EUCAST's hesitance to adopt new methods, a conversation should be had with the sponsor that EUCAST might not accept the method. Need specify what outcomes CLSI would or would not accept.
  - CLSI is cautiously optimistic pending further data. The sponsor can move forward, but it would be with some risk. CLSI is happy to arrange off cycle discussion/meetings before the June meeting to help keep this project moving forward and not hold it up for the June meeting. CLSI can engage the QCWG and EUCAST to discuss modified media. Note that EUCAST has not discussed this topic yet. They are very reluctant to accept a method change, and they will need very solid data.

A motion to support the agar dilution + PIH method for rifabutin testing of *Acinetobacter baumannii* as the reference method for Tier 1 and Tier 2 QC studies with approval of this specific method contingent on CLSI receiving further information that satisfies the Subcommittee concerns was made and seconded. Vote: 12 for, 2 against, 0 abstain, 0 absent (Pass)

Against Vote Reasoning:

- Method not ready yet to vote on.
- Wants to wait to see data in June 2024.

CEFIDEROCOL AD HOC WORKING GROUP REPORT

- Goals
  - 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.
  - Ease of reading and guidance for reading, especially with *Acinetobacter* (skips, trailing) and *P. aeruginosa* (skips, trailing). How to read the haze and trailing was a focus.
- Cefiderocol Powder Comparison
  - 145 CRE isolates from the SENTRY Antimicrobial Surveillance Program
  - Broth microdilution reference testing
  - Cefiderocol from Shionogi compared to Cefiderocol from Med Chem Express (MCE)
  - Testing performed with different inoculum and different days
  - No repeats performed for discrepancies
- Revised M100 Appendix H3 wording and pictures. "How to read" will be same between CLSI and EUCAST.

Step	Action	Comments
1.	Ensure the growth-control well demonstrates adequate growth in the form of a button of approximately ≥ 2 mm, or heavy turbidity.	Viewing devices intended to facilitate reading microdilution tests and recording results may be used as long as there is no compromise in the ability to discern growth in the wells.
1.	Compare the amount of growth in the wells containing cefiderocol with the amount of growth in the growth-control well containing ID-CAMHB (no antimicrobial agent). Read the MIC as the lowest concentration of cefiderocol (first clear well) where there is no trailing (button ≤ 1 mm) or light haziness observed. See Figures H1-H3. If reduced growth is observed, read the MIC as the lowest concentration of cefiderocol in which the reduction of growth compared to the growth-control well corresponds to: • a button of approximately ≤ 1mm (see Figure H2) or • a light haze or faint turbidity with a significant (eg 80%) reduction of growth compared to the growth-control well (see Figure H3)	Trailing growth can make endpoint determination difficult. Trailing occurs most frequently with Acinetobacter spp. and Pseudomonas aeruginosa. The laboratory may wish to perform repeat testing on isolates where trailing makes it difficult to determine an end point, especially if reduced growth is followed by an increase in growth at higher concentration. See Figure H2-C.
1.	Interpret the results.	Refer to the appropriate portion of Tables 2 for breakpoints.

- Future Activities: Continue work on reproducible manufacture of AST plates (other variables?) and appropriate QC

IDENTIFICATION OF QC ISOLATES TO ASSESS IRON-DEPLETED CATION-ADJUSTED MUELLER-HINTON BROTH (ID-CAMHB)

- Objective
  - Testing of cefiderocol requires the use of iron-depleted cation adjusted Mueller-Hinton broth
  - Current CLSI QC isolates for cefiderocol unable to assess iron depletion of media
    - MIC values of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 the same in ID-CAMHB and CAMHB
  - Identify QC isolates that can assess iron depletion of media needed for cefiderocol testing
    - Identify isolate(s) that show different MIC values in ID-CAMHB and CAMHB
- QC Strains Unable to Assess Iron Depletion of Media
  - Isolates do not show considerable MIC differences between ID-CAMHB and CAMHB
  - If MIC differences between CAMHB and ID-CAMHB are observed, MIC values with CAMHB still fall within QC range defined for ID-CAMHB
- Identification of Possible Additional QC Candidate Strains
  - 138 strains were evaluated
    - Isolates from CDC AR Bank (n=52), ATCC (n=30), *in vivo* efficacy (n=23), and surveillance studies (n=32)
  - MIC determinations were performed in iron-depleted cation adjusted Mueller-Hinton Broth (ID-CAMHB) and CAMHB using three brands of media (with multiple lots)
    - BD BBL (3 lots), BD Difco (3 lots), and Oxoid (2 lots)
    - Three different inoculum (total 9 or 6 replicates per media source)

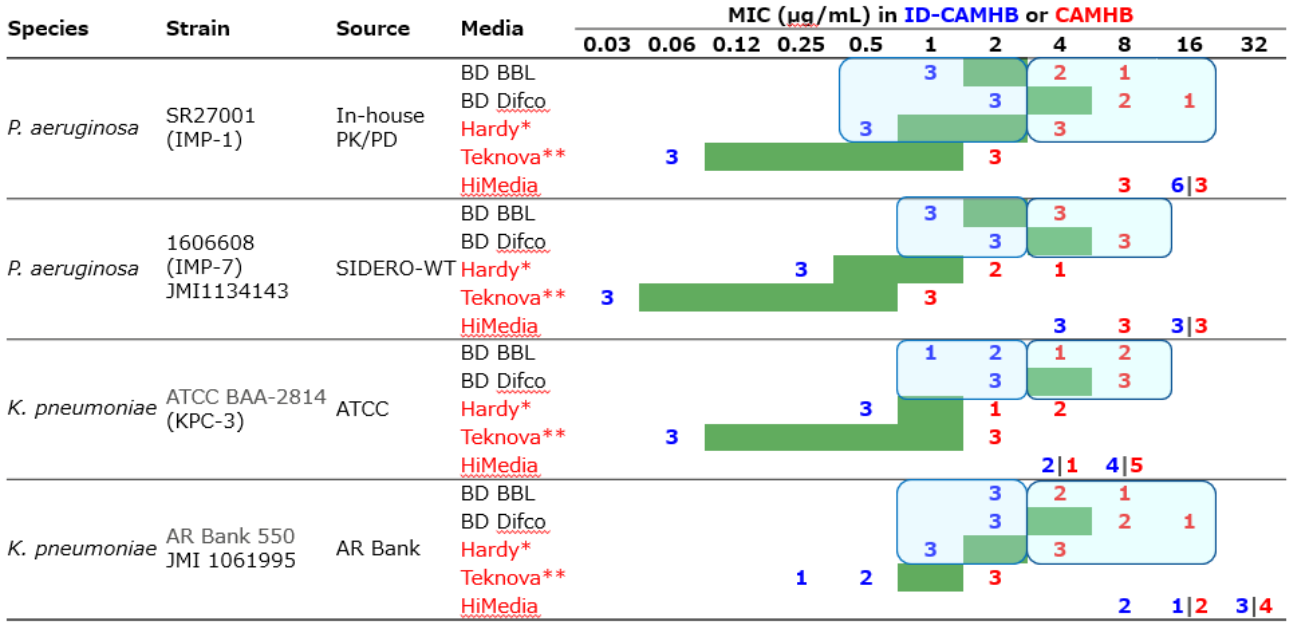
- Media iron content for all iron-depleted media confirmed as ≤0.03 mg/L
- Seven strains showed reproducible MIC differences between ID-CAMHB and CAMHB
- Four were further evaluated by JMI in five different media
  - CAMHB and ID-CAMHB from BD BBL, BD Difco, Hardy, HiMedia and Teknova
  - Triplicate from one inoculum

- Identification of Possible QC Candidate Strains
  - Seven isolates identified with MIC differences between ID-CAMHB and CAMHB for each individual media; two isolates showed good separation across three different media sources

Species	Strain No.	Source	Media	MIC (µg/mL) in ID-CAMHB or CAMHB								
				0.25	0.5	1	2	4	8	16	32	64
P. aeruginosa	SR27001 (IMP-1)	In-house PK/PD	BD BBL			4	5		9			
			BD Difco				7	2	1	8		
			Oxoid					5	1		6	
P. aeruginosa	1606608 (IMP-7)	SIDERO-WT	BD BBL		9				9			
			BD Difco			4	5		2	7		
			Oxoid					6		4	2	
P. aeruginosa	1260396 (IMP-7)	SIDERO-WT	BD BBL			7	2		9			
			BD Difco				4	5		7	2	
			Oxoid					1	5		6	
K. pneumoniae	VA-361 (KPC-2)	In-house PK/PD	BD BBL				8	1		9		
			BD Difco				1	7	1	7	2	
			Oxoid						6		6	
K. pneumoniae	VA-384 (KPC-2)	In-house PK/PD	BD BBL				9			9		
			BD Difco					7	2		9	
			Oxoid						5	1	2	4
K. pneumoniae	ATCC BAA-2814 (KPC-3)	ATCC	BD BBL			2	7		9			
			BD Difco				1	8	3	6		
			Oxoid					2	4	6		
K. pneumoniae	AR Bank 550	AR Bank	BD BBL				7	2		9		
			BD Difco					6	3	3	6	
			Oxoid						3|1	3|2	2	

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

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



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

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

- Summary
  - Several strains were identified that showed reproducible discrepant MIC values between ID-CAMHB and CAMHB prepared from different media sources
  - No isolate could be identified that showed discrepant MIC values between ID-CAMHB and CAMHB across all sources of media
    - Most isolates only show discrepant MIC values between ID-CAMHB and CAMHB for two media sources
  - Several isolates could be QC strain candidates to assess iron-depletion, but *K. pneumoniae* ATCC BAA-2814 would be preferred as this isolate is already used as QC isolate for other agents
- Proposal
  - Evaluate *P. aeruginosa* SR27001, *P. aeruginosa* 1606608, *P. aeruginosa* 1260396, *K. pneumoniae* VA-361 and *K. pneumoniae* ATCC BAA-2814 in CAMHB and ID-CAMHB prepared with BD-BBL, BD Difco and Oxoid media in M23 Quality Control Testing Tier 1 Study
    - 20 replicates (4 separate inoculums over 5 days) in one laboratory
  - Select one or two candidates for M23 Quality Control Testing Tier 2 Study
    - 10 replicates (individual inoculum, maximum 4 per day) in seven laboratories
    - CAMHB and ID-CAMHB prepared with BD-BBL, BD Difco and Oxoid media
  - Preliminary data indicate that it may be difficult to identify an isolate that shows discrepant MIC values between ID-CAMHB and CAMHB across all three sources of media

        - Most isolates show discrepant MIC values between ID-CAMHB and CAMHB for only two media sources
    - Would the working group accept a QC isolate based on results obtained with two media?
- MDSWG Discussion and Recommendation
    - Are their issues for MDSWG approval when 2/3 media are made by the same manufacturer (BD Difco and BD BBL)?
        - BD media are two different recipes/processes.
    - New modified Hardy media could also be explored.
    - Decision to be made after MDSWG sees study results.

SC DISCUSSION (MAIN POINTS)

- There was a powder comparison study for CRE where no difference was observed; however, they were looking at the QC results and they do look different. It is hard to comprehend how these two things can be true.
    - Clarification: This is one media and two different cefiderocol powders.
- Is there any discussion about how often this additional QC needs to be performed?
    - The working group did not have those discussions.
    - If this is to quality control the media and manufacturer, this could be as a sanity check for new lot or shipment.
- What was the original MIC performance? What media was used when the breakpoints were set? There is concern there is a lot of wiggle room around the breakpoint. Do we anchor around the existing breakpoint, or do we need to go back and look at our breakpoints?
- Should this be done on two media? Do not think it is acceptable to say, "I just want to see more data", it is not fair to the sponsor to ask for more data without planning to use that.
    - CLSI M23 says three lots and three manufacturers for media. Footnotes have been added before that a certain method has been developed on limited data and limited media.
    - Need to look at what media was used to set the breakpoint.
- Have a media variability issue. It would be easier to endorse Difco media. Can CLSI look at endorsing a specific media for this agent?
- Can FDA help investigate the media? All the media are supposed to be the exact same media.
    - FDA : This is a problem and what would be best is to name a specific media. If this is something CLSI wants to do.
- Do we want testing materials such that it gives us an MIC in that lower range? Thinking answer is no because that is not anchored on where the breakpoint is set.
- Should testing materials be consistent with where the breakpoint is? Yes, would accept two different media only.
- How to communicate this to the end user?
    - Need to look at our rules and see what is needed to address the media variation for this specific drug.
    - CLSI needs to be careful about writing off manufacturers based on one lot. The dehydrated beef could vary by cow type and hours of dehydration. This could be lot to lot variability.
- How do the AST voting members feel about the sponsor's question about if two media (from the same manufacturer) are acceptable?
    - There is concern how to communicate to the end user. Can we come up with other idea such as referencing a specific publication.
    - CLSI can make a statement about "established with limited manufacturers". Maybe say here is a QC that needs to work to be in line with how the breakpoints are set.
    - Overall, it sounds like willing to accept two kinds of media.
    - Must have no iron, this is very sensitive method. There is concern about QCing that level of detail for that right now.
- Would adding an extra media solve this?

- No, but the QC is not the answer either.
- Is NIST able to help?
- If two media work and there is a QC strain that works, maybe that is good enough.
- Perhaps it is not having the right strain for QC. Maybe need a better QC organism. Need a bigger MIC gap between the QC organisms because right now it is possible for them to overlap when considering plus or minus a doubling dilution for biological variability.
- Maybe measure zinc and heavy metals in some studies because the heavy metals are everywhere in water and all reagents, so the metal levels are all over the place in different reagents.
- Go back to the anchor, can we go back and say if the QC result is not behaving as wanted (eg, getting a mode that is off the target mode) then the test is not working. Should define the target QC mode.
- Does this have to be a standard range? Maybe define something else for the QC range? Define what the MIC mode should be.
- It is stated that iron content should be less than 0.03. State to confirm it. It is cleaner to define iron content.
  - FDA stated to tighten up a certain iron range before manufacturers put out the product.
  - The manufacturers confirm the iron in all these media is always less than 0.03, so there's some issue other than iron here. Also, it's hard to be accurate and define iron below 0.03.
  - Look at factors other than iron.
- CLSI is willing to accept two media; however, there are a lot of caveats and concerns. There is president to accept 2 media and the M23 has laid out a way to do this. CLSI is concerned about calling out specific media lots.
- There is going to be an overlap in the QC organism MIC range, so there is no reason the sponsor should spend the effort and the money to keep going with these QC stains. There is agreement that the QC strains here are not particularly helpful.
- Final message to the sponsor: CLSI does not encourage the sponsor to move forward with these organisms and QC strains because there is not enough separation.

PROPOSAL FOR AN AST METHOD FOR ZOSURABALPIN (RG6006)

- Zorsurabalpin Background
  - Belongs to a novel chemical class with a novel mechanism of action
  - Has a narrow spectrum of activity restricted to *Acinetobacter* species
  - No intrinsically resistant populations of *Acinetobacter* spp. have been identified to date: active against difficult-to-treat *A. baumannii*
  - Bactericidal with excellent activity in murine neutropenic thigh and pneumonia models
  - Currently in Phase I clinical studies
- The standard method is not well suited for zosurabalpin
  - MIC determinations for zosurabalpin is affected by aberrant readings (trailing, multiple skipped wells) in CAMHB making unambiguous MIC determination difficult.
  - Per study: ~50% of isolates are affected by aberrant readings, 5-10% are very difficult to read.
  - Own experience and feedback from multiple CROs: MIC determination in CAMHB is problematic.
- Summary of broth MIC method exploration for zosurabalpin
  - Roche investigated several conditions by testing methodologies problematic strains to assess the clarity of MIC reading.
  - Different testing media: CAMBH (including diluted CAMHB), Iron-depleted CAMHB, Lysogeny broth (LB), Tryptone soy broth (TSB), Roswell Park Memorial Institute (RPM) medium, Brain-heart infusion (BHI), Colombia medium + sheep blood, Brucella medium + sheep blood, hemin, and Vitamin K1

	○ Different additives to CAMHB ○ 20% horse serum supplementation reliably addresses interpretation issues for zosurabalpin MIC • Integration of feedback from CLSI and EUCAST ○ Refinement of the reference method for CAMHB ▪ Read no later than 20h post inoculation ▪ Read using the "substantial reduction of growth" criterion ▪ Sealing with adhesive film was considered as a tested condition given it provided marginal improvement ○ Considerations for CAMHB + horse serum ▪ Provide the recommendation for horse serum supplementation as a range ▪ Evaluate the impact of horse serum addition on *A. baumannii* growth • Tier 1: Parameter variation experimental setup ○ Media: CAMHB + 0%, 10% or 20% horse serum ○ 3 QC candidate strains of *A. baumannii* ▪ NCTC 13304 (no trailing) ▪ ROB07643 (Roche, trailer) ▪ ROB00867 (clinical isolate, trailer) ○ Assessed the parameters below for each strain to select most suitable QC strain. Each condition tested in triplicate. ○ *P. aeruginosa* ATCC 27853 to be used for meropenem to validate the study.

Time of incubation: 16h, 20h, 48h

Incubation environment:

- Plates covered with either hard cover or adhesive plastic film
- in ambient atmosphere, in anaerobic or microaerophilic conditions, in presence of 5% CO_2

Temperature variations: 30°C, 35°C and 42°C

Inoculum effects: 5×10^4, 5×10^5 and 5×10^6 CFU/mL

pH effects: 6.0, 7.2 -7.4, 8.0

Horse serum variation: 7 different suppliers, reference (Gibco) including heat-inactivated for the reference serum

CAMHB manufacturer variation: BD BBL, Oxoid (Thermo Fisher) and BD Difco

Divalent cation concentrations:

- Ca^{2+} (mg/L): <5, 25, 50
- Mg^{2+} (mg/L): <5, 12.5, 25

Fresh versus frozen and aged media (>7 days)

Addition of polysorbate-80 (Tween-80): 0.002% final concentration added to wells, and 0.004% added to compound diluent

Reference testing conditions are highlighted.

- Tier 2 broth parameter variation study summary
  - No systemic impact of any parameter (including medium manufacturers) on any of the tested methods
  - Clear endpoints were observed for all tested conditions for CAMHB + 10% horse serum and CAMHB + 20% horse serum
  - Addition of serum does not compromise method robustness and improved reproducibility
- Results
  - MIC in CAMHB read at substantial reduction can be highly variable
  - Disk zones correlate better to MIC determined with serum
- Summary and Proposal
  - Data show that supplementation of CAMHB with 10% or 20% horse serum results in:
    - Clear endpoint readings for all tested isolates
    - A reproducible method that is robust to parameter variation
    - Good correlations with disk diffusion zone of inhibition
  - Proposed zosurabalpin broth AST method: CAMHB + 10-20% (range) heat-inactivated horse serum at 100% inhibition
- MDSWG Discussion and Recommendation

- Any difficulties reading disks?
  - Must read outer zone
  - Reading guide required
- Should a range of horse serum be recommended versus a specific concentration?
  - Minimal differences in performance between 10% and 20% horse serum
  - One concentration of horse serum makes standardization more straightforward
  - 10% horse serum is less costly
  - Roche has done more testing and has more data using 20% horse serum
- Motion to accept zosurabalpin broth AST method of CAMHB + 10% heat-inactivated horse serum at 100% inhibition. WG Vote: 5-6-0-2.
- Motion to accept zosurabalpin broth AST method of CAMHB + 20% heat-inactivated horse serum at 100% inhibition. WG Vote: 11-0-0-2.

SC DISCUSSION (MAIN POINTS)

- Was 10% to 20% horse serum discussed? Why pick 20%?
  - When looking at the disk correlates most of the data was performed with 20%, so wanted to define a number that had data for disk correlates as well.
- How many manufactures/lots of horse serum were used? Answer: 7 lots/manufacturers
- How does this method compare with other compounds that use horse serum? Is there an opportunity to be consistent with existing methods to minimize the different number of reference methods?
  - The existing CLSI reference method has 25% horse serum but also DTT. The DTT does not work well with this compound. The 25% horse serum would not be a problem, but the DTT is.

A motion to accept the CAMHB + 20% heat inactivated horse serum read at 100% inhibition method for zosurabalpin (RG6006) broth MIC testing was made and seconded. Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

COAGULASE NEGATIVE *STAPHYLOCOCCUS* AD HOC WORKING GROUP REPORT

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

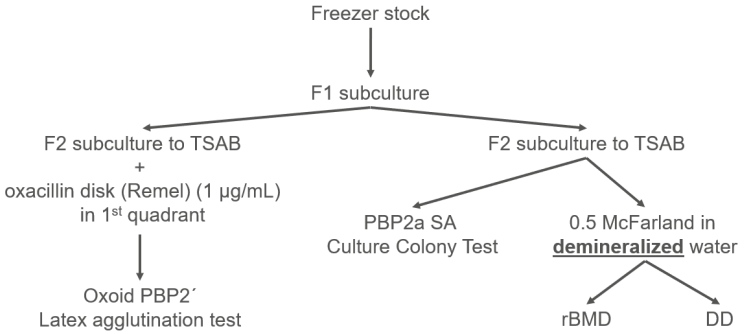
Year	Species	Recommendation	Reference
1986	All staphylococci	Publication of methicillin, nafcillin, and oxacillin MIC and DD susceptibility criteria in M100, first informational supplement	NCCLS, 1986
1999	SoSA	Establishment of oxacillin MIC and DD breakpoints in M100 that are different than those for *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 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*

- Methods
  - Reference method: mecA/C PCR
  - Reference broth microdilution (rBMD): frozen-form Sensititre custom panels:
    - Three different Cation-Adjusted Mueller-Hinton broth (CA-MHB) manufacturers:
      - Difco
      - BBL
      - Oxoid
    - Cefoxitin (Thermo Fisher Scientific) range: 0.015-32 µg/mL (read between 16-20 h)
    - Oxacillin (Toku-E) + 2% (w/v) NaCl range: 0.015-32 µg/mL (read at 24 h)
  - Disk diffusion (DD; read oxacillin between 16-18 h; read cefoxitin at 24 h):
    - Three different Muller-Hinton agar (MHA) manufacturers:
      - Becton, Dickinson and Company (BD)
      - Hardy Diagnostics
      - Remel (Thermo Fisher Scientific)
    - Three different cefoxitin (30 µg) and oxacillin (1 µg) disk manufacturers:
      - BD

- Hardy Diagnostics
- Remel (Thermo Fisher Scientific)

  - PBP2a immunoassays:
    - Oxoid PBP2´ Latex Agglutination Test (Thermo Fisher Scientific)
    - Clearview PBP2a SA Culture Colony Test (lateral flow immunoassay) (Abbott)

- Workflow



- *Staphylococcus saprophyticus* isolates included in the study

Countries of origin	Number of isolates	Number of *mecA*-negative isolates	Number of *mecA*-positive isolates
Belgium	1	1	0
Brazil	3	3	0
Canada	6	1	5
Hungary	1	0	1
Israel	2	1	1
Kuwait	1	1	0
Netherlands	1	1	0
Nigeria	1	0	1
Philippines	2	2	0
Portugal	1	1	0
Russia	2	1	1
South Africa	1	1	0
USA	30	15	15
Venezuela	1	0	1
Total	**53**	**28**	**25**

- CLSI breakpoints evaluated in this study

Breakpoint description	Susceptible breakpoint	Resistant breakpoint	Abbreviation
Oxacillin MIC, *S. aureus*/*S. lugdunensis*	≤2 µg/mL	≥4 µg/mL	OX MIC SA/SL
Oxacillin MIC, *Staphylococcus* species other than *S. aureus*/*S. lugdunensis*	≤0.5 µg/mL	≥1 µg/mL	OXA MIC STAPH
Oxacillin zone diameter, *S. epidermidis*, *S. pseudintermedius*, *S. schleiferi*	≥18 mm	≤17 mm	OX DD SE/SP/SS
Cefoxitin MIC, *S. aureus*/*S. lugdunensis*	≤4 µg/mL	≥8 µg/mL	FOX MIC SA/SL
Cefoxitin zone diameter, *S. aureus*/*S. lugdunensis*	≥22 mm	≤21 mm	FOX DD SA/SL
Cefoxitin zone diameter, *Staphylococcus* species other than *S. aureus*, *S. lugdunensis*, *S. pseudintermedius*, *S. Schleiferi*	≥25 mm	≤24 mm	FOX DD STAPH

- Overview of Results (12 mecA-negative isolates, 12 mecA-positive isolates)

Method	Antimicrobial	Breakpoint	Susceptible	Resistant	Categorical agreement (%)	Very major error (%)	Major error (%)
rBMD	Oxacillin	OX MIC SA/SL	≤2 µg/mL	≥4 µg/mL	76.4 (55 results/72 results)	38.9 (14 results/36 results)	8.3 (3 results/36 results)
rBMD	Oxacillin	OX MIC STAPH	≤0.5 µg/mL	≥1 µg/mL	87.5 (63 results/72 results)	13.9 (5 results/36 results)	11.1 (4 results/36 results)
DD	Oxacillin	OX DD SE/SP/SS	≥18 mm	≤17 mm	68.1 (147 results/216 results)	10.2 (11 results/108 results)	53.7 (58 results/108 results)
rBMD	Cefoxitin	FOX MIC SA/SL	≤4 µg/mL	≥8 µg/mL	62.5 (45 results/72 results)	69.4 (25 results/36 results)	5.6 (2 results/36 results)
DD	Cefoxitin	FOX DD SA/SL	≥22 mm	≤21 mm	74.5 (161 results/216 results)	47.2 (51 results/108 results)	3.7 (4 results/108 results)
DD	Cefoxitin	FOX DD STAPH	≥25 mm	≤24 mm	81.5 (176 results/216 results)	24.1 (26 results/108 results)	13.0 (14 results/108 results)

  - Categorical agreement = number of categorical result matches/total number of results
  - Very major error = number of very major error results/total number of resistant results by reference method
  - Major error rate = number of major error results/total number of susceptible results by reference method
- PBP2a Assays

FDA approved for *S. aureus* and SoSA		Oxoid PBP2´ Latex Agglutination Test	
		Negative	Positive
***mecA* PCR (gold standard)**	Negative	11 (TN)	1 (FP)
	Positive	3 (FN)	9 (TP)

- Sensitivity, 75% (95% confidence interval [CI] 42.8-94.5%)
- Specificity, 91.7% (95% CI 61.5-99.8%)

FDA approved for *S. aureus* only		PBP2a SA Culture Colony Test	
		Negative	Positive
***mecA* PCR (gold standard)**	Negative	7 (TN)	5 (FP)
	Positive	1 (FN)	11 (TP)

- Sensitivity, 94.7% (95% CI 61.5-99.8%)
- Specificity, 58.3% (95% CI 27.7-84.8%)

False-positive results with PBP2a SA culture colony test is unusual

- Conclusion and Next Steps
  - Based on these preliminary data, no single method/CLSI breakpoint appears to accurately differentiate between mecA-negative and mecA-positive *S. saprophyticus* isolates
  - Assay the remaining isolates using 0.5 McFarland made with demineralized water for both rBMD and DD
  - Confirm mecA status of isolates
  - Analyze data using CLSI and EUCAST breakpoints
  - Repeat testing with 0.85% (w/v) saline 0.5 McFarland for both rBMD and DD if needed
  - Present all data in June 2024 at next CLSI AST subcommittee meeting
- MDSWG Discussion and Recommendation
  - What method was used for mecA PCR?
    - In-house developed/validated method.
    - Repeats to use the same method and possibly a second commercial method (BCID?)
  - Were testing variations or other zone diameters considered?
    - Not yet.
  - Saline versus demineralized water for inoculum preparation
    - Group suggested to test using saline on subset of isolates to make sure there is no difference.

SC DISCUSSION (MAIN POINTS)

- There is no single CLSI method/breakpoint to accurately differentiate between mecA negative and mecA positive *S. saprophyticus* isolates.
  - This is a small study number; it would be difficult to make conclusions based on this data alone. More isolates need to be tested.
- Is the other mechanism for resistance to cefoxitin or oxacillin other than mecA in this *S. saprophyticus*?
  - Need to go back to the mecA lab developed PCR used and better understand it.

DIRECT BLOOD DISK DIFFUSION AD HOC WORKING GROUP REPORT

- Goals
  - Define disk diffusion breakpoints for applicable gram-negative rods direct from positive blood culture bottle broth
  - 16-18 hour (overnight reads) and 8-10 hour (early reads)
  - Review data from:
    - Direct Susceptibility Testing of Gram-negative Rods from Blood Cultures (ARLG DISK Study)
    - Seeded isolate testing (performed Fall 2020 to Spring 2021)
- Progress as of December 2023

	Enterobacterales 8-10h	Enterobacterales 16-18h	PA 8-10h	PA 16-18h	*Acinetobacter* 8-10h	*Acinetobacter* 16-18h
Ampicillin	AST SC approved new breakpoints 2/2022	AST SC approved current breakpoints 6/2020	N/A	N/A	N/A	N/A
Amp-sul	Unable to set breakpoints	Unable to set breakpoints	N/A	N/A	*Ad hoc* WG approved ---- breakpoints 12/2023	*Ad hoc* WG approved ---- breakpoints 12/2023
Aztreonam	AST SC approved current breakpoints 2/2021	AST SC approved current breakpoints 6/2020	Unable to set breakpoints	Unable to set breakpoints	N/A	N/A
Cefepime	*Ad hoc* WG approved new breakpoints (same as 16-18h direct) 11/2023	*Ad hoc* WG approved new breakpoints (same as 8-10h direct) 11/2023	Unable to set breakpoints 8/2023	AST SC approved current breakpoints 1/2023	AST SC approved current breakpoints 6/2023	AST SC approved current breakpoints 6/2023
Ceftazidime	AST SC approved current breakpoints 2/2021	AST SC approved current breakpoints 6/2020	Unable to set breakpoints 8/2023 but will present to AST SC	AST SC approved current breakpoints 6/2021	*Ad hoc* WG approved adoption of 16-18h breakpoints 8/2023	AST SC approved new breakpoints 6/2023
Ceftriaxone	AST SC approved current breakpoints 2/2021	AST SC approved current breakpoints 6/2020	N/A	N/A	AST SC approved current breakpoints 6/2023	AST SC approved new breakpoints 6/2023
Ciprofloxacin	AST SC approved new breakpoints 2/2022	AST SC approved new breakpoints 2/2022	AST SC approved new breakpoints 6/2021	AST SC approved current breakpoints 2/2021	AST SC approved current breakpoints 6/2023	AST SC approved current breakpoints 6/2023
Ertapenem	Unable to set breakpoints	Unable to set breakpoints	N/A	N/A	N/A	N/A
Meropenem	AST SC approved new breakpoints 2/2022	AST SC approved new breakpoints 2/2022	AST SC approved current breakpoints 2/2022	AST SC approved current breakpoints 2/2021	AST SC approved current breakpoints 6/2023	AST SC approved current breakpoints 6/2023
Pip-tazo	Unable to set breakpoints	Unable to set breakpoints	Unable to set breakpoints	Unable to set breakpoints	*Ad hoc* WG approved new breakpoints (same as 16-18h direct) 8/2023	*Ad hoc* WG approved new breakpoints (same as 8-10h direct) 8/2023
Tobramycin	AST SC approved current breakpoints 6/2023	AST SC approved current breakpoints 6/2023	AST SC approved current breakpoints 6/2023	AST SC approved current breakpoints 6/2023	AST SC approved current breakpoints 6/2023	AST SC approved current breakpoints 6/2023
Trimeth-sul	Unable to set breakpoints	AST SC approved current breakpoints 6/2020	N/A	N/A	AST SC approved current breakpoints 6/2023	AST SC approved current breakpoints 6/2023

Blue = recently voted on and passed by *Ad hoc* WG
Gray font = passed by AST SC
N/A = not applicable

- Comparison of Disk to Disk
  - Both disk and MIC results for Direct DISK study
  - After much discussion Winter 2021, MDSWG voted to compare direct DISK results to standard DD at the study site (STD DD SITE)-primary comparison
    - Secondary comparison would be DISK results to REF DD (performed at reference lab)
  - Discussed and agreed at AST Subcommittee Winter 2021
- Testing Procedure Comparison

DISK Study

1. Set up disk diffusion testing within 8 h of flagging positive
2. Four drops of blood culture broth (from a venting needle) applied to two Mueller-Hinton agar (MHA) plates
3. Subculture of the blood broth inoculated to blood agar plate
4. Plates incubated at 35°C in ambient air
5. Plates read at 8-10h
6. Plates read again at 16-18h
7. Standard disk diffusion was performed on isolated colonies at the study site (Std DD Site)
8. Isolates were shipped to reference lab for broth microdilution (MIC) and DD (Ref DD)

Seeded Study

1. Set up disk diffusion testing within 8 h of flagging positive
2. Four drops of blood culture broth (from a venting needle) applied to two Mueller-Hinton agar (MHA) plates
3. Subculture of the blood broth inoculated to blood agar plate
4. Plates incubated at 35°C in ambient air
5. Plates read at 8-10h
6. Plates read again at 16-18h
7. Standard disk diffusion was performed on isolated colonies at the study site (Std DD Site)
8. Isolates were shipped to reference lab for broth microdilution (MIC). No Ref DD performed.

- Seeded Study
  - Undertaken due to limited number of certain isolates in DISK study
  - Completed testing
    - 50 additional *P. aeruginosa*
    - 100 *Acinetobacter*
  - With much appreciation to BD, Accelerate, and ARLG

CEFEPIME FOR ENTEROBACTERALES

- Standard breakpoints (mm)

Standard breakpoints (mm)		
S	SDD	R
≥25	19-24	≤18

- Proposed breakpoints (mm)

Proposed breakpoints (mm)		
S	SDD	R
≥23	19-22	≤18

- Cefepime 8-10h vs. Std DD Enterobacterales for Proposed Zone Cutoffs

	Std DD			
8-10 hr	S	SDD	R	Grand Total
S	312	1		313
SDD	16	5		21
R	3	8	27	38
Grand Total	331	14	27	372

CA	344/372	92.7%
VME	0/27	0
ME	3/331	<1%
mE	25/372	6.7%

- Cefepime 8-10h vs. REF DD Enterobacterales for Proposed Zone Cutoffs

	REF DD			
8-10 hr	S	SDD	R	Grand Total
S	311	1		312
SDD	15	6		21
R	2	6	30	38
Grand Total	328	13	30	371

CA	347/371	93.5%
VME	0/30	0
ME	2/328	<1%
mE	22/371	5.9%

- Cefepime 16-18h vs. Std DD Enterobacterales for Proposed Zone Cutoffs

	Std DD			
16-18 hr	S	SDD	R	Grand Total
S	326	1		327
SDD	7	6		13
R		8	27	35
Grand Total	333	15	27	375

CA	359/375	95.7%
VME	0/27	0
ME	0/333	0
mE	16/375	4.3%

- Cefepime 16-18h vs. REF DD Enterobacterales for Proposed Zone Cutoffs

	REF DD			
16-18 hr	S	SDD	R	Grand Total
S	325	1		326
SDD	5	8		13
R		5	30	35
Grand Total	330	14	30	374

CA	363/374	97.0%
VME	0/30	0
ME	0/330	0
mE	11/374	2.9%

- MDSWG Discussion and Recommendation
  - Motion to accept the cefepime direct blood disk breakpoints for Enterobacterales (S≥23, I 19-22, R≤18 mm) for an 8-10h and 16-18h reading time. WG Vote: 9-1-1-2.
  - No vote: disagreement with CLSI cefepime breakpoints

SC DISCUSSION (MAIN POINTS)

- What is the difference between in reference vs. standard disk diffusion?
  - Disk diffusion is no longer a reference method; however, it is the reference used for this study.
  - This is terminology used for this specific study. "Reference disk diffusion" is testing performed at a reference lab for the study and "standard disk diffusion" is tested at a non-reference lab (the local study site lab).

A motion to accept the cefepime direct blood disk breakpoints for Enterobacterales (S≥23, I 19-22, R≤18 mm) for an 8-10h and 16-18h reading time was made and seconded. Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

CEFTAZIDIME FOR *ACINETOBACTER*

- Standard breakpoints (mm)

Standard breakpoints (mm)		
S	I	R
≥18	15-17	≤14

- Proposed breakpoints (mm)

Proposed breakpoints (mm)*		
S	I	R
≥17	15-16	≤14

*Match ceftazidime 16-18h read BPs approved by AST SC 6/2023

- Ceftazidime 8-10h vs. Std DD *Acinetobacter* for Proposed Zone Cutoffs

	Std DD			
8-10 hr	S	I	R	Grand Total
S	82	5		87
I				
R	1		19	20
Grand Total	83	5	19	107

CA	101/107	94.4%
VME	0/19	0
ME	1/83	1.2%
mE	5/107	4.7%

- MDSWG Discussion and Recommendation
  - Motion to accept the ceftazidime direct blood disk breakpoints for *Acinetobacter* (S≥17, I 15-16, R≤14 mm) for an 8-10h reading time. WG Vote: 10-0-1-2.

A motion to accept the ceftazidime direct blood disk breakpoints for *Acinetobacter* (S≥17, I 15-16, R≤14 mm) for an 8-10h reading time was made and seconded. Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

PIPERACILLIN-TAZOBACTAM FOR *ACINETOBACTER*

- Standard breakpoints (mm)

Standard breakpoints (mm)		
S	I	R
≥21	18-20	≤17

- Proposed breakpoints (mm)

Proposed breakpoints (mm)		
S	I	R
≥19	17-18	≤16

- Piperacillin-tazobactam 8-10h vs. Std DD *Acinetobacter* for Proposed Zone Cutoffs

	Std DD			
8-10 hr	S	I	R	Grand Total
S	73	7		80
I	2	4		6
R			20	20
Grand Total	75	11	20	106

CA	97/106	91.5%
VME	0/20	0
ME	0/75	0
mE	9/106	8.5%

- Piperacillin-tazobactam 16-18h vs. Std DD *Acinetobacter* for Proposed Zone Cutoffs

	Std DD			
16-18 hr	S	I	R	Grand Total
S	75	8		83
I		3		3
R			20	20
Grand Total	75	11	20	106

CA	98/106	92.4%
VME	0/20	0
ME	0/75	0
mE	8/106	7.5%

- MDSWG Discussion and Recommendation
  - Motion to accept the piperacillin-tazobactam direct blood disk breakpoints for *Acinetobacter* (S≥19, I 17-18, R≤16 mm) for an 8-10h and 16-18h reading time. WG Vote: 9-0-1-3.

A motion to accept the piperacillin-tazobactam direct blood disk breakpoints for *Acinetobacter* (S≥19, I 17-18, R≤16 mm) for an 8-10h and 16-18h reading time was made and seconded. Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

CEFTAZIDIME FOR *P. AERUGINOSA*

- Standard breakpoints (mm)

Standard breakpoints (mm)		
S	I	R
≥18	15-17	≤14

- 16-18h reads already approved previously using standard breakpoints
- Ceftazidime 8-10h vs. Std DD *P. aeruginosa* for Standard Zone Cutoffs

	Std DD			
8-10 hr	S	I	R	Grand Total
S	45			45
I	6	1	2	9
R		1	23	24
Grand Total	51	2	25	78

CA	69/78	88.5%
VME	0/25	0
ME	0/51	0
mE	9/78	11.5%

- Ceftazidime 8-10h vs. REF DD *P. aeruginosa* for Standard Zone Cutoffs

	REF DD			
8-10 hr	S	I	R	Grand Total
S	35			35
I	2			2
R			3	3
Grand Total	37		3	40

CA	38/40	95%
VME	0/3	0
ME	0/37	0
mE	2/40	5%

- MDSWG Discussion and Recommendation
  - Was not approved in AHWG since CA was below acceptance criteria.
  - Motion to accept the ceftazidime direct blood disk breakpoints for *P. aeruginosa* (S≥18, I 15-17, R≤14 mm) for an 8-10h reading time. WG Vote: 2-6-3-2.
  - No votes: 16-18h read available and CA below acceptance.
  - Motion to accept the ceftazidime direct blood disk breakpoints for *P. aeruginosa* (S≥18 and R≤14 mm) for an 8-10h reading time. WG Vote: 11-0-0-2.

SC DISCUSSION (MAIN POINTS)

- There are issues are around the intermediate but the susceptible and resistant data look good. So, can read the resistant or susceptible result at this 8-10hr reads and if it is intermediate, put it back in the incubator and read it at the later for the 16-18hr read.

A motion to accept the ceftazidime direct blood disk breakpoints for *P. aeruginosa* (S≥18, I 15-17, R≤14 mm) for an 8-10h reading time with a footnote that the I zone size would need to be reincubated and read at 16-18h was made and seconded. Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

RANGEFINDER FOR EARLY QC READS

- Ampicillin, Ampicillin-sulbactam, Ertapenem, Trimethoprim-sulfamethoxazole
- QC
  - Used standard inoculum and procedure for QC organisms
    - Read at 8-10h
  - Prior WG decisions
    - 10/2021: Direct Blood Culture DD Ad hoc WG approved RangeFinder for early QC ranges listed below
      - *E. coli* 25922 ciprofloxacin RangeFinder = 29-38 mm (matches CLSI std QC range)
      - *E. coli* 25922 tobramycin RangeFinder = 18-25 mm (CLSI std QC range 18-26 mm)
    - 1/2022: Methods Development and Standardization WG reviewed ciprofloxacin and tobramycin early QC ranges
    - 8/2023: Direct Blood Culture DD Ad hoc WG approved CLSI std QC ranges for early QC ranges listed below
      - *E. coli* 25922 ampicillin and trimethoprim-sulfamethoxazole
      - *E. coli* 35218 ampicillin-sulbactam
      - *P. aeruginosa* 27853 ertapenem
- Early Read QC Summary – CLSI vs RangeFinder

Antimicrobial Agent	QC	Current CLSI		RangeFinder	
		Range (mm)	% in range	Range (mm)	% in range
Ampicillin	*E. coli* 25922	15-22	96.3	14-21	96.8
Ampicillin-sulbactam	*E. coli* 35218	13-19	97.7	12-19	97.7
Ciprofloxacin	*E. coli* 25922	**29-38**	**98.7**	**29-38**	**98.7**
Ertapenem	*P. aeruginosa* 27853	13-21	96.5	12-21	97.2
Tobramycin	*E. coli* 25922	18-26	98.9	18-25	98.9
Trimethoprim-sulfamethoxazole	*E. coli* 25922	**23-29**	**98.5**	**23-29**	**98.5**

Green denotes the same ranges.

- MDSWG Discussion and Recommendation
  - Motion to accept the current CLSI ranges for early reads. WG Vote: 11-0-0-2.

SC DISCUSSION (MAIN POINTS)

- This is extra optional QC. Labs are not required to do this. The standard QC of the disk and materials is sufficient to confirm the quality of the materials, so standard QC is enough. For those who want an early read QC, we are providing an optional early read QC.

A motion to accept the current CLSI QC ranges for *E. coli* 25922 ampicillin (15-22 mm), *E. coli* 35218 ampicillin-sulbactam (13-19 mm), *P. aeruginosa* 27853 ertapenem (13-21 mm), and *E. coli* 25922 trimethoprim-sulfamethoxazole (23-29 mm) for direct blood disk early reads was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

DIRECT DISK EARLY READ QC EDITS

- Proposed edits

Test	Direct Disk Diffusion
QC recommendations - routine	• Perform QC according to the standard disk diffusion QC procedures per M02[1] (eg, daily or weekly).
NAME TITLE TBD	• Ranges have been established for early reading (8-10 hr) of select QC strain/antimicrobial agent combinations as shown below. This testing is performed using a 0.5 McFarland standardized inoculum (standard disk diffusion QC procedures per M02). • This testing may be used to train staff or assess competency for early reading but is not necessary for routine daily or weekly AST QC.

Antimicrobial Agent	Disk Content	Optional Early Read (8-10 hr) Ranges, mm		
		E. coli ATCC® 25922	*P. aeruginosa* ATCC® 27853	*E. coli* ATCC® 35218
Ampicillin	10 µg	XX-XX	-	-
Ampicillin-sulbactam	10/10 µg	-	-	XX-XX
Ciprofloxacin	5 µg	XX-XX	-	-
Ertapenem	10 µg	-	XX-XX	-
Tobramycin	10 µg	XX-XX	-	-
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	XX-XX	-	-

- MDSWG Discussion and Recommendation
  - Motion to accept the proposed direct blood disk early read QC table edits. WG Vote: 11-0-0-2.

SC DISCUSSION (MAIN POINTS)

- Can the wording on “daily or weekly QC” be removed to say “routine QC”?
  - To discuss offline with TTWG.
- Do we need to republish standard QC or just refer to them somewhere else?
  - Want to make sure this is a stand-alone method and keep this method separate.

A motion to accept the proposed direct blood disk early read QC table edits with wordsmithing the “daily or weekly QC” was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

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

STAPHYLOCOCCUS CONTENT

- SOSA = *Staphylococcus* other than *S. aureus*
  - Terminology now used in upcoming editions of M02/M07
  - TTWG agreed to use SOSA
  - Placement and use throughout TBD for June
- Consider use of *S. aureus* complex
  - *S. aureus*, *S. argenteus*, *S. schweitzeri*, *S. roterodami*, *S. singaporensis*
  - TTWG agreed not to use *S. aureus* complex throughout at this time
    - Except for *S. aureus* and *S. argenteus*, methods for susceptibility testing of the other complex members are not well-described
    - Will revise the general comment 3 in Table 2C Staphylococcus to help address likelihood ongoing addition of species to the complex
  - No objections from the Subcommittee on moving forward with this item
- Table 2C. *Staphylococcus* spp.

General Comments

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

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see 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, except for linezolid, which should be read with transmitted light (plate held up to light source). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter. For linezolid, any discernible growth within the zone of inhibition is indicative of resistance to the respective agent.

(3) *S. aureus* complex consists of the coagulase-positive species *S. aureus*, *Staphylococcus argenteus*, and *Staphylococcus schweitzeri*. 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]

TTWG will wordsmith this comment for June 2024.

- Update of *S. schleiferi* naming
  - Also coordinating with VET Staphylococcus AHWG liaison
  - *S. schleiferi* subsp. *coagulans* to *S. coagulans*
  - *S. schleiferi* subsp. *schleiferi* to *S. schleiferi*
  - EUCAST expanded to include both *S. schleiferi* and *S. coagulans*
  - TTWG will make the changes to relevant locations (eg, table in comment (6) and in Table 2C)
  - No objections from the Subcommittee on moving forward with this item
- Mupirocin testing clarity
  - Include a footnote attached to *S. aureus*
  - Indicating that this is for surveillance screening/ decolonization procedures
  - Not appropriate for all sources

- Sulfisoxazole comment (Staph and other sections)

Location	Comment	Proposed Changes
Table 2C Comment (24)	Is this comment still true?	Evaluate if retain or remove this comment

  - Comment exists in Tables 2A, 2B-5, 2C next to Sulfonamide row
  - Drug (alone) also listed in Table 2I *N. meningitidis* , for prophylaxis
  - QC Table 5A-1, Table 5B

SC DISCUSSION (MAIN POINTS)

- Does EUCAST know anything about sulfisoxazole availability outside of the US ?
  - No, EUCAST does not know who is using this drug.
  - Action item: Check if anyone uses sulfisoxazole.

PLACEMENT OF PROCEDURES WITH MODIFICATIONS TO THE REFERENCE METHOD

- Appendix I. Cefiderocol Broth Preparation and Reading Microdilution Minimal Inhibitory Concentration End Points
  - Originally used this Appendix as a temporary placeholder until cefiderocol could be added to M07
- Exebacase Procedure and Reading Instructions
  - Exebacase content in footnotes of Table 5A-1, 6A
- Decision from the TTWG: Single Appendix for non-standard methods
  - Combined into 1 common Appendix for modified methods (e.g. Appendix I-1, I-2, etc)
  - Mockup the Appendix for June

TABLE 3F-1 DIRECT DISK EARLY READ QC

- Added a new row to contain the early read zone diameters using proposed ranges

PROPOSED EDITS FOR REDUCED SUSCEPTIBILITY LANGUAGE

Supplemental Tests (Optional)

Supplemental Test	Organisms	Test Description	Optional for:	Table Locations
ESBL	• *E. coli* • *K. pneumoniae* • *Klebsiella oxytoca* • *Proteus mirabilis*	Broth microdilution or disk diffusion clavulanate inhibition test for ESBLs	Isolates meeting the criteria for testing as defined in Table 3A ~~demonstrating reduced susceptibility to cephalosporins~~ ~~Results that indicate presence or absence of ESBLs~~	3A
CarbaNP	• Enterobacterales • *P. aeruginosa*	Colorimetric assay for detecting carbapenem hydrolysis	Isolates that are not susceptible to one or more carbapenems ~~Results that indicate presence or absence of certain carbapenemases~~	3B
mCIM with or without eCIM	• mCIM only: Enterobacterales and *P. aeruginosa* • mCIM with eCIM: Enterobacterales only	Disk diffusion for detecting carbapenem hydrolysis (inactivation) eCIM add-on enables differentiation of metallo-β-lactamases from serine	Isolates that are not susceptible to one or more carbapenems ~~Results that indicate presence or absence of certain carbapenemases~~	3C

- These edits will take into consideration newly proposed language around carbapenemase testing presented by BPWG at this meeting or in June (if not finalized this meeting)

DEFERRED COMMENTS FOR OTHER WORKING GROUPS

Comments	Working Group
• Replace "*Parabacteroides* spp. only" with "associated genera" in various locations Note: presence of β-lactamases have been reported in this genus.	Anaerobe AHWG
• Should sulbactam-durlobactam be added to Appendix A for *Acinetobacter baumannii* complex as a Category I agent?	MAIWG
• Would suggest removing cefepime from the example of the *K. pneumoniae* tier reporting and just have the carbapenems listed as the example for that organism.	Table 1 AHWG
• May want to discuss adding a comment "Breakpoints may be applied to non-typhoidal Salmonella sp from urine if systemic infection has been ruled out". • Should it be suggested that "I" for this be considered as "SDD" (next round)? *S. pneumoniae* and nonmeningitis penicillin • Dosage request for *S. pneumoniae* and cefotaxime/ ceftriaxone. Current comment says "meningitis requires therapy with maximum dose"	BPWG
• Proposed changes to the Reference Method vs Commercial Method box	Reference Method AHWG

CONTENT REVIEW FOR M02/M07/M11/M100

- Concerns
  - Many laboratories read CLSI AST documents very carefully and may end up having to read the same content (message) appearing in several places
  - Even slight differences in format/wording may lead to confusion
  - Inadvertent editing errors may occur, resulting in differing content with same message
  - When there is a change in M100 in a year when M02/M07 is not updated, some M02/M07 content may be out of date leading to use of obsolete recommendations
- Brief History of M02/M07/M100
  - 1975- M02, 1980- M07 Standards documents
    - Indications for performing testing, Selecting agents for testing , Reagents, Procedure, Quality control
    - Table 1 (same as today), Table 2 (same as today), Table 3 (Control limits for monitoring precision and accuracy)
  - 1986- M100 Supplemental introduced (starting in 1997 published annually)
    - Contains the Tables formerly in M02/M07
    - Some additional information (QC, solvents, and diluents for stock solutions, preparing dilutions for agar and broth dilution tests, modifications of standard MIC methods for testing fastidious bacteria)
  - 1989-2003, tables loosely inserted into either M2 or M7 respectively
  - 2004 – present, M100 in separate bound document
- Guidance:
  - What content belongs in each document?
  - Need for overlap? If so, what?
  - Determine if any modifications in consolidation of content would be beneficial?
  - Consideration by SC to form an AHWG to:
    - Re-evaluate the core content necessary across documents (prior charges to the AHWG were tasked to 'update' the current content)
    - Identify improvements in how information is provided to users
    - Harmonize content, where necessary
  - This is particularly relevant when considering the different review cycle timelines: M100 – yearly; M02/M07/M11 – every 3-5 years

SC DISCUSSION (MAIN POINTS)

- Will there still be a printable version with the new Edaptive tables?
  - Yes, a printable version will be available.
- CLSI is in favor of forming a new AHWG to address the duplicate information and the Edaptive editing platform. CLSI will defer to TTWG's timeline/process for when they want to start an AHWG.
- Historically, there was no place for comments, so should M02 and M07 just be procedural on how to perform the test. It is better to have labs go to one document rather than two.
- What is the goal of the AHWG? Is it to inform the Subcommittee?

	○ It is to inform the Subcommittee that we are removing content from M02 and M07. For example, there is not a need to have D testing in multiple documents. The goal is to remove duplicate information. The standards documents have how to perform testing, and the M100 has what to test.

6. **M45 WORKING GROUP (T. SIMNER)**

PROCESS FOR SETTING M45 "BREAKPOINTS"

- Literature review on MIC distributions, PK-PD, antimicrobial resistance mechanisms, cases studies/series and clinical outcomes
- Accumulate MIC data from publications and reference laboratories
    - Prioritize reference methods
    - Evaluate all data including all non-reference method data
- Run data through ECOFF Finder
- Create histograms with MIC data and compare to current M45 breakpoints, breakpoints from related organisms, any PK-PD/clinical data (rarely available) and EUCAST non-species-specific PK-PD breakpoints
- Complete template
- Update/create M45 tables
- Provide next steps for future M45 updates
- Transparency about data utilized to set "breakpoints" with follow-up publications on MIC distributions/ posting on the CLSI website

CRITERIA FOR M100 VS M45

	Data Required	Available for M45	Available for M100
Breakpoint	• ECV • Non clinical PK-PD cutoff • Clinical exposure-response cutoff • Clinical cutoff	No	Yes
ECV	• Collecting & merging data from a range of sources to define the upper-limit of the WT distribution • Need to use a recognized reference method • Data from ≥ 3 labs • MICs should be on scale	Maybe (but usually "No")	Yes
MIC distribution data with or without a reference method	• Data from one or more laboratories • Data may be generated using non reference MIC methods (e.g., lyophilized MIC panels) or using a non-standard method (e.g. *Capnocytophaga* species)	Yes	Not applicable; as ECVs usually available

ORGANISM-SPECIFIC AREAS FOR EVALUATION

Table	Potential revisions/needs
Table 2. *Aerococcus*	Growth failures with current method; add disk diffusion breakpoints
Table 3. *Aeromonas*	FQ failures / low level resistance (update breakpoint?); mCIM testing to detect *cphA* as carbapenem breakpoint low already
Table 3. *Bacillus* spp.	Assess impact of adding related genera in last edition; Address penicillin resistance-revisited *B. anthracis* breakpoints
Table 5. *Campylobacter jejuni/coli*	Look at other species, add a meropenem breakpoint and disk correlates
Table 6. *Corynebacterium* spp.	Revisit penicillin BP with aerotolerant *Actinomyces*
Table 7. *Gemella* spp.	Add other catalase negative GPC; Study to evaluate adding daptomycin and linezolid
Table 9. HACEK	Assess differences with EUCAST Impact of testing methods added in last edition
Table 10. *Helicobacter pylori*	Assess differences with EUCAST; Time for breakpoints?
Table 12. *Lactococcus* spp.	Add doxycycline; Add a comment about endocarditis with penicillin → apply viridans strep breakpoints despite essentially placing all MICs in the intermediate category
Table 13. Leuconostoc	Add linezolid and daptomycin breakpoints; consider adding *Weisella* spp
Table 15. *Micrococcus* spp.	Test nitrocefin & penicillin; Separate out *Kocuria* spp?
Table 16. *Moraxella catarrhalis*	Expand to *Moraxella* spp.
Table 17. *Pasteurella* spp.	Re-evaluate disk correlates

NEW ORGANISMS

Table	Additions
Capnocytophaga species	ARUP data using custom lyophilized sensititre panel, BHI + LHB, 35°C, elevated CO_2, 24-120h incubation. Consider recommending β-lactamase test at minimum to laboratories as media may be difficult for laboratories to obtain
Non-*aeruginosa Pseudomonas*	Perform a BMD study to define MIC distribution, define intrinsic resistance, disk-to-MIC, evaluate gradient diffusion & mCIM (include CRO subset); evaluate FQ breakpoints
Achromobacter species	Perform a BMD study to define MIC distribution, define intrinsic resistance, disk-to-MIC, evaluate gradient diffusion & mCIM (include CRO subset); evaluate FQ breakpoints
~~Non-Enterobacterales~~	~~Move to M45 – At minimum, the non-Enterobacterales tables should be reviewed to potentially align with the updated *Enterobacterales/P. aeruginosa* breakpoints.~~

- Will not pursue non-influenzae/parainfluenzae *Haemophilus* with this edition

COMPLETED STUDIES

Panels	# of panels	Location of Panels	Organisms	# of Isolates	Study	# of testing sites	# of panels for testing	# of panels for QC
GNB CAMHB panel (IHMA)	550	JHU	*Achromobacter* species	100	Disk-to-MIC & GD & mCIM, include CRO subset; FQ	1	200	10
			Non-*aeruginosa Pseudomonas*	100	Disk-to-MIC & GD & mCIM, include CRO subset; FQ*	1	200	10
			Aeromonas species	50	Disk-to-MIC & mCIM, FQ	1	100	20
LHB panel (IHMA)	450	VUMC	*Aerococcus* species	100	Disk-to-MIC	1	200	20
			Pasteurella spcies	50	Disk-to-MIC	1	100	10
			Gamella species & other catalase negative GPC	?	Add linez/dapto BPs	1		
			Leuconostoc species	?	Add linez/dapto BPs	1		
			Weisella species	?		1		
GP CAMHB (Thermo)	350	VUMC	*Micrococcus* species	?	Test nitrocefin & penicillin	1		
			Kocuria, Dermacoccus,	?	Eval as alternative media type?	1		
			Kytococcus, etc	100	Eval as alternative media type?	1		
			Aerococcus speices	50		1		
			Pasteurella species					

TABLE 1: *ABIOTROPHIA* AND *GRANULICATELLA* SPP.

- Will remove reference to the old terminology of "Nutritionally Deficient Streptococci"
- Confirmed pyridoxal content is correct in M45
- Proposed changes:
  - Proposal to increase the penicillin BP from S:0.12/0.25-2/4 to S:2/R:4
  - ECV: 2 ug/ml
  - Passed BPWG and AST SC vote
- Add linezolid BP of S: ≤2 ug/ml
- Daptomycin MIC distribution similar to enterococci – decided to not add a BP
  - Add a comment about higher MICs with daptomycin in supplemental testing information
- Updates to supplemental information section
  - Discuss differences in beta-lactam susceptibility
  - The need to speciate for identification

- Do not use chocolate agar

TABLE 2: *AEROCOCCUS* SPP.

- Performed a disk correlate study
  - Grows better on solid media (BMHA) than broth (CAMHB); differences observed between media
  - Disk correlates – issue with disks likely due to disk content
  - Add penicillin, ciprofloxacin, levofloxacin, nitrofurantoin, tetracycline and vancomycin disk correlates
- Penicillin breakpoints
  - Proposal to increase the penicillin BP to S:2/I:4/R: 8 with associated disk correlate
- Add ampicillin breakpoint to reflect updated penicillin breakpoint to S only ≤2 ug/ml
  - Ampicillin disk correlate with 2µg disk looks good
  - Alternative: No disk for ampicillin and infer amoxicillin from ampicillin similar to EUCAST
- Cefotaxime, ceftriaxone, meropenem – same MIC BP but no disk BP
  - Test via a reliable MIC method
- Nitrofurantoin
  - Update breakpoint to S:≤32 ug/ml, R: ≥64 ug/ml
- Tetracycline alters intermediate range to be in line with M23 guidance
- Remove TMP-SMX BP altogether

TABLE NEW: *ACHROMOBACTER* SPP.

- Contemporary data reviewed
- Intrinsic resistance to aminoglycosides, cephalosporins and aztreonam
- A disk correlate study was completed by testing 92 *Achromobacter* species isolates obtained from 2 U.S. centers and the CDC by comparing reference broth microdilution results to disk diffusion results
- Proposed breakpoints are in Table 1
  - SDD – Match dosing to M100
- BPs for ceftazidime, fluroquinolones and minocycline were not defined due to a high proportion of isolates with elevated MICs

TABLE 4: *BACILLUS* SPP.

- Added related genera including, *Brevibacillus*, *Lysinibacillus* and *Paenibacillus*
- Breakpoint Changes:
  - Remove ampicillin/penicillin breakpoint as most isolates test resistant
  - Remove gentamicin and amikacin in line with *Staphylococcus* species removal
  - Clindamycin:
    - ECV: 1ug/ml
    - MIC distribution suggests: 1/2/4
    - Update breakpoint
  - Meropenem breakpoint set at S:≤4, I:8, R≥16 based on 2012 M100 *Staphylococcus* spp BP
    - Discussions to evaluate PK data and likely lower in line with gram-negative meropenem BPs/EUCAST

- Keep breakpoints as currently for levofloxacin, rifampin, TMP-SMX, vancomycin, erythromycin, ciprofloxacin
- Add comment at a footnote right below the table about *B. cereus biovar anthracis* and *Bacillus tropicus*

TABLE 6: *CORYNEBACTERIUM* SPP.

- Proposal to increase the penicillin BP from S:≤0.12/R:≥0.25 to S:2/I:4/R:8 with a dosing comment
  - ECV: 0.12 ug/ml
- Ceftriaxone distributions to be evaluate by species, discussions about removal; EUCAST does not have a BP
- TMP-SMX – *C. diphtheriae* and *C. ulcerans* looks good; evaluate by species
  - EUCAST has BPs for *C. diphtheriae*/*C. ulcerans* only
- Emergence of resistance to daptomycin while on therapy – BP to be removed and add comment to testing notes
- Quinipristin-dalfopristin and gentamicin BPs to be removed
- Keep breakpoints as currently for erythromycin, ciprofloxacin, clindamycin, linezolid, vancomycin, rifampin, tetracycline, doxycycline, meropenem

TABLE 10: *HELICOBACTER PYLORI*

- Currently only have a clarithromycin BP
- Add the following breakpoints:
  - Metronidazole: S:8/I:16/R:32
    - Add comment that *in vitro* resistance is not an absolute predictor of eradication failure
  - Levofloxacin: S:1/I:2/R:4
    - Follow-up about QC with QCWG
  - Amoxicillin: S only: ≤0.125
- Decided to hold off on setting BPs for tetracycline and rifampin/rifabutin
  - Review again with 5th Edition of M45

TABLE 11: *LACTOBACILLUS* SPP.

- Update based on nomenclature changes; split into 25 genera
  - Review vancomycin intrinsic resistance comment relative to new genera
- Proposal to update penicillin/ampicillin breakpoints
  - Current BP S only: ≤8
  - Update to 2/4/8 to align with other organisms
    - Passed BPWG and AST SC vote
- Update daptomycin from S only: ≤8 to 2/4/8 to align with *Enterococcus* spp
- Linezolid – S only: 2 ug/ml –align with *Pediococcus*/*Leuconostoc* and PK/PD demonstrating 2ug/ml is the highest MIC that can be achieved
- No need to change any other breakpoints based on review of the data

TABLE 12: *LACTOCOCCUS* SPP.

- Penicillin breakpoint
  - Current :1/2/4

  - ECV: 2 ug/ml
  - Proposed: 2/4/8
    - Passed BPWG and AST SC vote
- Ampicillin no new data –align with penicillin
- Doxycycline
  - ECV: 1
  - Change BP to S: 2/I:4/R:8 based on contemporary MIC data

TABLE 13: *LEUCONOSTOC* SPP.

- Reviewing data to see if *Weisella* spp. can be added
- Update penicillin breakpoint from S: 8ug/ml to S: 4 ug/ml
  - Passed BPWG and AST SC vote
- Clindamycin (listed as an alternative treatment option) set new breakpoint of S:0.5/I:1/R:2
- Linezolid - Wild type for linezolid exceeds MICs typically achievable by PK/PD ; remove breakpoint and add comment

TABLE 14: *LISTERIA MONOCYTOGENES*

- No updates to breakpoints required
- Testing notes and references were updated

TABLE 15: *MICROCOCCUS* SPP.

- Revisit penicillin BP
  - ECV: 0.5ug/ml
  - No *blaZ* detected in a contemporary set of >150 *Micrococcus* isolates
  - Current breakpoint: S: ≤0.12/R: ≥0.25
  - Update to 2/4/8
    - Passed BPWG and AST SC vote
- Add daptomycin BP
  - ECV: 1 ug/ml
  - Add BP of S:1 ug/ml based on contemporary MIC distribution data and aligns with *Staphylococcus* breakpoint
- Add trimethoprim-sulfamethoxazole BP
  - ECV: 1ug/ml
  - Proposed BP: S:2/R:4
- Doxycycline
  - ECV: 1 ug/ml
  - MIC distribution suggests S:1/I:2/R:4 but *Staphylococcus* BP at S:4/I:8/R:16
  - Decided to use the lower breakpoint based on MIC distribution and limited PK-PD and clinical outcomes data
- *Ketococcus*/*Kocuria* behave differently than Micrococcus spp and was decided not to include in this table. A comment will be added.

TABLE 17: *PASTEURELLA* SPP.

- Contemporary data reviewed
  - No changes to current MIC BPs
  - Ceftriaxone non-susceptible isolates encountered
- Disk correlate study performed
  - Ampicillin disk correlate completed with 2 µg disk content
  - Disk correlates to be updated for amoxicillin-clavulanate, penicillin and trimethoprim-sulfamethoxazole
- CA-MHB evaluated as an alternative media type

Antimicrobial agent	Current susceptible disk diffusion BP (mm)	Proposed susceptible disk diffusion BP (mm)
Amoxicillin-clavulanate	≥27	≥21
Penicillin	≥25	≥23
Ampicillin	≥27	No update
Ceftriaxone	≥34	No update
Levofloxacin	≥28	No update
Tetracycline	≥23	No update
Trimethoprim-sulfamethoxazole	≥24	≥17

TABLE 18: *PEDIOCOCCUS* SPP.

- Reviewed penicillin distribution data
  - ECV: 1 ug/ml
  - Current BP: 8 ug/ml -> no treatment failures
  - Lower breakpoint to S:2/I:4/R:8
    - Passed BPWG and AST SC vote
- Add new breakpoints for daptomycin, linezolid and levofloxacin
- Up for discussion:
  - Clindamycin: -> Ask for feedback from the subcommittee
    - ECV: 0.12 ug/ml
    - Pediatrics may use? Add breakpoint or not?
    - WG leaning toward not adding breakpoint
- Reviewed data but decided not to add breakpoints for ceftriaxone (MICs shifted right), meropenem (high MICs), doxycyline (high MICs), erythromycin (very low MICs; but no clinical use), gentamicin (used more as combination therapy; most MICs beyond PK-PD BP), rifampin (use is rare, hardware infections, use in combo), trimethoprim-sulfamethoxazole (decided not to add due to differences in the distributions)

TABLE NEW: *PSEUDOMONAS NON-AERUGINOSA*

- Disk correlate study performed
- Top 3 species

- *P. fluorescens* group, *P. putida* group, and *P. stutzeri*
- Align with *Pseudomonas* breakpoints
  - Match *Pseudomonas aeruginosa*
    - Disk zones widened to address M23 criteria compared to *P. aeruginosa*
  - Meropenem – set a different disk zone diameter as the distribution is different
- TMP-SMX, amikacin and minocycline were evaluated but not included
- Dosing comments to be added similar to *Pseudomonas aeruginosa*

Table 1. Proposed MIC and Disk Diffusion Breakpoints for Non-*aeruginosa Pseudomonas* species	MIC (µg/ml)			Disk Diffusion (mm)		
	S	I	R	S	I	R
Piperacillin-tazobactam	≤16/4	32/4	≥64/4	≥22	17-21	≤16
Ceftazidime	≤8	16	≥32	≥19	15-18	≤14
Cefepime	≤8	16	≥32	≥19	14-18	≤13
Imipenem	≤2	4	≥8	≥20	15-19	≤14
Meropenem	≤2	4	≥8	≥18	14-17	≤13
Tobramycin	≤1	2	≥4	≥21	15-20	≤14
Ciprofloxacin	≤0.5	1	≥2	≥25	19-24	≤18
Levofloxacin	≤1	2	≥4	≥22	15-21	≤14
S: susceptible, I: intermediate, R: resistant						

TABLE 19: *ROTHIA MUCILAGINOSA*

- Doxycycline
  - ECV: 1 ug/ml
  - WG discussed changing BP to S:1/I:2/R:4
  - Limited PK/PD and clinical data
- Reviewed daptomycin data
  - ECV: 4 ug/ml
  - Discussion to change to S:2/I:4/R:8 based on contemporary MIC data and align with *Enterococcus* species BP
  - Decided to not include breakpoint because off-label use and other treatment options available
  - Publish MIC distribution data

TABLE 20: *VIBRIO* SPP.

- Update and adopt M100 Enterobacterales breakpoints for third generation cephalosporins, fluoroquinolones, piperacillin-tazobactam and meropenem
- Update azithromycin breakpoint to align with EUCAST
- TMP-SMX: Keep BP as is. Not necessary to lower breakpoint to align with EUCAST

	• Doxycycline/Tetracycline:
	  ○ WG discussed changing BP to S:0.5/I:1/R:2 based on contemporary MIC distributions and aligns with EUCAST
	  ▪ Note: Cuts into distribution of *V. fluvialis*
	  ○ Limited PK/PD data and clinical outcome data support this
	  ○ Adopt EUCAST disk correlates for tetracycline
	  ○ Add comment that disk is unreliable for doxycycline
	
	FOLLOW-UP ITEMS
	• Penicillin and tetracycline breakpoint standardization presented to the Breakpoint Working Group
	• There will be no big changes of Tables being moved from the M100 to the M45 for this Edition of the document
	• After this edition, the AST subcommittee will create a checklist and define the M100 vs M45 criteria to make the changes moving forward in the 5th Edition
	• M45 WG working toward completing our updated tables by February 23rd, 2024
	
	SC DISCUSSION (MAIN POINTS)
	• Please include clindamycin for *Pediococcus*. The ECV is 0.12 and there are limited on oral options to treat this organism. Having clindamycin is helpful.
	• If there is no difference between *P. aeruginosa* and *P. non-aeruginosa* breakpoints, then why have two tables? EUCAST has one table.
	  ○ The difference is the data. There is no PK/PD data for *P. non-aeruginosa*.
	  ○ The M45 working group wants *P. aeruginosa* to be able to go into the M100 because there is enough data for that species.
	  ○ This will apply to all *P. non-aeruginosa* species.
	• Action Item: Please work with the anaerobe working group for *Lactobacillus*. They are working on clarifying information for labs around this organism.
	  ○ Is *Corynebacterium* moving to the *Actinomyces* group? Please work with anaerobic working group on this topic.
	• Need to work together between M100, M45, and the fungal documents for the definition of intrinsic resistance.
	• Were different manufacturers of media and disks used to set the disk correlates?
	  ○ No, multiple manufacturers were not looked at.
	• Is there data about the prevalence of *erm* genes in *Pediococcus*?
	  ○ There have been some *erm* genes detected, but it has all been described as constitutive. There is not any data to indicate inducible resistance at this time.

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

PLENARY ATTENDEES

Plenary 1	Plenary 2	Plenary 3
Abbott April	Abbott April	Abbott April
Abdul Azim Ahmed	Abdul Azim Ahmed	Abdul Azim Ahmed
Abdulrahman Marwah	Abdulrahman Marwah	Abdulrahman Marwah
Adams Jennifer K.	Abera Dawit	Abera Dawit
Alby Kevin	Adams Jennifer K.	Adams Jennifer K.
Anastasiou Diane	Alby Kevin	Alby Kevin
Antonara Stella	Anastasiou Diane	Anastasiou Diane
Arbefeville Sophie	Antonara Stella	Arbefeville Sophie
Asempa Tomefa	Arbefeville Sophie	Asempa Tomefa
Atkinson Dunn Robyn	Asempa Tomefa	Atkinson Dunn Robyn
Babcock Ken	Atkinson Dunn Robyn	Babcock Ken
Balbuena Rocio	Babcock Ken	Balbuena Rocio
Barnett Katie	Bala Shukal	Barnett Katie
Berger Jane	Balbuena Rocio	Berkow Elizabeth
Berkow Elizabeth	Barnett Katie	Bhatnagar Amelia
Bharat Amrita	Berger Jane	Bhavnani Sujata M.
Bhatnagar Amelia	Berkow Elizabeth	Bhowmick Tanaya
Bhavnani Sujata M.	Bharat Amrita	Black Kelley
Bhowmick Tanaya	Bhatnagar Amelia	Blosser Sara
Black Kelley	Bhavnani Sujata M.	Bobenchik April M.
Blosser Sara	Bhowmick Tanaya	Boswell Malcolm
Bobenchik April M.	Black Kelley	Bowden Robert
Boswell Malcolm	Blosser Sara	Boyer Jennifer
Bowden Robert	Bobenchik April M.	Bradford Patricia
Boyer Jennifer	Boswell Malcolm	Brandt Maryann
Bradford Patricia	Bowden Robert	Breton John
Brandt Maryann	Boyer Jennifer	Brown Carrine
Breton John	Bradford Patricia	Bryan, MD, PhD Andrew
Brown Carrine	Brandt Maryann	Bryant Kendall
Bryan, MD, PhD Andrew	Breton John	Bryowsky Jason
Bryant Kendall	Brown Carrine	Bryson Alexandra Lynn
Bryowsky Jason	Bryan, MD, PhD Andrew	Burbick Claire R.
Bryson Alexandra Lynn	Bryant Kendall	Burnham Carey-Ann
Burbick Claire R.	Bryowsky Jason	Bush Karen
Burnham Carey-Ann	Bryson Alexandra Lynn	Butler Deborah
Burwell Rebecca	Burbick Claire R.	Butler-Wu Susan
Bush Karen	Burnham Carey-Ann	Caidi Hayat
Butler Deborah	Bush Karen	Campbell Davina
Butler-Wu Susan	Butler Deborah	Campeau Shelley
Caidi Hayat	Butler-Wu Susan	Canton Rafael

Campbell Davina
Campeau Shelley
Canton Rafael
Capraro Gerald A.
Carpenter Darcie E.
Carvalhaes Cecilia
Castagna Katharine
Castanheira Mariana
Castillo-Martinez Nydia
Ceric Olgica
Chandler Courtney
Chantell Christina
Chaturvedi Sudha
CHEN YAMIN
Cicala Katherine
Cole Nicolynn
Cullen Sharon K.
D'Angelo Jessica
Danielsen Zhixia
Debabov Dmitri
DeDonder Keith
DeJonge Boudewijn
DeStefano Ian
Diaz-Campos Dubraska V.
Dien Bard Jennifer
Dingle Tanis
Donohue Lindsay
Drammeh Kaddijatou
Dressel Dana C.
Dumm Rebekah
Duncan Elaine
Dwivedi Hari P.
Edelstein Paul
Esparza German
Ewald-Saldana Gina L.
Ferrell Andrea L.
Fisher Mark A.
Flemming Laurie
Fratoni Andrew
Friedrich Lawrence V.
Fritz Heather
Fuller Jeff
Gaddah Latifa Hassan

Campbell Davina
Campeau Shelley
Canton Rafael
Capraro Gerald A.
Carpenter Darcie E.
Carvalhaes Cecilia
Castagna Katharine
Castanheira Mariana
Castillo-Martinez Nydia
Ceric Olgica
Chandler Courtney
Chantell Christina
Chaturvedi Sudha
CHEN YAMIN
Cicala Katherine
Cole Nicolynn
Cullen Sharon K.
D'Angelo Jessica
Debabov Dmitri
DeDonder Keith
DeJonge Boudewijn
DeStefano Ian
Diaz-Campos Dubraska V.
Dien Bard Jennifer
Dingle Tanis
Donohue Lindsay
Drammeh Kaddijatou
Dressel Dana C.
Dumm Rebekah
Duncan Elaine
Edelstein Paul
Esparza German
Ewald-Saldana Gina L.
Ferrell Andrea L.
Fisher Mark A.
Flemming Laurie
Fratoni Andrew
Friedrich Lawrence V.
Fritz Heather
Galas Marcelo F.
Gancarz Barb
Garg Rahul
Garner Cherilyn D.

Capraro Gerald A.
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Carvalhaes Cecilia
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Galas Marcelo F.
Gancarz Barb
Garg Rahul
Garner Cherilyn D.
Garrett Elizabeth
Giske Christian G.
Glaser Laurel
Glasgow Heather
Goldstein Beth P.
Gomez Emily J.

Galas Marcelo F.
Gancarz Barb
Garg Rahul
Garner Cherilyn D.
Garrett Elizabeth
Giffen Samantha
Giske Christian G.
Glaser Laurel
Glasgow Heather
Goldstein Beth P.
Gomez Emily J.
Grande Roche Kerian K.
Gray Alice
Gray Kamisha
Greninger Alex
Griffin Natasha
Gutierrez Carlos
Hackel Meredith
Haddock Christopher
Hamula Camille
Hawser Stephen
Hernandez Esther
Herrera Elide
Hilligoss Danielle
Hindler Janet A.
Hirsch Elizabeth
Hoffard Rita
Holliday Nicole
Holzknecht Barbara Juliane
Hsiung Andre
Humphries Romney M
Hunt Lauren
Huse Holly
Iarikov Dmitri
Jean Sophonie
Jimenez Antonieta
Johnson Kristie
Jorgensen James H.
Justo Julie Ann
Karlowsky James
Karlsson Asa
Karlsson Maria
Kaspar Heike

Garrett Elizabeth
Giffen Samantha
Giske Christian G.
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Kaspar Heike
Kersh Ellen N.
Khalid Haziq
Khan Ayesha
Killian Scott B.

Grande Roche Kerian K.
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Khan Ayesha
Killian Scott B.
Kirn Thomas J.
Klavins Anna
Koeth Laura M.
Kulwicki Amanda
Kuperus Amanda L.
Kuti Joseph
Lam Christine M.
LaVoie Stephen
Lee Sang

Kersh Ellen N.
Khalid Haziq
Khan Ayesha
Killian Scott B.
Kirn Thomas J.
Klavins Anna
Koeth Laura M.
Kulwicki Amanda
Kuperus Amanda L.
Kuti Joseph
Lam Christine M.
LaVoie Stephen
Lee Sang
Lemon Jamie
Leung Beth
Lewis James S.
Li Liang
Li Xian-Zhi
Liesman Rachael
Limbago Brandi
Lisboa Luiz
Litchfield Niki
Livesay Hannah
Lonsway David
Lozano Sergio
Luna Brian
Lutgring Joseph
Macedo Nubia
Malysa Michelle
Margadonna Robert
Martin Isabella
Master Ron
Mathers Amy J
Matuschek Erika
McCloskey Lynn
McCurdy Sandra
McLeod Sarah
Mendes Rod
Miller Jennifer
Miller Linda A.
Miller William
Min Sharon
Mindel Susan
Kirn Thomas J.
Klavins Anna
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Mendes Rod
Miller Jennifer
Miller Linda A.
Miller William
Min Sharon
Mindel Susan
Mirasol Ruel
Mitchell Stephanie L.
Mizusawa Masako
Mochon Brian
Lemon Jamie
Leppanen Sarah Blaine
Leung Beth
Lewis James S.
Li Liang
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Liesman Rachael
Limbago Brandi
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McLeod Sarah
Mendes Rod
Miller Jennifer
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Miller William
Min Sharon
Mindel Susan
Mirasol Ruel
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Mizusawa Masako
Mochon Brian
Moeck Greg
Moore Nicholas M.
Moore Sarah
Morales Yesenia
Morrissey Ian
Motyl Mary R.
Mudenda Timothy
Mwanamoonga Leocrisia
Myers Michelle
Narayanan Navaneeth

Mirasol Ruel
Mitchell Stephanie L.
Mizusawa Masako
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Moeck Greg
Moore Nicholas M.
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Morales Yesenia
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Mwanamoonga Leocrisia
Myers Michelle
Naccache Samia N.
Narayanan Navaneeth
Nigg Benjamin
North Michael
O'Rourke Susan
Ohkusu Kiyofumi
Orazi Giulia
Otima Evans
Otterson Linda G.
Oyarzun Sebastian Cifuentes
Palavecino Elizabeth
Panen Sherle
Patterson Logan
Perez Katherine
Pham Cau Dinh
Pierce Jessica
Pierce Virginia M.
Pillar Chris
Puttaswamy Sachidevi
Quinn Brigit
Rajeev Lara
Ramos Karl Anthony
Redell Mark A
Richter Sandra S.
Roberts Kristen
Rodriguez Veronica
Rossi Flavia
Rotunno Will
Ruhe Zachary
Sabour Sarah
Moeck Greg
Moore Nicholas M.
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Morales Yesenia
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Sabour Sarah
Satlin Michael
Scangarella-Oman Nicole
Scheetz Marc H.
Nigg Benjamin
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Quinn Brigit
Rajeev Lara
Ramos Karl Anthony
Re David
Redell Mark A
Rice Felicia
Richter Sandra S.
Roberts Kristen
Rodriguez Veronica
Rossi Flavia
Rotunno Will
Sabour Sarah
Satlin Michael
Scangarella-Oman Nicole
Scheetz Marc H.
Schneider Cynthia
Schuermeyer Linda
Schuetz Audrey N.
Selby Ashley
Seyedmousavi Amir
Shannon Samantha
Sharp Susan
Shawar Ribhi M.
She Rosemary
Sheehy Michael
Shier Kileen

Satlin Michael
Scangarella-Oman Nicole
Scheetz Marc H.
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Selby Ashley
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Sheehy Michael
Shier Kileen
Shurland Simone M
Silva Marissa
Simner Patricia J.
Slaughter Jennifer
Smart Jennifer
Snippes Vagnone Paula M.
Staats Dylan
Steenbergen Judith
Stone Gregory G.
Stuart Keira
Takemura Miki
Tamma Pranita D.
Tedesco John
Tekle Tsigereda
Tenllado Jolyn
Tesfa Tewodros
Thomson Susan
Thrupp Lauri D.
Trabold Peter
Trauner Andrej
Truman Lauren
Turng Ben
Usongo Valentine
Van Tam T.
Vercelli Cristina
Wang Yun F (Wayne)
Weingarten Rebecca
Weinstein Melvin P.
Weir Susan
Wenzler Eric

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Westblade Lars F.
Whitman Charles
Wikler Matthew A.

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Westblade Lars F.
Whitman Charles
Wikler Matthew A.
Worden Lacy
Yamano Yoshinori
Yamashiro Hidenori
Yan S. Steve
Yarbrough Melanie
Yee Cheung
Zimmer Barbara L.

Westblade Lars F.
Whitman Charles
Wikler Matthew A.
Worden Lacy
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Yan S. Steve
Yarbrough Melanie
Yee Cheung
Young Katherine
Zappas Kristie
Zimmer Barbara L.

Worden Lacy
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