Meeting Title:	**Subcommittee on Antimicrobial Susceptibility Testing (AST)**	**Contact:**	egomez@clsi.org
Meeting Location:	Orlando, Florida, USA		
Meeting Dates and Times: All times are Central (US) time.	**Plenary 1:** Monday, 23 January 2023, 7:30 AM – 12:00 PM **Plenary 2:** Monday, 23 January 2023, 1:00 – 5:00 PM **Plenary 3:** Tuesday, 24 January 2023, 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 (34th).		
Requested Attendee(s):	SC Chairholder, Vice-Chairholder, 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**
Jean B. Patel, PhD, D(ABMM) Expert Panel on Microbiology Chairholder	**Beckman Coulter, Inc.**
Members Present:	
Sharon K. Cullen, BS, RAC	Beckman Coulter, Inc. Microbiology Business
Tanis Dingle, PhD, D(ABMM), FCCM	Alberta Precision Laboratories
Marcelo F. Galas, BSc	Pan American Health Organization
Romney M. Humphries, PhD, D(ABMM), FIDSA	Vanderbilt University Medical Center
Thomas J. Kirn, MD, PhD	Rutgers Robert Wood Johnson Medical School
Brandi Limbago, PhD	Centers for Disease Control and Prevention
Virginia M. Pierce, MD, FIDSA	University of Michigan Medical School
Sandra S. Richter, MD, D(ABMM), FIDSA	Mayo Clinic (Jacksonville, FL)
Michael Satlin, MD	Weill Cornell Medicine
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:	
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), MT(ASCP)	Penn State Hershey Medical Center
Shelley Campeau, PhD, D(ABMM)	Scientifc and Medical Affairs Consulting, LLC
Mariana Castanheira, PhD	JMI Laboratories
Sanchita Das, MD, D(ABMM)	National Institutes of Health
German Esparza, MSc	Proasecal SAS
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
Joe Kuti, PharmD, FIDP	Hartford Hospital
Joseph D. Lutgring, MD	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.
Navaneeth Narayanan, PharmD, MPH	Rutgers University
Kiyofumi Ohkusu, PhD	Tokyo Medical University
Elizabeth Palavecino, MD	Wake Forest Baptist Medical Center
Robin Patel, MD	Mayo Clinic
Samir Patel, PhD, FCCM, D(ABMM)	Public Health Ontario
Eric Wenzler, PharmD, BCPS, AAHIVP	University of Illinois at Chicago
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, 23 January 2023 (In-person/Hybrid) 7:30 AM – 12:00 PM All Times listed are Eastern (US) Time			
Time	**Item**	**Presenter**	**Page**
7:30 AM – 7:35 AM (5 min)	Opening Remarks	J. Lewis	6
7:35 AM – 7:40 AM (5 min)	September 2022 AST SC Virtual Meeting Minutes Approval	J. Lewis	6
7:40 AM – 7:50 AM (10 min)	CLSI Update	B. Jones	6
7:50 AM – 8:00 AM (10 min)	EUCAST Update	C. Giske	7
8:00 AM – 8:10 AM (10 min)	VET AST Update	R. Bowden	8
8:10 AM – 8:40 AM (30 min)	M45 Update	T. Simner	10
8:40 AM – 9:00 AM (20 min)	Table 1 AHWG Update	T. Simner	14
9:00 AM – 9:30 AM (30 min)	Outreach WG	J. Hindler A. Schuetz	15
9:30 AM – 9:50 AM (20 min)	Break		
9:50 AM – 12:00 PM (2 hr 10 min)	Breakpoints WG: Part 1	N. Narayanan M. Satlin	18

PLENARY AGENDA: Session 2 Monday, 23 January 2023 (In-person/Hybrid) 1:00 PM – 5:00 PM All Times listed are Eastern (US) Time			
Time	**Item**	**Presenter**	**Page**
1:00 PM – 2:00 PM (1 hr)	Breakpoints WG: Part 2	N. Narayanan M. Satlin	27
2:00 PM – 3:20 PM (1 hr 20 min)	Methods Application and Interpretation WG	T. Kirn B. Limbago	39

3:20 PM – 3:40 PM (20 min)	Break		
3:40 PM – 5:00 PM (1 hr 20 min)	Quality Control WG	S. Cullen C. Pillar	45

PLENARY AGENDA: Session 3
Tuesday, 24 January 2023 (In-person/Hybrid)
7:30 AM – 12:00 PM
All Times listed are Eastern (US) Time

Time	Item	Presenter	Page
7:30 AM – 8:00 AM (30 min)	Joint CLSI-EUCAST WG	J. Hindler E. Matuschek	58
8:00 AM – 9:30 AM (1 hr 30 min)	Methods Development and Standardization WG: Part 1	D. Hardy B. Zimmer	63
9:30 AM – 9:50 AM (20 min)	Break		
9:50 AM – 11:30 AM (40 min)	Methods Development and Standardization WG: Part 2	D. Hardy B. Zimmer	63
11:30 AM – 11:50 AM (20 min)	Text and Tables WG	A. Bobenchik S. Campeau	79
11:50 AM – 12:00 PM (10 min)	Closing Remarks	J. Lewis	80

Summary of Voting Decisions and Action Items

Summary of Passing Votes			
#	**Motion Made and Seconded**	**Results**[a]	**Page**[b]
1.	To approve the September 2022 AST SC virtual meeting summary minutes.	14-0-0-0	6
2.	To remove the ceftazidime MIC breakpoints for *Stenotrophomonas maltophilia*.	14-0-0-0	20
3.	To approve the minocycline MIC breakpoints (S≤1, I 2, R≥4) for *Stenotrophomonas maltophilia* based on a dosage of 200 mg q12h. Note: Disk diffusion breakpoints to be reviewed in June 2023.	14-0-0-0	22
4.	To add the ceftriaxone dosing comment for MSSA stating that susceptibility is based on a dosage of 2g q12h and "Current data suggest that ceftriaxone may not be adequate for all MSSA infections. ID consult suggested.".	13-1-0-0	25
5.	To approve the tedizolid *S. aureus* disk breakpoints (S≥19 mm, I 16-18 mm, R≤15) with reflected light.	13-0-1-0	28
6.	To approve the tedizolid disk QC range for *S. aureus* (19-25 mm) with reflected light.	14-0-0-0	29
7.	To approve the linezolid *S. aureus* disk breakpoints (S≥26 mm, I 23-25 mm, R≤22 mm) with reflected light and remove the comment for confirmation with an MIC method for resistant *S. aureus* disk results.	10-3-1-0	31
8.	To approve the tedizolid beta-hemolytic *Streptococcus* disk breakpoint (S≥15 mm) with reflected light.	13-0-0-1	34
9.	To approve the tedizolid *Streptococcus anginosus* group disk breakpoint (S≥18 mm) with reflected light.	14-0-0-0	35
10.	To approve adding the aztreonam and ceftazidime-avibactam broth disk elution method for Enterobacterales and *Stenotrophomonas* in Table 3.	11-1-2-0	44
11.	To approve the SPR206 QC ranges for *E. coli* ATCC 25922, *E. coli* NCTC 13846, and *P. aeruginosa* ATCC 27853.	12-0-2-0	46
12.	To approve the Polymyxin B QC range for *E. coli* NCTC 13846.	14-0-0-0	48
13.	To approve the Imipenem-XNW4107 QC ranges for *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *K. pneumoniae* ATCC BAA-1705, and *P. aeruginosa* ATCC 27853.	14-0-0-0	50
14.	To approve the "Procedure for Confirming the Acceptability of the Mueller-Hinton Agar Sources for Subsequent use in CLSI and/or EUCAST Studies to Establish Disk Diffusion QC Ranges" as an encouraged (not required) procedure.	13-0-0-1	62
15.	To retain the current cefepime *P. aeruginosa* disk diffusion zone cutoffs (S≥18, I 15-17, R≤14) with a comment to confirm intermediate readings with an additional testing method for the 16-18h direct blood disk diffusion method.	12-0-0-2	70
16.	To approve the exebacase broth microdilution MIC testing of *Staphylococcus* species other than *S. aureus* for CAMHB-HSD media with the 5% CO2 and 20-24 hours incubation modifications with the contingency to confirm the acceptability of QC performance and range prior to publication.	11-1-0-2	71

[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.

2023 JANUARY AST MEETING SUMMARY MINUTES PLENARY 1: Monday, 23 January 2023 (In-person/Hybrid) 7:30 AM – 12:00 PM Eastern (US) Time	
#	**Description**
1.	**OPENING REMARKS (J. LEWIS)** Dr. Lewis opened the meeting at 7:30 AM Eastern (US) time by welcoming the participants to the hybrid CLSI meeting in Orlando, Florida.
2.	**SEPTEMBER 2022 AST SC VIRTUAL MEETING SUMMARY MINUTES APPROVAL (J. LEWIS)**
	A motion to approve the September 2022 AST SC virtual meeting summary minutes was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)
3.	**CLSI UPDATE (B.JONES)** Ms. Jones presented the CLSI Excellence in Standards Development Award to German Esparza. German Esparza, MSc has been a CLSI volunteer since 2008 and currently is a member of the Expert Panel on Microbiology and an advisor on the CLSI Antimicrobial Susceptibility Testing Subcommittee. Mr. Esparza is a professor of infectious diseases at Universidad del Rosario in Bogotá, Colombia. He is a consultant on antimicrobial resistance at the Pan American Health Organization in Bogotá and he leads the PROASECAL proficiency testing program in clinical microbiology. He is dedicated to educating health care professionals such as physicians, medical technologists, and pharmacologists about the importance of microbiology standards. Ms. Jones shared a career story about the impact CLSI has on the medical community. She thanked the CLSI volunteers for the work completed for the mission of CLSI.

4.	**EUCAST UPDATE (C. GISKE)**
	Dr. Giske provided an update on the activities of EUCAST. The main points included:
	• Revision of fosfomycin breakpoints
	  ○ Revision of fosfomycin MIC breakpoints for *E. coli* (S≤8 mg/L, R>8 mg/L) and *S. aureus* (S≤(32) mg/L, R>(32) mg/L) for the daily dose of at least 16g.
	  ○ The EUCAST breakpoints proposed for *E. coli* pertain to the use of intravenous fosfomycin in monotherapy for infections originating in the urinary tract.
	  ○ The proposed breakpoints do not contradict, fail to acknowledge, or discourage from the use of intravenous fosfomycin in combination therapy for other infections. However, the correlation between the *in vitro* susceptibility of a pathogen to fosfomycin and the efficacy of adding fosfomycin to combination therapy has not been formally studied. Consequently, it is not possible to propose a breakpoint for use in any combination regimen.
	  ○ ECOFFs can be used to determine if a strain belongs to the wild type, although this information does not predict efficacy in combination therapy. For S. *aureus* the ECOFF is 32 mg/L, for *K. pneumoniae* it is 64 mg/L, for *P. aeruginosa* it is 256 mg/L, and for *P. mirabilis* it is 8 mg/L. For other species: insufficient data.
	• Proposed revision of chloramphenicol MIC breakpoints for Enterobacterales, *Staphylococcus* spp., *Streptococcus* groups A, B, C, G, and *S. pneumoniae*.
	• Ongoing discussion for Cephs vs *S. aureus*
	  ○ Cefotaxime and ceftriaxone: both indicated as high exposure
	  ○ No current consensus on further restriction to “non-severe infections” for ceftriaxone
	  ○ Work is ongoing with a guidance document to explain caveats with ceftriaxone
	• New guidance on the implementation of revised aminopenicillin breakpoints for Enterobacterales
	• New changes to *Enterococcus* spp. and *Corynebacterium diphtheriae* and *C. ulcerans* breakpoint tables
	• 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

5.	**VET SUBCOMMITTEE (VAST) UPDATE (R. BOWDEN)**
	Mr. Bowden provided an update on the activities of the Subcommittee on Veterinary Antimicrobial Susceptibility testing. The main points included:
	• WG on Aquatic Animals
	  ○ 13 labs are working with the FDA Center for Veterinary Medicine (CVM) to develop ECVs for multiple agents for *Streptococcus iniae, Yersinia ruckeri,* and multiple *Aeromonas* spp., *Edwardsiella* spp., and *Vibrio* spp.
	  ○ International harmonization of incubation temp and time to create standard for future testing
	  ○ ECVs are expected to be presented at the winter 2024 meeting
	  ○ Issue delaying testing is *Streptococcus* spp. Incubation: ISO and EUCAST = 16-20h, CLSI = 20-24h
	  ○ QC ranges at 16-20h vs. 20-24h will be compared and the study plans to perform readings at 16, 20, and 24h
	  ○ Additionally, some of the more fastidious streptococci require CAMHB w/ LHB + NAD for growth
	  ○ Discussed using MHF broth for all streps, but sourcing is a concern, and testing must begin soon
	• Animal Health WG on Molecular AST
	  ○ Formed December 2022
	  ○ Purpose: Develop recommendations for veterinary research and diagnostics application
	  ○ Goal: Encourage appropriate testing and interpretation, and outline applications that are inappropriate
	  ○ Deliverables: 1) Create a VET01S table similar to M100 Appendix H, but targeted for vet application 2) White paper (possible collaboration with AVMA) on use of sequencing in routine vet diagnostics
	  ○ M100 Appendix H will be reviewed to see what information can be included in VET01S
	  ○ Subgroups will form to look at specific organism groups
	  ○ Seeking to have collaboration with SC on AST
	• VET05 Generation, Presentation, and Application of AST Data for Bacteria of Animal Origin
	  ○ VET05 is primarily focused on larger surveillance study design and incorporation of WGS data
	  ○ Project proposal for a 2nd edition was approved at the winter 2022 plenary
	  ○ As of winter 2023: several changes in membership, insufficient volunteers, approved to archive
	• VET06 Methods for AST of Infrequently Isolated or Fastidious Bacteria Isolated From Animals
	  ○ Major aim is to enable further studies of these organisms to be conducted in a standardized manner, generating data sufficient for the methods and BPs to be moved to the VET01 and VET01S documents
	  ○ May focus on ECVs rather than PK-PD, due to lack of sufficient applicable data
	  ○ Challenges for data acquisition due to identification methods having varied between publications
	• VET09 Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings
	  ○ 2nd edition underway, with many revisions
	  ○ Includes/expands on sections describing how BPs are set, importance of PK-PD, critically important agents
	  ○ 2 new chapters: poultry and how to approach extrapolating to animal species for which there are no BPs
	• Education WG
	  ○ Collaboration with Ohio State University to develop online trainings focused around CLSI documents
	  ○ Goal is to eventually become a hybrid course, with a twinning program for labs in US and Caribbean
	  ○ Manuscript with recommended guidelines for reviewers and editors to ensure proper vocabulary and breakpoints are used when CLSI is referenced in manuscript drafts
	  ○ Revitalize VAST newsletter efforts

- WG on PD Targets for Establishing Breakpoints (Subgroup of VET02 WG)
  - Discussion and approval that future urine-specific BPs should be based on clinical cutoff values (CO_{CL}) and wild type cutoff values (CO_{WT}) and not pharmacodynamic cutoff values (CO_{PD})

Antimicrobial Agent	PK-PD Target
Penicillins	*f*T>MIC = 50%
Cephalosporins	*f*T>MIC = 50%
Carbapenems	*f*T>MIC = 40%
Fluoroquinolones	*f*AUC/MIC = 72
Tetracyclines	*f*AUC/MIC = 25
Chloramphenicol	*f*AUC/MIC = 40

- WG on Generic Drugs
  - 3 BPs approved at winter 2023 meeting
  - Revision of 2 FQ BPs for dogs
  - Creation of canine-specific BPs for chloramphenicol
- WG on VAST Breakpoints/Editorial Tables (VET01S)
  - Broadened BP applicability from *E. coli* to Enterobacterales:
    - canines: amikacin, amoxicillin-clavulanate, ampicillin, cefazolin, cephalexin, enrofloxacin, doxycycline, and minocycline
    - equines: amikacin, ampicillin, cefazolin, enrofloxacin, doxycycline, and minocycline
    - felines: ampicillin, amoxicillin-clavulanate, enrofloxacin
    - bovines: ampicillin
  - Broadened BP applicability from *S. aureus* to *Staphylococcus* spp.:
    - equines: enrofloxacin, doxycycline, minocycline
  - Broadened BP applicability from *S. pseudintermedius* to *Staphylococcus* spp.:
    - canines: doxycycline, minocycline
  - Broadened BP applicability to *Streptococcus* spp. except *S. pneumoniae*:
    - equines: ampicillin
  - Broadened BP applicability to *Streptococcus* Beta-hemolytic group:
    - equines: enrofloxacin
- WG on VAST Breakpoints/Editorial Tables (VET01S)
  - Oxacillin BP changes in M100 Table 2C vs. VET01S Table 2C-1
    - Concern from the SC on VAST regarding adoption of the new M100 Oxacillin BP of <=0.5 "S" into VET01S
    - Unknown if the new BPs may substantially under call resistance in veterinary diagnostic labs (non-BMD)
    - The VET01S WG's *Staphylococcus* subgroup was tasked with examining the issue
    - Findings: new M100 BPs greatly under calls mecA for *S. pseudintermedius* if using commercial methods
    - At this time, VET01S will not adopt the revised M100 oxacillin MIC BPs for *S. pseudintermedius* or *S. schleiferi*
    - VET01S will adopt the new M100 BPs for other *Staphylococcus* spp.
    - Work continues...potential for collaboration with AHWG on CoNS

6. **M45 UPDATE (T. SIMNER)**

Dr. Simner provided an update on the M45 Revision. The main points included:

- Process to date:
  - Five teleconferences to date; meet monthly until June 2023 meeting
  - Organism groups assigned to members and reviewed with committee
  - Updating guidance tables for M45, 4th Edition
  - Ongoing evaluation vs. EUCAST guidance, new clinical data, and testing issues
- 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
  - Consideration of intrinsic resistance tables for M45 organisms
  - Provide next-steps for future M45 updates
- Current forward for M45 (page ix): "The working group used a thorough search of the published literature in conjunction with the clinical expertise of its members to apply or adapt interpretive criteria from CLSI document M100 to the interpretation of tests for organisms in this document. Users of the guideline should be aware that the very extensive microbiological, clinical, and pharmacodynamic databases normally used for setting breakpoints by CLSI do not exist for the collection of "orphan" organisms described in this document."
- Defining "Breakpoints" in M45
  - M45 is a guideline while M100 is a standard

	Data Required	Available for M45
Breakpoint	• ECV • Non clinical PK-PD cutoff • Clinical exposure-response cutoff • Clinical cutoff	No
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")
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

- Current Approach
  - Transparency about data utilized to set “breakpoints” with follow-up publications on MIC distributions/ posting on the CLSI website
  - Should these continue to be called “breakpoints”?
  - Options discussed for reporting M45 breakpoints:
  - Breakpoints with caveats well defined throughout the document
    - Investigational breakpoints
      - M100 definition: Includes antimicrobial agents where the breakpoints are investigational for the organism group and have not yet been approved by the FDA for use in the US
      - Does not apply to all organism/antimicrobial groups in the document
    - ECVs
      - Official ECVs not possible for great majority of organism/agent pairs due to lack of data
      - “Investigational ECVs”, or “Tentative” ECV?
  - Consensus from the committee continue to refer to them as breakpoints and create an optional comment for laboratories to append to reports. Example: “Presumptive breakpoints established with limited data”
  - M45 is a guideline (not a standard)
  - Guidance from AST subcommittee on how to define the “breakpoints” published in M45 was asked (see discussion below)
- Setting Non-Species Related PK-PD Breakpoints was discussed at the Breakpoints Working Group meeting
- 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?

- Studies being pursued. Work with the CDC on a joint initiative.

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 xyloxosidans*	100-150	Disk-to-MIC & GD & mCIM, include CRO subset; FQ	1	200	10
			Non-*aeruginosa Pseudomonas*	100-150	Disk-to-MIC & GD & mCIM, include CRO subset; FQ*	1	200	10
			Aeromonas species	100	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					

- Isolates needed

Required Isolates	Shipping Address
Achromobacter species Non-*aeruginosa Pseudomonas* *Aeromonas* species	Medical Microbiology Attention Dr. Simner/Tsige 600 N. Wolfe Street Meyer Building, B-121 Baltimore, Maryland 21287-0005
Aerococcus species *Pasteurella* spcies *Gemella* species & other catalase negative GPC (e.g., *Facklamia, Dolosigranulum, Globicatella, Dolosicuccus, Helcoccus, Tetragenococcus*) *Leuconostoc* species *Weisella* species *Micrococcus* species, *Kocuria* spp., *Nesterenkonia* spp., *Dermacoccus* spp., *Kytococcus*, etc	Vanderbilt University Medical Center Attention: Dr. Humphries 1161 21st Avenue South Medical Center North, CC3309 Nashville, TN 37232

- Follow-up Items
  - Make M45 freely available
  - Create an online "living" document
  - Move Non-Enterobacterales to the M45?
  - Create a M45 working group that reports to an established working group

SC DISCUSSION (MAIN POINTS)

- Concern with the reporting of the comment since many labs send these organisms to reference labs for testing and are not able to perform in house. Suggestion to keep the comments simple for laboratories to interface into their systems.
- Concerns that a comment in a report will eventually get ignored and be confusing.
- Suggestion to develop an innovative brand new nomenclature or endpoints for these breakpoints.
- Question asked if there were breakpoint discussions with previous M45 editions. There were not previous discussions.
- Since S/I/R is the output, the M45 results will be reported as breakpoints; therefore, a comment makes sense to indicate that these are presumptive breakpoints.
- Anaerobes in M100 are the same issue.
- Suggestion to bring affirmative M45 breakpoints into M100 and leave the rest in M45.
- Suggestion to convey in the interpretive criteria (S/I/R) that the breakpoints are not as robust. Possibly add a symbol to the interpretive criteria, similar to I^, such as a question mark or exclamation mark.
- Suggestion for a mini rationale document for a reference to users. FDA partially recognizes some M45 organism drug groups. Question if rationale documents already exist.
- Suggestion to report MIC_{50}, MIC_{90}, and ranges instead of S/I/R.
- Concern with the legality of the comment.
- Suggestion to modify M100 breakpoint definition to make fit for M45 organisms or create a new term and definition.
- Overall, agreement that the M45 breakpoints are different and the difference needs to be pointed out in a comment.
- Education to clinicians and laboratories is needed (M45 webinar).

7.	**TABLE 1 AHWG REPORT (T. SIMNER)**
	Dr. Simner provided an update on the activities of the Table 1 AHWG. The main points included:
	• Table 1 Revision History
	  ○ AHWG formed in January 2019
	  ○ June 2019: Presented initial re-assignment of agents without additional group.
	  ○ September/October 2020: The concept of an additional "Group" passed AST SC vote (9-2-1).
	  ○ January 2021: The use of Tiers as a replacement to Groups was accepted. The horizontal format was favored over the previous vertical format.
	  ○ June 2021: Presented placement of the antimicrobial agents for each organism table with the addition of the 4th category and change to the horizontal format. Received feedback from the plenary.
	  ○ Fall/Winter 2021: WG met to incorporate feedback from plenary and refined placement of antimicrobial agents. Submitted revised Instructions for Use (IFU) and Tables 1A to 1Q.
	  ○ Winter Plenary 2022: The IFU and concept of the new Table 1 passed (13:0) and Tables 1D to 1O passed (13:0). Additional feedback on Enterobacterales Table1 A-C and anaerobe Tables P and Q.
	  ○ May 2022: The AHWG met to form final recommendations for Table1 A-C and anaerobe Tables P and Q.
	  ○ June 2022: Final approval for publication in M100-S33.
	• What Changed in M100 Tables A in 2023?
	  ○ Format from vertical to horizontal
	  ○ 3 multi-organism tables to 16 single organism/organism group tables
	  ○ 3+ Groups to 4+ Tiers (added one new tier) Urine, Other, and Investigational – no change
	  ○ Expanded definitions of selective and cascade reporting
	  ○ Expanded suggestions for use of selective/cascade reporting; added examples
	  ○ Intense reevaluation of placement of antimicrobial agents in specific "tier" and added several new footnotes
	  ○ Further emphasized labs must work with ASP and follow institutional guidelines
	• Additional Items to Address
	  ○ Need to address *Neisseria meningitidis*: No Table 1 currently. All agents listed as Group C.
	  ○ Ensure consistency between Tables 1 and 2 with agents being found in the same or different boxes
	  ○ Should *Salmonella* and *Shigella* have their own Table 2? Differences in breakpoints for fluoroquinolones and azithromycin. Aminoglycosides, 1st- and 2nd generation cephalosporins and cephamycins are not effective.
	  ○ Provide suggested reporting comments to support ASP initiatives
	
	• Next Steps
	  ○ Create resources to help laboratories with implementation
	  ○ Education on the Tables (Education Symposium, JCM Minireview, ASM Microbe Symposia)
	  ○ Start to develop the tables for other geographic areas (South America)
	
	SC DISCUSSION (MAIN POINTS)
	• Support for the separation of *Salmonella* and *Shigella* into a different table (eg, Table 2).

8. **OUTREACH WG (ORWG) REPORT (J. HINDLER)**

WEBINARS/PRESENTATIONS

- CLSI-SIDP ACCP Annual Webinar
  - The Laboratory-Stewardship Partnership: Putting Susceptibility Testing Results for Gram-Negative Organisms into Practice
  - July 14, 2022
  - Samuel Aitken, PharmD and Tanis Dingle, PhD, D(ABMM)
  - 498 attendees
- CAP-CLSI Annual Webinar
  - What's New in Susceptibility Testing of Mycobacteria?
  - May 4, 2023
  - Barbara Brown-Elliot and Marie-Claire Rowlinson, PhD, D(ABMM)
- CLSI Annual Update (20th)
  - What's New in the 2023 CLSI Standards for Antimicrobial Susceptibility Testing (AST)?
  - April 5 and 6, 2023
  - April Bobenchik, PhD, D(ABMM) and Romney Humphries, PhD, D(ABMM)
- Suggested Webinars
  - Table 1
  - Source specific reporting: Urine? Other?
- January 2023 CLSI New Member Orientation on the CLSI website and YouTube
- ASM Microbe 2023
  - CLSI Tables for Antimicrobial Reporting- A New Look!
  - June 16, 2023
  - Virginia Pierce, MD

M100 EDUCATIONAL PROGRAM

- Available on the CLSI website
- No fee
- Provides 1.5-hour CEU ($30)
- Will be updated to 33rd edition

ORWG NEWS UPDATE

- Winter 2023 Edition
  - Feature: Aminoglycosides breakpoints
  - Case: Aminoglycosides use
  - Practice Tips: Cefiderocol testing
  - Hot Topic: Intrinsic resistance – antifungals
- Revamp ORWG News Update
  - Fall 2022 survey of ORWG members outcome

- Place background information in separate location on CLSI website
- Highlight main articles (features, cases, etc.) on separate webpage

- Future News Update Main Content Suggestions
  - M100 Table 1
  - Differences between "O" and "Inv" tiers
  - Antifungal reporting by body site

AST SC MEETING WORKSHOPS

- January 2023
  - Guiding Stewardship with Thoughtful Antimicrobial Reporting – An Updated Approach for 2023!
  - Patricia J. Simner, PhD, D(ABMM), Nathan P. Wiederholder, PharmD, and Stephen Cole, VMD, MS, DACVM
  - Will be available for on-demand viewing and CE credit
- June 2023
  - Standardization of Reference Standard AST Methods
  - Will include discussions of global standardization of reference methods; variations of reference methods to accommodate various agents and organisms
  - Speakers: Clinical lab, Industry –diagnostics, Industry -pharma

PUBLICATIONS (PEER-REVIEWED LITERATURE)

- Minireview – M100 32nd and 33rd Editions
  - Table 1
  - Aminoglycoside Breakpoints
  - Pip-tazo - P. *aeruginosa* Breakpoints
  - Breakpoint Update Guidance
- Cascade reporting (point-counterpoint)

VOLUNTEER OPPORTUNITIES

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

BREAKPOINT IMPLEMENTATION AD HOC WG REPORT

- Goals

- Identify needs of clinical laboratories to ensure they are using current CLSI, FDA and/or EUCAST breakpoints (BPs)
- Provide resources to assist clinical laboratories to determine:
  - What BPs are currently used in their laboratory at the AST instrument, LIS and EHR levels
  - Which BPs require updating
  - A plan for updating BPs
- Develop ongoing mechanism for communicating with clinical laboratories any new information about BPs.
- Collaborate with APHL, ASM, and CAP in development of resources

- Meetings
  - March 2022
    - Organized as part of ORWG
  - June 2022
    - Posted BP in use template on CLSI website
    - Modify BP Additions/Revisions Table in M100 into two separate sections
    - Updating BP article in June 2022 CLSI News Update – CAP requirements
    - Workshop at AST SC Meeting
    - Updating Breakpoints - Challenges and Solutions for Various Stakeholders
  - January 2023
    - Finalize 2023 Breakpoint Implementation Toolkit (BIT) for posting
    - Review CDC FDA AR Bank status of isolates for validations
    - Revise "commercial AST system – BP" discussion in front of M100
    - Discuss proposal for CLSI validation guideline
- Agenda January 2023
  - Review the 2023 Breakpoint Implementation Toolkit
  - Provide suggestions to enhance guidelines for users of CDC FDA AR Bank isolates for AST and AST BP verifications and validations
  - Proposed edit to M100, page xxxi last paragraph: "Following discussions with the antimicrobial stewardship team and other relevant institutional stakeholders), newly approved or revised breakpoints may be implemented on a commercial device after appropriate validation testing."
  - New CLSI Guideline Proposal "Validation of Alternative AST Breakpoints on a Verified Commercial AST System" to be submitted soon

SC DISCUSSION (MAIN POINTS)

- Suggestion to include supplementary QC strains as a source verifications and validations for other isolates.
- Concerns that laboratories outside of the US have issues acquiring AR Bank isolates. AR Bank has mechanisms in place to help international laboratories. Reach out to Maria Machado if you have any issues.
- Question was asked as to whether the pre-populated breakpoints in the 2023 Breakpoint Implementation Toolkit spreadsheets are set to the current M100 edition. The answer is yes. If the breakpoints change, the spreadsheet will need to be updated. The M100 edition and STIC website date will be provided at the top of the spreadsheet.
- Any changes to M100 page xxxi, please communicate to the M02/M07 AHWG in order to include in the newest edition (publishing in January 2024). These changes can be added to M02/M07 after the June 2023 AST SC meeting.

9. **BREAKPOINTS WG (BPWG) REPORT PART 1 (N. NARAYANAN AND M. SATLIN)**

STENOTROPHOMONAS AD HOC WG REPORT

- *Stenotrophomonas maltophilia* Breakpoint History
  - Opportunistic, environmental, non-fermenting Gram-negative rod with increasing prevalence, especially among critically ill and immunocompromised patients
  - Early 2000's work done to establish specific breakpoints of *Stenotrophomonas maltophilia* and *Burkholderia cepacia* (previously Table 2B *Pseudomonas aeruginosa* and Other Non –Enterobacteriaceae 2004 est. specific BP and 2006 established separate table)
  - After assessing intrinsic resistance work largely focused on methods, reproducibility and wild-type distribution (little to no PK/PD available at that time especially for these organisms)
- Differences in Recognized Breakpoints for *Stenotrophomonas maltophilia*

	CLSI			EUCAST		FDA	
	Category	MIC (µg/mL)	DD (mm)	MIC (mg/L)	DD (mm)	MIC (µg/mL)	DD (mm)
Ticarcillin-clavulanate	O	S ≤16/2, I 32/2-64/2, R ≥128/2	---	XX	XX	XX	XX
Ceftazidime	B	S ≤8, I 16, R≥32	---	XX	XX	S ≤8, I 16, R≥32	--
Cefiderocol#	B	S ≤1	S ≥15	S ≤ 0.001 mg/L "off scale" breakpoint (IE).	≥20 mm corresponds with MIC≤2	XX	XX
Minocycline	A	S ≤4, I 8 R ≥16	S ≥19, I 15-18, R ≤14	XX	XX	XX	XX
Levofloxacin	A	S ≤2 I 4 R ≥8	S ≥17, I 14-16, R ≤13	XX	XX	XX	XX
Trimethoprim-sulfamethoxazole	A	S ≤2/38, R≥4/76	S ≥16 I 11-15 R ≤10	S=0.001, I ≤2, R>4 mg/L*	S>50 mm* I16-50 R<16 mm	XX	XX
Chloramphenicol	C	S ≤8, I 16, R≥32	---	XX	XX	XX	XX

Breakpoints are based on PK/PD properties, MIC distributions, and limited clinical data. *Reading guide provided trimethoprim component only. MICs ≤2 as intermediate, which requires the use of a higher dosing regimen, 240 mg (trimethoprim component) intravenously every 12 hours

STENOTROPHOMONAS CEFTAZIDIME MIC BREAKPOINTS

- History
  - 1993: *Xanthomonas maltophilia* and *Stenotrophomonas maltophilia*
  - 2007: First updated ceftazidime label on drugs at FDA site
  - 2020: Ceftazidime label updated to remove STIC, refers to website
  - Unclear whether any organisms in clinical studies leading to ceftazidime initial FDA approval were *Stenotrophomonas maltophilia*, possible that up to 16 isolates included as *Pseudomonas* species

- No available/accessible data describing basis for original susceptibility testing interpretive criteria
- Modern knowledge of resistance mechanisms (eg, L1/L2) for *S. maltophilia* not incorporated when originally establishing STIC

- Microbiology Laboratory Data Summary
  - Reproducibility of susceptibility testing is problematic across reference methods and commercial systems
  - L1 and/or L2 ß-lactamases thought to be present in essentially all isolates
  - Acquired mutations in an efflux pump leads to higher ceftazidime MICs following drug exposure
  - Current breakpoints may split the wild type distribution
- PK/PD Data Summary
  - 3 papers and 1 abstract evaluated
  - *In vitro* 1-compartment (chemostat) model: Garrison et al. AAC 1996 and Zelenitsky et al. DMID 2005
  - Animal model: Chen et al. AAC 2019 (plus ASM Microbe 2019 abstract)
- Clinical Outcomes Data Summary
  - No high-quality comparative studies of ceftazidime vs other antimicrobials for *Stenotrophomonas maltophilia*
  - Sparse data published for clinical outcome by MIC
  - Development of resistance during treatment reported
  - Outcome not always correlated with susceptibility interpretation
- AHWG Proposal and Rationale
  - Proposal: Remove the ceftazidime breakpoints
  - AHWG vote: 7-0-0-1
  - *S. maltophilia* is not in the FDA approved indication for ceftazidime
  - Cannot find solid data supporting the establishment of the current breakpoint
  - The current breakpoint may split the wild type distribution
  - Reproducibility of susceptibility testing by reference methods and commercial methods is problematic
  - Lack of PK/PD data to validate current breakpoint (only 1 isolate with MIC of 8 mg/L used in thigh infection model)
  - No high-quality comparative studies of ceftazidime vs other antimicrobials for *Stenotrophomonas maltophilia*
  - Sparse data published for clinical outcome by MIC
  - Limited examples of successful treatment with ceftazidime monotherapy without removable foci of infection/surgical intervention
  - Development of resistance during treatment reported
  - Outcome not always correlated with susceptibility interpretation
  - Defer to the intrinsic working group to assess data as whether appropriate to list as intrinsically resistant
- BPWG Discussion and Vote
  - Questions to FDA
    - Atypical to have FDA recognize CLSI M100 for organism not in labeling? Not common but happens (eg, *Acinetobacter*/meropenem)
    - Why have breakpoints/STIC for ceftazidime but not other drugs? No standard reason, to be reconciled
  - Concern about removing one of few drugs for *Stenotrophomonas*. Drug is active against isolates in preclinical models.
  - Removing only FDA recognized breakpoint would eliminate reason for commercial AST manufacturers to pursue testing against *Stenotrophomonas*
  - If intrinsic resistance, would be always resistant and no MICs. Not recommended to pursue.

- Motion to remove ceftazidime MIC breakpoints for *Stenotrophomonas maltophilia*. Pass: 9-0-1-1

SC DISCUSSION (MAIN POINTS)

- Suggestion of a breakpoint of 4 based on the PK/PD value. There were a number of other issues that removed this option. The reference method is not reproducible to a level that is reliable. Issues with the chromosomal mediated resistance. Issues with the wild-type distribution.
- Concerns with clinicians requesting and using ceftazidime even if removed.
- Little data was present to set the breakpoints to begin with.
- EUCAST does not have ceftazidime breakpoints because of these same issues discussed at this meeting.
- Removing the ceftazidime breakpoints may put the AST device manufacturers in a predicament. The FDA STIC website currently recognizes the breakpoints. If CLSI removes it, there were concerns with what will happen on the STIC website.
- Concerns that if FDA agrees and removes ceftazidime, there are no *Stenotrophomonas* breakpoints that the FDA recognizes. Option for manufacturers to report an MIC with no interpretation. Most devices report limitations already. May not make economic sense for manufacturers to test.
- Suggestion to report ceftazidime susceptible only breakpoint with a comment to treat with ceftazidime-avibactam combination. Issues if the ceftazidime is actually susceptible.

A motion to remove the ceftazidime MIC breakpoints for *Stenotrophomonas maltophilia* was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

STENOTROPHOMONAS MINOCYCLINE MIC BREAKPOINTS

- Microbiology Laboratory Data Summary
  - Susceptibility reproducibility is acceptable
  - Based on current breakpoints almost all isolates are classified as susceptible
  - Dilution ranges on commercial systems may limit breakpoint changes
- PK/PD Data Summary
  - 2 papers evaluated
  - Fratoni et al: neutropenic murine thigh infection model
  - Wei et al: Monte Carlo simulation
- Clinical Outcomes Data Summary
  - Retrospective observational data
  - Majority of isolates from a respiratory source, many polymicrobial
  - Within these limitations, rates of failure with minocycline and TMP/SMX in these studies appear to be similar
  - One study that looked at minocycline MICs in relation to therapy found MICs of 4 mg/L were more frequent in patients with clinical failure
- AHWG Discussions
  - Option #1: Set susceptible breakpoint at ≤0.5 µg/mL
    - Based on 200 mg q12 h
    - Vote: 2-5-1
    - Reasons for no votes: splits too far into wildtype and 0.5 ug/ML is below the MIC range on some commercial platforms

S (or SDD)	I	R
≤0.5	1	≥2
200 mg Q12H	200 mg Q12H	-
PTA 1-log kill >90%	PTA stasis >90%	PTA Stasis <70%
~65-70% isolates	~20% isolates	~10-15% isolates

- Option #2: Set breakpoint as I≤1 and R≥2 µg/mL
  - Based on 200 mg q12 h and recommendation to be used only in combination
  - Vote: 0-7-1
  - Reasons for no votes: do not like intermediate only because providers will assume they cannot use the drug

I		R
≤1		≥2
200 mg Q12H		-
PTA stasis >90%		PTA Stasis <70%
~85% isolates		~15%

- Option #3: Set breakpoint as S≤1, I 2, and R≥4 µg/mL
  - Based on 200 mg q12 and recommendation to be used only in combination
  - Vote: 7-0-1
  - Reasons for no votes: PTA for stasis >90% but less than ideal for 1-log kill, but most commercial systems should be able to accommodate down to 1 ug/mL.

S	I	R
≤1	2	≥4
200 mg Q12H	200 mg Q12H	-
PTA stasis >90%	PTA stasis ~70%	PTA Stasis <20%
~85-90% isolates	~8% isolates	~2% isolates

- BPWG Discussion and Vote

- Questions on tolerability of 200 mg q12h. Data from phase 1 PK studies (reasonably tolerated up to 300 mg q12h).
- Differences in ELF penetration in humans and mice? No data.
- FDA is ok with 200 mg q12h (included in labeling) and basing a breakpoint on stasis PK/PD endpoint
- Levofloxacin breakpoint stayed the same (suboptimal PK/PD PTA) but comment added to not use as monotherapy
- Stasis vs 1-log kill endpoint for organism that causes serious infections
- Discussion on scope of CLSI to include comments on need for combination therapy. Differences in "recommending" that vs stating the clinical data is primarily combination therapy for the particular drug.
- Motion to revise the breakpoints to S/I/R of ≤1/2/≥4 based on a dosage of 200 mg q12h. Pass: 9-0-1-1
- No motion on further comments (recognized issues with having levofloxacin comment but context is different than minocycline data)
- Deferred disk diffusion breakpoints to June to examine more contemporary data

SC DISCUSSION (MAIN POINTS)

- Confirmed that the Acumen study was with the 100 mg dose. This was a single dose PK study with a 200 mg dose.
- Levofloxacin breakpoint was not lowered. PK/PD is very different from minocycline based on stasis.
- Concerns with needing the minocycline MIC breakpoint since the commercial systems have limitations.
- Data with minocycline and *Acinetobacter* show similar results and support the need for a lower susceptible MIC.
- Question if there should there be concerns with low coefficient of determination (R^2). No, the R^2 is the PK variability.
- There is no ECV. EUCAST has an ECV set as 1.
- Question about which drugs to use for combination therapy. IDSA recommendations are to treat *Stenotrophomonas* with combination therapy using two of the following drugs: trimethoprim-sulfamethoxazole, levofloxacin, minocycline, ceftazidime-avibactam + aztreonam, and cefiderocol.
- Concern how to communicate that the breakpoints are based on the higher dose when most institutions use the lower dose. Dosing comments will be pulled into Appendix E.
- Concern with the intermediate breakpoint and the dose. Consensus is that an intermediate is needed for technical variability.

A motion to approve the minocycline MIC breakpoints (S≤1, I 2, R≥4) for *Stenotrophomonas maltophilia* based on a dosage of 200 mg q12h was made and seconded. Note: Disk diffusion breakpoints to be reviewed in June 2023. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

CEFTRIAXONE/*STAPHYLOCOCCUS AUREUS* (MSSA) MIC BREAKPOINT REASSESSMENT

- M100 Table 2C *Staphylococcus* spp. History
  - Then:

	S	I	R	Comment
CLSI (pre-June 2012)	≤8	16-32	≥64	
FDA (pre-2013-2015?)	≤4	8	≥16	Based on 2 g IV q12-24h

    - FDA breakpoint reduced based on reassessment of ceftriaxone PD profile. Unable to achieve >90% PTA for MSSA at MIC of 8 mcg/mL.
    - S ≤4 mcg/mL -> 2g IV q24h
    - S ≤2 mcg/mL -> 1g IV q24h
  - Now:

- No breakpoints for most beta-lactams (BLBLIs, oral and parenteral cephalosporins, carbapenems) but inferred from oxacillin/cefoxitin
- Considered susceptible based on clinical efficacy, site of infection, and appropriate dosing

(13) Oxacillin (or cefoxitin) results can be applied to the other penicillinase-stable penicillins (cloxacillin, dicloxacillin, methicillin, and nafcillin). For agents with established clinical efficacy and considering site of infection and appropriate dosing, methicillin (oxacillin)-susceptible staphylococci can be considered susceptible to:

- β-lactam combination agents (amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam)
- Oral cephems (cefaclor, cefdinir, cephalexin, cefpodoxime, cefprozil, cefuroxime, loracarbef)
- Parenteral cephems including cephalosporins I, II, III, and IV (cefamandole, cefazolin, cefepime, cefmetazole, cefonicid, cefoperazone, cefotaxime, cefotetan, ceftizoxime, ceftriaxone, cefuroxime, ceftaroline, moxalactam)
- Carbapenems (doripenem, ertapenem, imipenem, meropenem)

- FDA Labeling Today
  - No ceftriaxone/*S. aureus* breakpoint/STIC
  - "Susceptibility of staphylococci to ceftriaxone may be deduced from testing only penicillin and either cefoxitin or oxacillin" (FDA labeling)
  - *S. aureus* included in indications for: LRTI, SSTI, bacterial septicemia, bone and joint infections
  - Adult dosing (Sandoz PI): MSSA recommended daily dose of 2 to 4 grams, in order to achieve >90% target attainment
- Clinical Use and Guidelines
  - IDSA Vertebral Osteomyelitis Guidelines: MSSA ceftriaxone 2 g IV daily
  - IDSA Prosthetic Joint Infection Guidelines: MSSA ceftriaxone 1-2 g IV daily
    - Not a consensus on the use of ceftriaxone as a single agent. Panel recognizes that there are retrospective cohort data with short duration of follow-up available to support its use.
  - IDSA Community-Acquired Bacterial Pneumonia Guidelines: ceftriaxone 1-2 g IV daily
  - IDSA *Staphylococcus aureus* Bacteremia Guidelines are in production
- Microbiology Data Summary
  - Probability of ceftriaxone MIC ≥8 µg/mL is 1.6%, 3.9%, 17.5%, and 48.7% for an oxacillin MIC of ≤0.25, 0.5, 1, and 2 µg/mL, respectively
  - Oxacillin MIC ≤0.5 µg/mL -> ≥2 g/day
  - Oxacillin MIC of 1 or 2 µg/mL -> 2 g q12h
- PK/PD Concerns for Ceftriaxone
  - Lower potency (increased MIC_{90}/ECV for S. *aureus)*
  - High protein binding leads to relatively low free drug concentrations
  - What is the appropriate dose to treat S. *aureus*?
- Clinical data summary was presented.
- EUCAST v_12.0 (*Staphylococcus* spp.)
  - No breakpoints for cephalosporins (except ceftaroline, ceftobiprole)
  - Note: Susceptibility of staphylococci to cephalosporins is inferred from the cefoxitin susceptibility except for cefixime, ceftazidime, ceftazidime-avibactam, ceftibuten and ceftolozane-tazobactam, which do not have breakpoints and should not be used for staphylococcal infections. For agents given orally, care to achieve sufficient exposure at the site of the infection should be exercised. If cefotaxime and

ceftriaxone are reported for methicillin-susceptible staphylococci, these should be reported "Susceptible, increase exposure" (I). See table of Dosages.

Standard Dosage	High Dosage	Special Situations
2 g IV daily	2 g IV BID or 4 g IV daily	*S. aureus*: High dose only

  - Per EUCAST website: General consultation 26 September – 7 November 2022. EUCAST response being prepared.
  - Also report ceftriaxone as "suitable only for non-serious infection"
  - EUCAST Consultation Conclusion: Available clinical data and PK/PD analyses support the use of cefazolin and cefepime with the currently listed dosage regimens. PK/PD analysis support the use of cefuroxime iv, but published experience with its use is limited and high dosages are required. PK/PD analyses suggest that cefotaxime may not be a reliable agent, especially in serious infections. This is also the case for ceftriaxone, although there is ongoing controversy in the literature about is role and efficacy [32].
- Summary
  - Ceftriaxone MICs for MSSA: MIC_{90} (4 mcg/mL), ECV (8 mcg/mL)
  - FDA recommends 2-4 g/day for MSSA (based on 2011 PD analysis). Discordance with clinical guidelines that include ceftriaxone for MSSA.
  - Assuming MIC breakpoint of 4 mcg/mL (previous FDA): Dosage of 2 g q12h (maybe 2 g daily) necessary for adequate PTA
  - Clinical data is extremely limited in quality but most dosing is 2 g daily
  - EUCAST recommends high dose (2 g q12h) and currently in consultation to add note for use in non-serious infections only
- BPWG Discussion and Vote
  - Labs tell clinicians that susceptibility can be inferred but no information on what dose the susceptibility is based on –can be misleading
  - Favor to add dosing comment (Jim), do it now given data presented (Amy) and needed as most clinicians use 2g QD (likely for convenience of QD dosing)
  - Options:
    - Make dosing comment based on data today
    - Form AHWG to determine comment(s)
    - Form AHWG to review dosing of all pertinent beta-lactams for MSSA
  - Issue adding dosing comment when no breakpoint but dosing comments are all in Appendix E now so ok
  - Motion to add dosing comment for ceftriaxone that susceptibility is based on dosage of 2g q12-24h. Pass: 7-2-1-1
  - Reason for no votes: believe it should be 2g q12h, not q24h

SC DISCUSSION (MAIN POINTS)

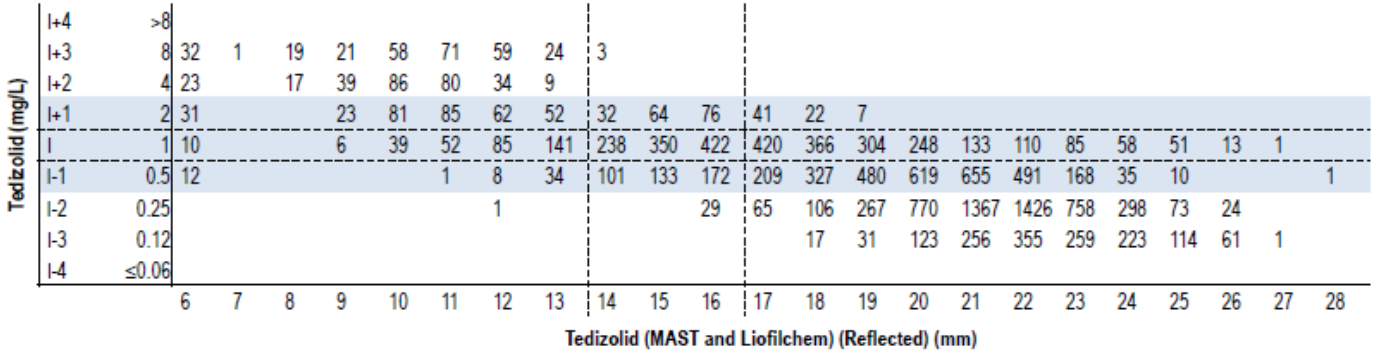
- Concern to not encourage treatment of mild MSSA infections with ceftriaxone in multi-drug resistant gram-negative areas.
- Concern that 2g daily is not a sufficient dosage for systemic MSSA infections.
- Concern with confusion when using cefoxitin MIC breakpoint for *S. aureus* for the use of ceftriaxone and using the correct dose. Suggestion to review interfering comments in M100.
- Multiple suggestions for 2g q12h.
- Education and guidance are needed. Communication needed to IDSA guideline committees.
- Concern about losing ceftriaxone therapy for relevant MSSA infections.
- CLSI is not a dosing organization. CLSI's responsibility is to state what dosage was used to set the breakpoints.

	• Suggestion to add a warning/comment to M100 comment 12 (inferring comment) stating that a consultation is needed with an ID pharmacist. Concern the dosing comment will not be seen by the laboratory. • Suggestion to add to comment 13 at the end of the parental cephems bullet: "For systemic infections, ceftriaxone susceptibility for MSSA is based on a dosage of 2g q12h." • Comment 13 is in the general *Staphylococcus* spp. comments, not specifically S. *aureus* or MSSA. If the comment was added in Table 2, it would be in the S. *aureus*/S. *lugdunensis* row. • Concern that consistency is needed with all drug dosages. • Concern that adding wording to existing comments will encourage the use of ceftriaxone for MSSA. • Suggestion to add a comment, "Current data suggest that ceftriaxone may not be adequate for all MSSA infections. ID consult suggested." A similar comment exists for daptomycin and *E. faecium*. • BPWG and TTWG will work on a comment for Table 2 to include the ID consult and systemic infections for MSSA to present in June 2023.
	A motion to add ceftriaxone dosing comment for MSSA stating that susceptibility is based on a dosage of 2g q12h and "Current data suggest that ceftriaxone may not be adequate for all MSSA infections. ID consult suggested." was made and seconded. Vote: 13 for, 1 against, 0 abstain, 0 absent (Pass)
	Against Vote Reasoning: • CLSI is not a dosing organization. IDSA should be making these decisions.
10.	**ADJOURNMENT** Dr. Lewis thanked the participants for their attention. The meeting was adjourned at 12:15 PM Eastern (US) time.

2023 JANUARY AST MEETING SUMMARY MINUTES PLENARY 2: Monday, 23 January 2023 (In-person/Hybrid) 1:00 PM – 5:00 PM Eastern (US) Time	
#	**Description**
1.	**OPENING** Dr. Lewis opened the meeting at 1:00 PM Eastern (US) time.

2.	**BREAKPOINTS WG (BPWG) REPORT PART 2 (N. NARAYANAN AND M. SATLIN)**
	
	TEDIZOLID S.*AUREUS* DISK BREAKPOINTS WITH REFLECTED LIGHT
	• Background: Setting tedizolid and re-evaluating linezolid disk diffusion breakpoints
	  ○ No prior disk diffusion breakpoints for tedizolid
	  ○ Prior linezolid breakpoints using transmitted light
	  ▪ June 2022: data presented and approved that should use reflected light for both tedizolid and linezolid because easier, more reproducible, and harmonized with EUCAST
	  ▪ With reflected light, the M100 32nd edition linezolid/*S. aureus* disk diffusion breakpoints were inaccurate
	  ○ New QC ranges set for linezolid and *S. aureus* (24-30 mm) with reflected light
	  ○ At a prior meeting, the tedizolid QC range of 19-25 mm was proposed but not passed
	• Methods
	  ○ Multicenter study of tedizolid and linezolid disk correlates for S. aureus
	  ○ BMD testing: JMI produced frozen panels
	  ○ Disk testing:
	  ▪ 2 µg tedizolid disks: 2 manufacturers (Liofilchem, Mast) and 2 lots were used for each disk
	  ▪ 30 µg linezolid disks: 1 manufacturer (BD), 1 lot
	  ▪ 4 sites, 2 readers per site
	  ▪ 3 brands of agar plates (Hardy, BD, BBL, Remel)
	  ▪ Zone diameters read by reflected light
	  ○ Isolates: 25 linezolid-susceptible and 25 linezolid-resistant isolates
	• Disk Diffusion Breakpoint Discussion
	  ○ CLSI MIC breakpoints: S: ≤0.5; I: 1; R: ≥2
	  ○ EUCAST MIC breakpoints: S: ≤0.5; R: ≥1
	  ○ EUCAST disk correlates (same disk mass): S: ≥20 mm; R: ≤16 mm
	  ▪ Problem: minor errors would be out of acceptable range in M23
	  ○ Rodrigo Mendes (JMI) proposed: S: ≥19 mm; I: 16-18 mm; R: ≤15 mm
	  ○ Concern about QC range (proposed 19-25 mm) so close to intermediate zone. QC range in intermediate zone not a contraindication

Disk breakpoints	Range	Number	Error rates		
			Very major (%)	Major (%)	Minor (%)
≥17mm (S)/≤13mm (R)	≥I+2	576	0	N/A	3 (0.52)
	I+1 to I-1	7,164	70 (0.98)	55 (0.77)	2,700 (37.69)
	≤I-2	6,624	N/A	1 (0.02)	29 (0.44)
	Total	14,364	70 (0.49)	56 (0.39)	2,732 (19.02)
≥18mm (S)/≤14mm (R)	≥I+2	576	0	N/A	0
	I+1 to I-1	7,164	29 (0.4)	156 (2.18)	2,635 (36.78)
	≤I-2	6,624	N/A	1 (0.02)	94 (1.42)
	Total	14,364	29 (0.2)	157 (1.09)	2,729 (19.0)
≥19mm (S)/≤15mm (R)	≥I+2	576	0	N/A	0
BPWG and Rod recommendation	I+1 to I-1	7,164	7 (0.1)	289 (4.03)	2,771 (38.68)
	≤I-2	6,624	N/A	1 (0.02)	217 (3.28)
	Total	14,364	7 (0.05)	290 (2.02)	2,988 (20.8)
≥20mm (S)/≤16mm (R)	≥I+2	576	0	N/A	0
EUCAST	I+1 to I-1	7,164	0	461 (6.43)	3,128 (43.66)
	≤I-2	6,624	N/A	30 (0.45)	486 (7.34)
	Total	14,364	0	491 (3.42)	3,614 (25.16)



- BPWG Proposed Tedizolid *S. Aureus* Disk Breakpoints: S: ≥19 mm; I: 16-18 mm; R: ≤15 mm. Pass: 8-0-1-2

A motion to approve tedizolid *S. aureus* disk breakpoints (S≥19 mm, I 16-18 mm, R≤15 mm) with reflected light was made and seconded. Vote: 13 for, 0 against, 1 abstain, 0 absent (Pass)

Reason for abstention:

- Absent from room during discussion.

TEDIZOLID S. *AUREUS* QC DISK BREAKPOINTS WITH REFLECTED LIGHT

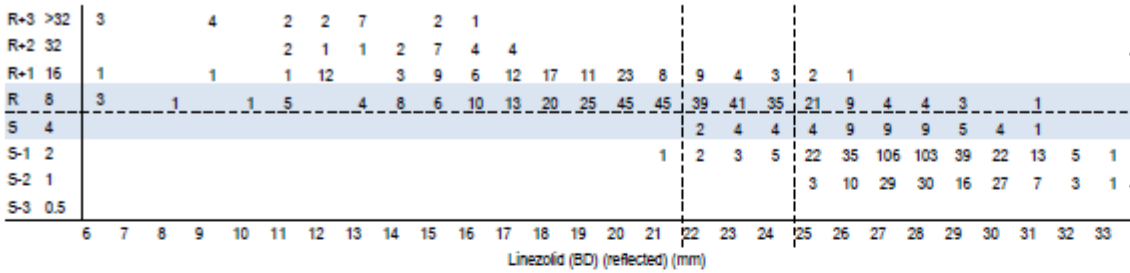
- Current Ranges
  - CLSI range with transmitted light: 18-24, footnote h: read using transmitted light
  - EUCAST range (2 mcg disk): NA for *S. aureus* 25923, for *S. aureus* 29213 19-25, target 22
- Recommendation: 19-25 (7) 99.0%

Study	Media	Disk	Labs	Gavin	Range Finder	Comments
Tier 2: Slide 10 2019 QCWG_6A_Tedizolid_Tier 2_2mcg	21, 21, 22	22, 22	20,3@21,3 @22,23	18-25mm 99.6% 8mm,	19-25mm, 99.0% 7mm,	Lab variability, some media variability
JMI 4 lab: Linezolid Disk Diffusion Testing September 2022 Presentation	Stats not available	NA	NA	NA	NA	Disks: MAST and Liofilchem, no effect Media: Remel, Hardy, BD, no effect Labs: Small variability Zones smaller with transmitted light compared to reflected light 100% of results within range 18-24 (very few at 18 and 19)

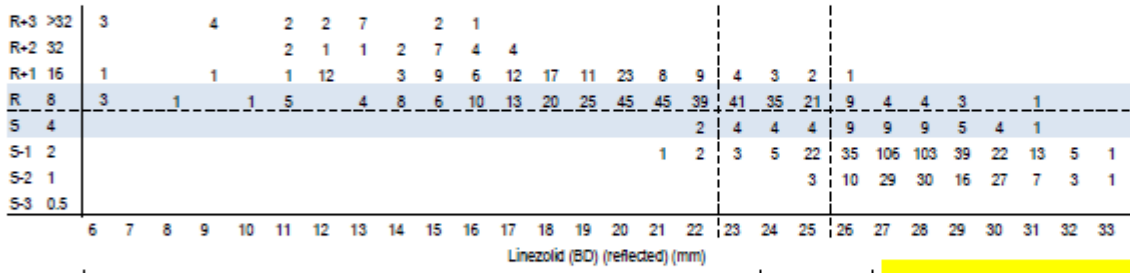
A motion to approve tedizolid disk QC range for *S. aureus* (19-25 mm) with reflected light was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

LINEZOLID S. *AUREUS* DISK BREAKPOINTS WITH REFLECTED LIGHT (VOTE #1)

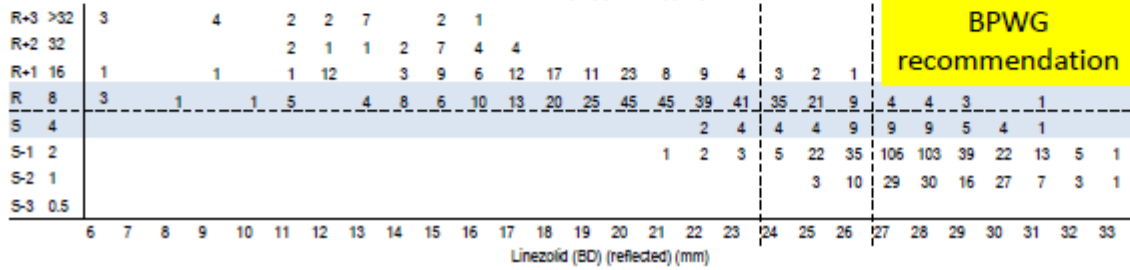
- Disk Diffusion Breakpoint Discussion
  - CLSI and EUCAST MIC breakpoints: S: ≤4; R: ≥8
  - EUCAST linezolid disk content is different than CLSI
  - Rodrigo Mendes (JMI) proposed: S: ≥26 mm; I: 23-25 mm; R: ≤22 mm
  - Precedent for S/I/R disk diffusion breakpoints with S/R MIC BPs
    - CAZ-AVI/Enterobacterales: no intermediate disk category, but "confirmatory MIC testing is indicated for isolates with zones of 20-22 mm to avoid reporting false-susceptible or false-resistant results"
    - TMP-SMX and all organisms that have disk breakpoints
  - Current comment: "Organisms with resistant results by disk diffusion should be confirmed using an MIC method"
  - Preference to minimize very major errors compared to minor errors



MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥R+1	165	3 (1.8)	N/A	16 (9.7)
S+R	394	42 (10.6)	0 (0.0)	125 (31.7)
≤S-1	483	N/A	1 (0.2)	10 (2.1)
Total	1,042	45 (4.3)	1 (0.1)	151 (14.5)



MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥R+1	165	1 (0.6)	N/A	9 (5.4)
S+R	394	21 (5.3)	2 (0.5)	109 (27.7)
≤S-1	483	N/A	3 (0.6)	33 (6.8)
Total	1,042	22 (2.1)	5 (0.5)	151 (14.5)



MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥R+1	165	0 (0.0)	N/A	6 (3.6)
S+R	394	12 (3.0)	6 (1.5)	82 (20.8)
≤S-1	483	N/A	6 (1.2)	75 (15.5)
Total	1,042	12 (1.2)	12 (1.2)	163 (15.6)

- BPWG Proposed Linezolid *S. aureus* Disk Breakpoints: S: ≥27 mm; I: 24-26 mm; R: ≤23 mm with current comment about confirming resistant organisms with an MIC method. Pass: 8-0-1-2
- Reason for no vote: would prefer comment changed to indicate organisms with resistant or intermediate results by disk diffusion should be confirmed by an MIC method

SC DISCUSSION (MAIN POINTS)

- Concern with 15% minor error rate.
- Concern with recommendation to confirm resistant organisms with a major error rate only being 1.2%. Burden to laboratory if not needed.
- There is a large number of intermediate disks.
- Suggestion for a comment to state, "If you get an intermediate with disk, you may consider testing with MIC." MIC may test as susceptible.
- Only enriched data set was used. Concern that it is not the reality in the clinical setting.

- Current confirmation of resistant comment is attached to all *Staphylococci*.
- Suggestion to use S≥26 because of the decrease of the minor error rate and the MIC of 2.

A motion to approve linezolid *S. aureus* disk breakpoints (S≥27 mm, I 24-26 mm, R≤23 mm) with reflected light and remove the comment for confirmation with an MIC method for resistant *S. aureus* disk results was made and seconded. Vote: 0 for, 14 against, 0 abstain, 0 absent (Fail)

Against Vote Reasoning:
- Like S≥26 mm over S≥27 mm.
- Intermediate disk should be confirmed with MIC.
- Confusion about interpretation of data set.
- Only applies to S. *aureus* but not other *Staphylococcus* species. Confusing if reflected light will only be used for *S. aureus* or for all *Staphylococcus* species.

LINEZOLID S. *AUREUS* DISK BREAKPOINTS WITH REFLECTED LIGHT (VOTE #2)

SC DISCUSSION (MAIN POINTS)
- Error rate bound method should be used for an enriched population. Need to be more relaxed with very major errors when using an enriched population.

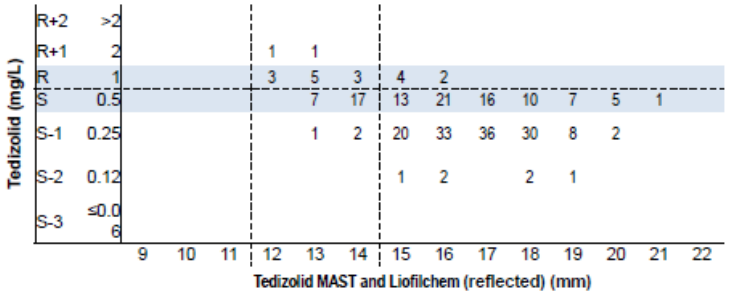
A motion to approve linezolid *S. aureus* disk breakpoints (S≥26 mm, I 23-25 mm, R≤22 mm) with reflected light and remove the comment for confirmation with an MIC method for resistant *S. aureus* disk results was made and seconded. Vote: 10 for, 3 against, 1 abstain, 0 absent (Pass)

Against Vote Reasoning:
- Uncomfortable with removing confirmation comment with the enriched data.
- Intermediate disk should be confirmed with MIC.
- Confusion about interpretation of the data set.
- Comment is based on transmitted light and not reflected light. Suggestion to look into *Staphylococcus* species reflected light data. Concerns reflected light will be used to read transmitted *Staphylococcus* species breakpoints. JMI will try to perform a small study.

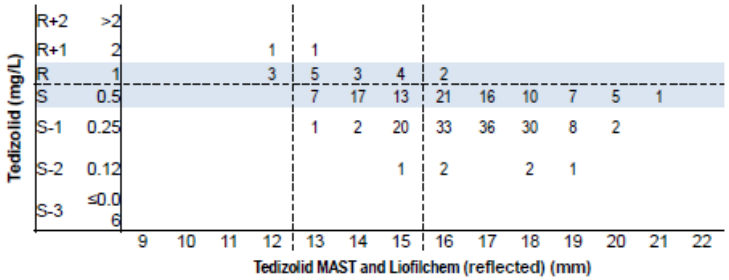
TEDIZOLID AND LINEZOLID *ENTEROCOCCUS* DISK BREAKPOINTS WITH REFLECTED LIGHT
- Methods for *Streptococcus* and *Enterococcus*
  - 2 laboratories (ACM Global and JMI)
  - BMD testing: CA-MHB as testing medium; 2.5-5% lysed horse blood used for streptococci: 1 replicate
    - JMI: own panels
    - ACM: Sensititre panels from ThermoFisher
  - Disk diffusion: 1 replicate
    - 2 brands of disk (Liofilchem, Mast)
    - 2 brands of agar (BBL, Remel)

- Isolates: phase 3 clinical trials and SENTRY (2010-2018)
- Results from 3 different technicians

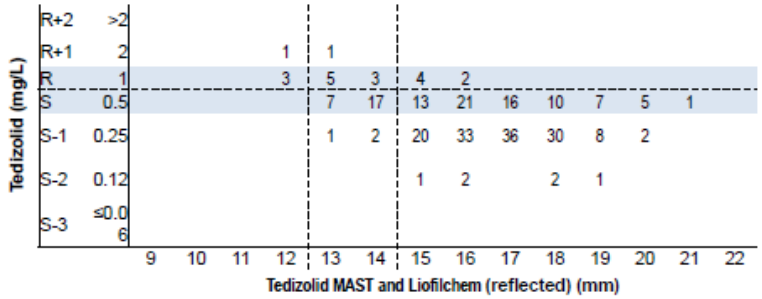
- Tedizolid MIC/disk correlations for *E. faecalis*



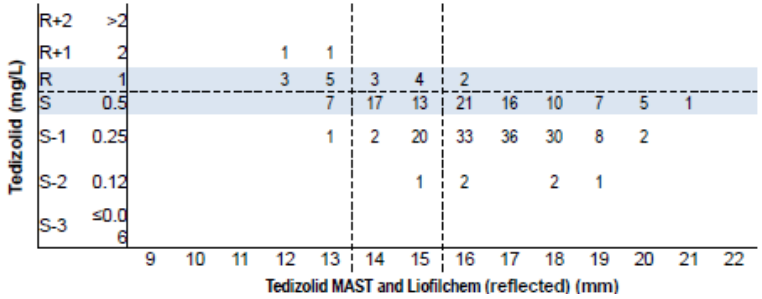
MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥R+1	2	0	N/A	2 (100.0)
S+R	114	6 (0.88)	0	35 (30.7)
≤S-1	138	N/A	0	3 (2.2)
Total	254	6 (0.4)	0	21 (8.3)



MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥R+1	2	0	N/A	1 (50.0)
S+R	114	2 (1.8)	0	49 (43.0)
≤S-1	138	N/A	0	24 (17.4)
Total	254	2 (0.8)	0	74 (29.1)

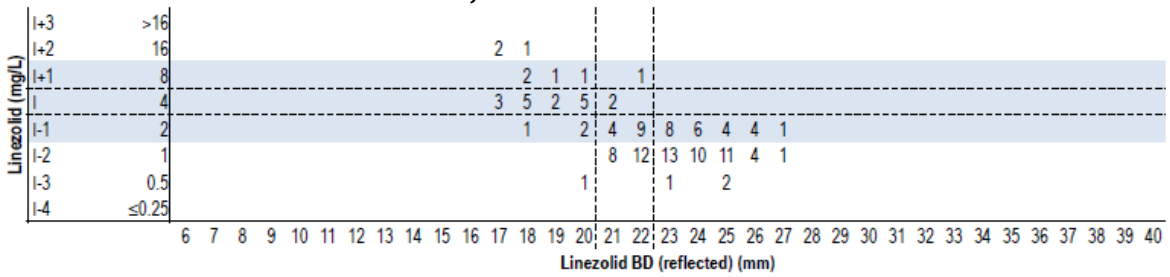


MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥R+1	2	0	N/A	1 (50.0)
S+R	114	6 (0.88)	0	32 (28.1)
≤S-1	138	N/A	0	3 (2.2)
Total	254	6 (0.4)	0	21 (8.3)



MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥R+1	2	0	N/A	0 (0.0)
S+R	114	2 (1.8)	7 (6.1)	37 (32.5)
≤S-1	138	N/A	1 (0.7)	23 (16.7)
Total	254	2 (0.8)	8 (3.1)	60 (23.6)

- Linezolid MIC/disk correlations for E. *faecalis*



MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥I+2	3	0	N/A	0
I+1 to I-1	61	0	3 (4.9)	16 (26.2)
≤I-2	63	N/A	1 (1.6)	20 (31.7)
Total	127	0	4 (3.1)	36 (28.3)

Neither linezolid nor tedizolid disk diffusion breakpoints proposed for enterococci

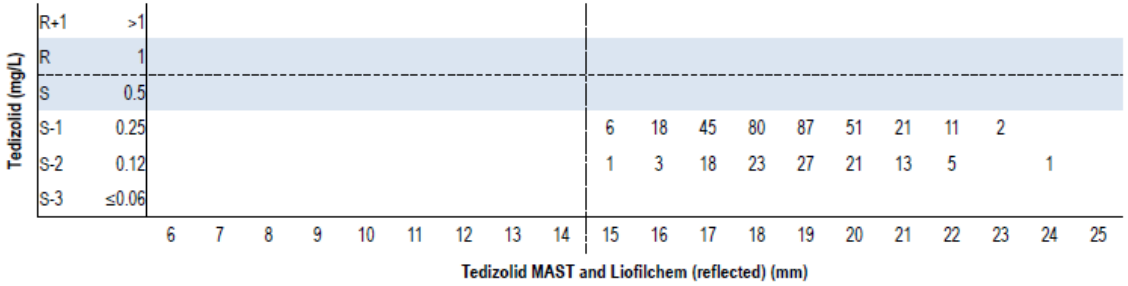
- Decision: No linezolid nor tedizolid disk breakpoints proposed MIC/disk correlations for *Enterococci*

SC DISCUSSION (MAIN POINTS)

- Current linezolid breakpoints for *Enterococcus* are based off of reflected light. No changes will be made based on the data.

TEDIZOLID BETA-HEMOLYTIC *STREPTOCOCCI* DISK BREAKPOINTS WITH REFLECTED LIGHT

- Data



MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥R+1	0	0	N/A	0
S+R	0	0	0	0
≤S-1	433	N/A	0	0
Total	433	0	0	0

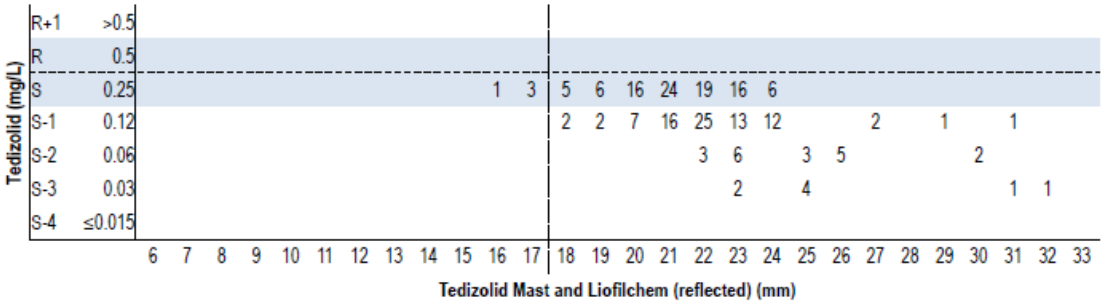
Motion: S: ≥15 mm
- **Passed: 8 Yes, 0 No, 1 Abstain, 2 Absent**

- BPWG Proposed Tedizolid Beta-Hemolytic *Streptococcus* Disk Breakpoints: S: ≥15 mm. Pass: 8-0-1-2

A motion to approve tedizolid beta-hemolytic *Streptococcus* disk breakpoint (S≥15 mm) with reflected light was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

TEDIZOLID *STREPTOCOCCI ANGINOSUS* DISK BREAKPOINTS WITH REFLECTED LIGHT

- Data



MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥R+1	0	0	N/A	0
S+R	96	0	0	4 (4.2) •
≤S-1	108	N/A	0	0
Total	204	0	0	4 (2.0)

Motion: S: ≥18 mm
- **Passed: 8 Yes, 0 No, 1 Abstain, 2 Absent**

- Match EUCAST breakpoints; tedizolid only for *S. anginosus* among viridans group streptococci

- BPWG Proposed Tedizolid *Streptococcus anginosus* Disk Breakpoints: S: ≥18 mm. Pass: 8-0-1-2

SC DISCUSSION (MAIN POINTS)
- Breakpoints are for S*treptococcus anginosus* group.
- Only an MIC breakpoint for *Streptococcus anginosis* group in M100.
- Data is *Streptococcus anginosus* group.
- Suggestion to make S≥16 mm. Observations were small at 16 mm. Harmonization with EUCAST at 18 mm.

A motion to approve tedizolid *Streptococcus anginosus* group disk breakpoint (S≥18 mm) with reflected light was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

OXA-48 PRODUCING ENTEROBACTERALES

- Background
  - Common in North Africa, Middle East, and Southern Europe
  - Test resistant to carbapenem, but hydrolytic activity less than other carbapenemase (can have relatively low MIC values)
  - Test susceptible to extended-spectrum cephalosporins but often have ESBLs that confer extended-cephalosporin resistance
- Challenges with new ß-lactam/ß-lactamase Inhibitors vs OXA-48-producing Enterobacterales

	CLSI Interpretive Categories and MIC Breakpoints, mg/L		
	Susceptible	Intermediate	Resistant
Ceftazidime	≤ 4	8	≥16
Ceftazidime/avibactam	≤ 8/4	-	≥ 16/4
Imipenem	≤ 1	2	≥4
Imipenem/relebactam	≤ 1/4	2/4	≥4/4
Meropenem	≤ 1	2	≥4
Meropenem/vaborbactam	≤ 4/8	8/8	≥16/8

  - Relebactam and vaborbactam have limited to no OXA-48 carbapenemase inhibitory activity as noted in product label
  - Meropenem/vaborbactam susceptible breakpoint is 2-dilutions higher than meropenem alone
    - Based on higher dose (2 g q8h vs. 1 g q8h) AND prolonged infusion (over 3 hours instead of 30 minutes)
- Concern: Ineffective therapy with meropenem-vaborbactam vs. OXA-48-producing Enterobacterales?
  - OXA-48-producing Enterobacterales often have meropenem-vaborbactam (MEM-VAB) MICs of 2-4 µg/mL
  - Labs may not know a carbapenem-resistant Enterobacterales (CRE) isolate is an OXA-48-producer and report these isolates as susceptible
  - Patients may be treated with MEM-VAB for OXA-48-producing Enterobacterales with MICs of 2-4 µg/mL
  - Is MEM-VAB effective in vivo for OXA-48-producing isolates with MIC values of 2-4 µg/mL?
- Summary and Recommendations to BPWG
  - Summary: MEM-VAB does not reliably achieve 1-log killing in the neutropenic thigh model vs. OXA-48-producing Enterobacterales with MEM-VAB MICs of 2-4 µg/mL (even though these organisms are considered susceptible)
    - In contrast, MEM-VAB achieved 1-log-kill for KPC-producing Enterobacterales and CAZ-AVI achieved 1-log kill for OXA-48-producing Enterobacterales with MIC of 8 mg/mL
  - Ask: Lower MEM-VAB susceptibility breakpoint from susceptible ≤4 µg/mL to ≤1 µg/mL to avoid ineffective therapy of OXA-48-producing Enterobacterales with MEM-VAB
- BPWG Discussion
  - Sponsor provided ample data to support S: ≤4 µg/mL
  - Concern that if lowered MEM-VAB susceptible BP from ≤4 to ≤1 µg/mL, might take away this drug in situations where might be effective
  - Discussions on KPC-producing Enterobacterales MIC distributions
    - Data presented at MIC_{90} ≤1 µg/mL so would not affect this
    - Others thought seeing more KPC+ isolates with MICs of 2-4 µg/mL
  - BPWG motion: Establish an AHWG (with Hartford group) to review additional data and revisit for June 2023. Pass: 9-0-0-2

SC DISCUSSION (MAIN POINTS)

- Question asked if June 2023 is a reasonable time for the Hartford group to gather data to present. Answer is yes.
- Concern with harmonization with the FDA. Study was funded through an FDA grant.
- Question regarding species and the distribution. Hartford group can look into the species specific data.
- Concerns that lowering the breakpoint will lose a portion of KPC producing isolates.
- Guidance of testing and reporting with a known mechanism of resistance is needed.
- Suggestion to present the known mechanism of resistance in June 2023.

- CLSI imipenem-relebactam breakpoint appears adequate for indicating non-suceptibility for OXA-48 organisms.
- Mechanisms of resistance in gram negative organisms is changing because of the hydrolytic capacity of the enzymes. May be a need to create breakpoints based off PK/PD target attainments and solely MIC.
- OXA-48 is a concern internationally.

CONSIDERATIONS FOR PK/PD BREAKPOINTS FOR M45

- How it might work
  - Obtain reliable MIC
    - Ampicillin = 0.5 µg/mL
    - Penicillin = 1.0 µg/mL
    - Ceftriaxone = 8.0 µg/mL
  - Compare this to the ECV/literature
    - Very little data, appears in line with other reports
  - Compare this to the PK/PD breakpoint (EUCAST, current)
    - Ampicillin, ≤2 / >8 mg/L
    - Penicillin, ≤0.25 / >2 mg/L
    - Ceftriaxone, ≤1/>2 mg/L
  - If ALL these point in the same direction, it can be reassuring.
  - If PK/PD is lower than MIC obtained, more caution may be needed
- EUCAST PK/PD Breakpoints
  - Not species-specific
  - To be applied to species with a lack of data to support a clinical breakpoint
  - Based on conservative PK-PD targets for Gram-positive (or Gram-negatives)
  - If tested MIC > PK/PD breakpoint, EUCAST advised against use of agent (“futility exercise”)
  - If tested MIC < PK/PD breakpoint, EUCAST advised to use with caution
  - Provides MIC, but not an ”S” or “R” interpretation
  - Many limitations: variability across species, lack of clinical data, lack of specific PK-PD target for organism, might bisect ECV (often don’t know it)
- BPWG PK/PD Discussions
  - Reasonable consensus to “start slow” with common antibiotics where there are likely to be ample PK-PD data
  - Agreement not to report S/I/R, but to report an MIC with comment
  - Concern that PK/PD breakpoints might be misused in hospitals without ID specialists
  - Practical constraints for commercial manufacturers (FDA does not allow submission of MIC reporting only without interpretation)
  - BPWG motion that PK/PD breakpoints are worth pursuing with a limited scope. Pass: 10-0-0-1
  - BPWG motion that a PK/PD breakpoint ad hoc working group under BPWG will be formed. Pass: 10-0-0-1

SC DISCUSSION (MAIN POINTS)

- Not work the M45 WG will take on. There will be a BPWG AHWG.
- EUCAST has a document and CLSI needs to evaluate if we should have one too.

- If there was an accurate method used to test the M45 organisms needs to be included. Testing the M45 organisms is needed using a standardized condition.
- Suggestion to consider gram-positive and gram-negative non-specific PK/PD breakpoints.
- Concern with the ability of laboratories to perform an MIC test without a commercial system and with no interpretive criteria. Could this be done without an agreement with FDA.
- Question if the M45 organisms can be sent to reference laboratories. Answer is yes, they could be.
- Outside the US, companies evaluate the organisms that are likely to be PK/PD and establish the EA and bias according to that standard to report the MIC only.

ANAEROBE AD HOC WORKING GROUP METRONIDAZOLE TESTING UPDATE

- Background
  - Discrepancy between metronidazole breakpoints for anaerobes between EUCAST and CLSI
  - Anaerobe WG proposed metronidazole breakpoint decrease in the past by one doubling dilution (S ≤4 µg/mL rather than S ≤8 µg/mL)
  - AST SC did not support given lack of clinical or PK/PD supportive data
- New metronidazole MIC distribution data and AHWG discussion
  - 8699 isolates by agar dilution at Mayo Clinic from 2020-2021
  - Sprandelet al. DMID 2006 and Child et al. J Ped Infect Dis Society 2019 were mentioned as PK/PD metronidazole studies
  - Issues with studies: no consideration of active hydroxyl metabolite and unclear justification for AUC/MIC targets evaluated (based on single *in vitro* chemostat model)
  - No apparent clinical data
  - Decision of Anaerobe AHWG: no data to justify changing breakpoints currently

3.	**METHODS APPLICATION AND INTERPRETATION WG (MAIWG) REPORT (T.KIRN)**
	
	CEFEPIME VS CARBAPENEM ENTEROBACTERALES AST INTERPRETATION
	• Study Methodology
	  ○ Total of 131 genotypically characterized clinical isolates were acquired
	  ○ Isolates were molecularly characterized by laboratory-developed RT-PCR, Cepheid Xpert Carba-R assay, or whole-genome sequencing
	  ○ Conducted phenotypic testing by mCIM, meropenem/ertapenem MIC by manual BMD, cefepime MICs by manual BMD, AST (BD Phoenix), disk diffusion
	  ○ Murine PK studies: Observed cefepime murine concentration-time profile. Dosing regimen matched human exposure.
	  ○ Murine efficacy studies: Used neutropenic murine thigh infection model
	• *In vivo* Study Results Summary
	  ○ Among CRE isolates that test as cefepime-S or cefepime-SDD
	  ▪ Significant blunting of cefepime *in vivo* activity among CP-CRE isolates vs non-CP CRE despite having similar MICs and receiving the same cefepime 2g q8h HSR
	  ▪ Cefepime antimicrobial activity in CRE does not meet 1-log kill threshold indicative of clinical efficacy
	  ○ Among non-CRE (ESBL-like) isolates that test as cefepime-S or cefepime-SDD
	  ▪ In contrast to CRE isolates, administration of cefepime 2g q8h HSR resulted in >2 log kill among non-CRE isolates with cefepime-S indicative of clinical efficacy
	  ▪ However, similar activity was observed among non-CRE and non-CP CRE isolates that tested as cefepime-SDD
	• MAIWG Discussions and Motions
	  ○ Add to or modify current Appendix H to encourage suppression/conversion to R of cefepime results for CREs. Pass: 10-0-0-0
	  ○ Add a comment to Table 2 to encourage suppressing/conversion to R cefepime for CREs. Pass: 10-0-0-0
	  ○ Review criteria for consideration of breakpoint review for cefepime vs Enterobacterales. Pass: 10-0-0-0
	
	PROPOSED CEFEPIME CARBAPENEM ENTEROBACTERALES TABLE H3 (APPENDIX H) REVISION (VOTE #1)

Table H3. (Continued)

Indication	Resistance Mechanism	Method	Specimen Type	Results: Resistance Mechanism Detected	Results: AST (if tested)	Suggestions for Resolution	Report as:	Comments[a]
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP carbapenemases	NAAT, microarray, phenotypic methods such as those described in Tables 3b and 3c	Colony, blood culture	Any carbapenemase(s)	Susceptibility (S or SDD) to 3rd- and/or 4th-generation cephalosporins but intermediate or resistant to at least one carbapenem tested	Repeat resistance mechanism test(s) and AST.	If the discrepancy is not resolved, repeat AST should be performed using a reference method, and the conflicting resistance mechanism and reference AST results should both be reported along with a comment advising caution: "Current clinical and laboratory evidence is insufficient to conclude whether cephalosporin therapy of carbapenemase-carrying strains with an MIC in the S/SDD range will be effective."	1-4, 12-14

- Option #1: If the discrepancy is not resolved, repeat AST should be performed using a reference method, and the conflicting resistance mechanism and reference AST results should both be reported, with cefepime reported as R, along with a comment advising caution: "Current evidence suggests cefepime therapy may not be effective against carbapenem-resistant and/ or carbapenemase-producing strains. Clinical and laboratory evidence is insufficient to conclude whether cephalosporin therapy with other cephalosporins against of carbapenemase-carrying strains with an MIC in the S range will be effective."
- Option #2: If the discrepancy is not resolved, repeat AST should be performed using a reference method, and cefepime S/SDD results should not be reported. The remaining conflicting resistance mechanism and reference AST results should both be reported, along with a comment advising caution: "Current evidence suggests cefepime therapy may not be effective against carbapenem-resistant and/ or carbapenemase-producing strains. Clinical and laboratory evidence is insufficient to conclude whether cephalosporin therapy with other cephalosporins against of carbapenemase-carrying strains with an MIC in the S range will be effective."

SC DISCUSSION (MAIN POINTS)

- Question if significant MIC heterogeneity or variability was seen for cefepime for carbapenemase producing and non-producing isolates. No issues seen.
- Concern that this should be restricted to KPC producers since other mechanisms were not reviewed.
- Should keep to cefepime and not include other cephalosporins.
- Concern that if the laboratory does not know the enzyme it is difficult to know which drug should or should not be reported.
- Concern that resistance mechanism testing is not present in all laboratories.

- If the cefepime breakpoint is dropped to the EUCAST breakpoint, it does this resolve the problem.
- Concern that not all laboratories are currently reporting as resistance.
- Suggestion to not perform the reference method and report as resistance.
- Suggestion to recommend reporting as resistant or to not report.
- Should use genotyping for drugs that have been designed specifically for resistance mechanisms.
- Concern that only making cefepime resistant and not the other cephalosporins may be confusing on the report.
- Suggestion to make cefepime a separate row in the Table H3.
- Concern that the comment is confusing.
- Revision of header for Table H3 from "Molecular Target Results" to "Resistance Mechanism Detected" will be confirmed by MAIWG for M100 34th edition.

A motion to approve Table H3 (Appendix H) edit to report cefepime as resistant for carbapenemase-producing Enterobacterales with no caution comment was made and seconded. Vote: 4 for, 10 against, 0 abstain, 0 absent (Fail)

Against Vote Reasoning:
- Leave option to the laboratory director and not force a resistance reporting.
- Did not agree with removing the caution comment.
- Liked the suggestion of cefepime in a separate row.
- Cefepime would be an effective drug for OXA-48 organisms.
- Preferred the option #2 and remove the reference method recommendation.
- Confusion with the third generation cephalosporins.
- More work needs to be figured out the MAIWG.

PROPOSED CEFEPIME ADDITIONAL TABLE 2A COMMENT

- Additional comment options for cefepime in Table 2A:
  - Option #1: If carbapenem resistance or carbapenemase production (Table 3H) is detected, cefepime should be reported as R.
  - Option #2: For isolates that test carbapenem not susceptible and/or a carbapenemase is detected (Table 3H), cefepime S/SDD results should not be reported.

SC DISCUSSION (MAIN POINTS)
- Consensus supports adding a comment to Table 2A. MAIWG will review.
- Concerns with cefepime breakpoints with Enterobacterales. BPWG will review.
- Suggestion to add a comment to refer to molecular testing.
- Suggestion to state comment as "If carbapenem resistance is detected, cefepime should not be reported as S or SDD."
- Suggestion that a similar type of comment should in the carbapenem row because users would not reflex to the cefepime suppression unless carbapenem resistance is tested.
- Suggestion for location of this information in Appendix A instead of Table 2A.

AZTREONAM AND CEFTAZIDIME-AVIBACTAM BROTH DISK ELUTION STUDY

- Background
  - MBL hydrolyze all beta-lactams, except aztreonam
  - Avibactam inhibits concomitant production of other beta-lactamases
  - Aztreonam and ceftazidime-avibactam is recommended as a preferred or alternative treatment for certain multidrug-resistant gram-negative infections where there are limited therapeutic options (MBL-producing CRE and *Stenotrophomonas maltophilia*)
  - RUO methods: disk proximity, gradient diffusion, etc
  - ARLN labs offer aztreonam-avibactam AST for MBL-producing Enterobacterales
- Disk Broth Elution Study Summary
  - 3 testing sites: JHU, VUMC, and UIC
  - Compared aztreonam and ceftazidime-avibactam (ATM -CZA) Broth Disk Elution (BDE) Test to reference broth microdilution (BMD) aztreonam and ceftazidime-avibactam AST results
  - Isolates Used:
    - Phase 1: 59 Enterobacterales from the CDC AR Bank (56 susceptible (≤4 µg/ml) to ATM-CZA and 3 not susceptible (>4 µg/ml) to ATM-CZA)
    - Phase 2: Metallo-beta-lactamase producing Enterobacterales, *P. aeruginosa,* or *S. maltophilia* clinical isolates at each site (147 total: 125 susceptible, 22 not susceptible)
- Disk Broth Elution Study Data

Phase of Study	Antimicrobial Agent	% Categorical Agreement (N)	% Major Errors (N)	% Very Major Errors (N)
Phase 1	ATM	97.1% (170/175)	6.7% (3/45)	1.5% (2/132)
	CZA	97.1% (170/175)	1.9% (2/105)	4.2% (3/72)
	ATM-CZA	98.3% (172/175)	1.8% (3/169)	0%
Phase 2	ATM	100% (147/147)	0%	0%
	CZA	98.0% (144/147)	10% (1/10)	1.5% (2/137)
	ATM-CZA	97.9% (144/147)	2.4% (3/125)	0%
Overall – Phase 1 & 2 combined	ATM	98.4% (317/322)	5.5% (3/55)	0.7% (2/269)
	CZA	97.5% (314/322)	2.6% (3/115)	2.4% (5/209)
	ATM-CZA	98.1% (316/322)	2.0% (6/294)	0%

- Manufacturer Comparison Study Summary
  - Across all sites, various manufacturers and lot numbers were used throughout the study

- A manufacturer comparison study was conducted to assess accuracy across manufacturers and lots and to determine if there are discrepancies in broth disk elution results dependent on the manufacturer
- All possible combinations of available reagents were tested to evaluate individual performance

- Manufacturer Comparison Study Data

Escherichia coli AR0348

CA- MHB	Hardy				BBL				Thermo			
ATM Disks	O	B	O	B	O	B	O	B	O	B	O	B
CZA Disks	H	B	B	H	H	B	B	H	H	B	B	H
ATM Result	+	+	+	+	+	+	+	+	+	+	+	+
CZA Result	-	-	-	-	+	-	-	+	+	+	+	+
ATM-CZA Result	-	-	-	-	+	-	-	+	+	+	+	+
Interpretation	S	S	S	S	NS	S	S	NS	NS	NS	NS	NS

O: Oxoid; B: Becton Dickinson (BD); H: Hardy Diagnostics

- Conclusions
  - BDE is a precise and effective methodology to determine susceptibility to combination ATM-CZA
  - Not susceptible results should be confirmed by BMD method
  - Manufacturer of CZA disks and CA-MHB important for test efficacy. Not susceptible control is required to ensure accuracy of results.
- MAIWG Discussions and Motions
  - ATM-CZA is not suggested for *Pseudomonas aeruginosa*; should focus on Enterobacterales and *Stenotrophomonas* only
  - Reading guidance for the tubes, look for visible turbidity
  - Performance differences between combinations of broths/disks from different manufacturers – how to provide guidance?
  - Would be in Table 3
  - What to say on how to interpret the results without a breakpoint; using aztreonam breakpoint. Mupirocin is an example of this.
  - MAIWG motion to add this method of Table 3 for Enterobacterales and *Stenotrophomonas*. Pass: 10-0-0-0

SC DISCUSSION (MAIN POINTS)

- Question about study isolates used. Phase 1 was AR Bank isolates with all three sites looking at reproducibility. Phase 2 was metallo-beta-lactamase producing, including *Pseudomonas aeruginosa*. MAIWG only recommending for Enterobacterales and *Stenotrophomonas*.
- Question if the reporting aligns with how public health laboratories are reporting. Public health labs only report MIC and metallo-beta-lactamase producing Enterobacterales. CLSI reporting would be susceptible and not susceptible for Enterobacterales and *Stenotrophomonas*.

- Concern with including *Stenotrophomonas* based on the small resistant isolate data set.
- Suggestion to report as growth (*in vitro* inhibition) and no growth (no *in vitro* inhibition).
- Suggestion to report MIC only and provide further guidance as needed.
- Concern with test performance variability based on media. Important to use the controls. No lot to lot variability with the same manufacturer.
- Errors resolved on repeat testing.

A motion to approve adding the aztreonam and ceftazidime-avibactam broth disk elution method for Enterobacterales and *Stenotrophomonas* in Table 3 was made and seconded. Vote: 11 for, 1 against, 2 abstain, 0 absent (Pass)

Against Vote Reasoning:
- Need more resistance isolate data for S*tenotrophomonas*.
- Not enough time to review the data set prior to the vote.

ANAEROBE AD HOC WORKING GROUP REPORT
- Metronidazole Breakpoint Update: Breakpoint re-evaluation request was change to an informational presentation were made at the Breakpoints Working GroupJanuary meeting. Two additional PK/PD publications were found. These were reviewed with Joe Kuti. The PK/PD data did not necessarily support a change in breakpoint; overall, there are few PK/PD data for metronidazole.
- EUCAST Anaerobe Disk Testing Discussion: Discussed the method. EUCAST continuing to work to expand the offering. Darcie to discuss with EUCAST about participation/collaboration of CLSI member on next testing project. Working group is requesting to present this method to the methods working group at the June 2023 meeting
- Antibiogram: Appendix D – no progress made

4. **QUALITY CONTROL WG (QCWG) REPORT (S. CULLEN)**

CLSI TIER 2 QC

SPR206

- Background

Drug: SPR206	**Abbreviation** (Glossary II & III): No approved abbreviations.	**Previous ID**: SPR01206, CA1263
Solvent (Table 6A): sterile distilled water	**Diluent (Table 6A):** sterile distilled water	**Preparation(Table 6C combination agents):** N/A
Route of administration (Glossary II): IV	**Class (Glossary I & II):** Lipopeptide	**Subclass (Glossary I & II):** Polymyxin
Study Report by: JMI Laboratories (18-SPT-03)	**Pharma Co**: Spero Therapeutics	**Control Drugs**: Colistin and Polymyxin B
Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, cations, zinc, surfactants, etc): • *In vitro* effect studies completed by Micromyx. Stability study completed on the drug powder. • **Equivalency of agar dilution to broth dilution**: No. • **ISO/TS 16782 assessment of Tier 2 study materials**: All 3 CAMHB media lots met ISO/TS 16782:2016 criteria.	
Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):** Additional footnotes needed. No.	
Discussion	Colistin and Polymyxin B used as controls. ≥95% in range with *E. coli* ATCC 25922 and *E. coli* ATCC 13846. This provides evidence that materials and testing process was in control per M23. Only 93.8% in range with Colistin and 92.2% in range with Polymyxin B with *P. aeruginosa* ATCC 27853. Add to Tier 3 list for reassessment	

- Proposed QC Ranges

QC Strain	Range	% In	Mode	Dil	Shoulder	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
E. coli **ATCC 25922**	0.06 - 0.25	95.9	0.12	3	<30%	3@0.12	8@0.12	0.06-0.25 (3)	0.06-0.25 (3)	
E. coli **NCTC 13846 (mcr-1)**	1 - 4	99.6	2	3	>30%	3@2	8@2	1 - 4 (3)	1 - 4 (3)	
P. aeruginosa **ATCC 27853**	0.12-0.5	99.6	0.25	3	<30%	3@0.25	8@0.25	0.12-0.5 (3)	0.12-0.5 (3)	

- QCWG Discussions and Motions
  - QCWG motion to approve the SPR206 proposed QC ranges. Pass: 11-0-1-2

A motion to approve the SPR206 QC ranges for *E. coli* ATCC 25922 (0.06-0.25), *E. coli* NCTC 13846 (1-4), and *P. aeruginosa* ATCC 27853 (0.12-0.5) was made and seconded. Vote: 12 for, 0 against, 2 abstain, 0 absent (Pass)

Abstention Vote Reasoning:
- Not in the room for majority of the presentation.

COLISTIN

- Background

Drug Name:	Colistin					Votes:	No vote (current QC ranges) (For, Against, Absent, Abstain)			
QC Strain	Range	% In	Mode	Dil	Shoulder	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
E. coli ATCC 25922	0.25-2	98.8	0.5	4	46.0% @0.25	1@0.25 2@0.5	8@0.5			Current CLSI range
E. coli NCTC 13846	1-4	100	4	3	94.3% @2	1@2 2@4	3@2 5@4			Current CLSI range. Mode at top of range. Note: these data are from one panel lot and agent known to be impacted by production processes/plastics. Combine with Tier 3 data and reassess in June 2023.
P. aeruginosa ATCC 27853	0.5-4	93.8	0.5	4	64.0% @1	2@0.5 1@1	5@0.5 3@1			Current CLSI range. <95% in range. 15 results out low @0.25. Mode at bottom of range Reassess in future with Tier 3

- QCWG Discussions
  - Multiple QCWG member reported similar results with *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

POLYMYXIN B

- Background and Proposed QC Ranges

QC Strain	Range	% In	Mode	Dil	Shoulder	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
E. coli ATCC 25922	0.25-2	99.2	0.5	4	56.7% @0.25	1@0.25 2@0.5	8@0.5			Current CLSI range Mode at low end of range
E. coli NCTC 13846	1-4	100	2	3	<30%	3@2	8@2			No current range. Proposed new range.
P. aeruginosa ATCC 27853	0.5-2	92.2	1	3	70.2% @0.5	1@0.5 2@1	1@0.5 1@0.5,1 6@1			Current CLSI range <95% in range. Mode at low end of range. Reassess in future with Tier 3

- QCWG Discussions and Motions
  - Multiple QCWG members reported similar results with *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

- QCWG motion to approve the Polymyxin B QC range for *E. coli* NCTC 13846 (1-4). Pass: 11-0-1-2

SC DISCUSSION (MAIN POINTS)

- NCTC strain has the MCR1 resistance mechanism.

A motion to approve the Polymyxin B QC range for *E. coli* NCTC 13846 (1-4) was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

IMIPENEM-XNW4107 (NEW NAME IMIPENEM-FUNOBACTAM)

- Background

Drug: Imipenem-XNW4107 (fixed 8 mg/L) New name Imipenem-funobactam	**Abbreviation (Glossary II & III):** IPF	**Previous ID:**
Solvent (Table 6A): Phosphate buffer pH 7.2 (0.01 M) for imipenem and DMSO for XNW4107	**Diluent (Table 6A):** Phosphate buffer pH 7.2 (0.01 M) for imipenem and sterile distilled water or CAMHB for XNW4107	**Preparation (Table 6C combination agents):** Prepare 10× starting concentration of imipenem at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of XNW4107 160 µg/mL to each of the diluted tubes. For a starting concentration of 16/8 µg/mL in the panel, prepare a 10× stock concentration of imipenem at 320 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of XNW4107 at 160 µg/mL. Then add an equal volume of the XNW4107 160 µg/mL solution to each diluted tube of imipenem. For example, 5 mL of 320 µg/mL imipenem + 5 mL of 160 µg/mL XNW4107 = 10 mL of 160/80 µg/mL imipenem-XNW4107. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.
Route of administration (Glossary II): IV	**Class (Glossary I & II):** β-lactam combination agents	**Subclass (Glossary I & II):** None
Study Report by: JMI Laboratories (21-SNV-02)	**Pharma Co:** Evopoint Biosciences	**Control Drugs:** Imipenem and Piperacillin-tazobactam

Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, cations, zinc, surfactants, etc): • Yes, JMI study reports 21-SNV-02, 21-SNV-03, 21-SNV-04, and 21-SNV-05. • **Equivalency of agar dilution to broth dilution**: No. • **ISO/TS 16782 assessment of Tier 2 study materials**: All 3 CAMHB media lots met ISO/TS 16782:2016 criteria.
Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):** No.
Discussion	Controls: Piperacillin-tazobactam (1 media lot 100% in range) and Imipenem (3 media lots with multiple QC organisms). Imipenem: Media lot C (Oxoid) contributed to 18 or the 20 out of range results with *K. pneumoniae* ATCC BAA-1705 (92.3% in range) and 53 of 70 for *K. pneumoniae* ATCC BAA-2814 (75.2% in range). >95% in range with Media lots A and B. Mode for all media lots are upper end with these QC strains. Both strains are recommended to check QC strain integrity. Note: Out of range high results have been observed with multiple drugs with QC integrity check strains suggesting variable expression. These QC ranges should be reassessed with Tier 3 (potentially as > instead of a specific range).

Drug Name:	Imipenem	Votes:	No votes (For, Against, Absent, Abstain).

QC Strain	Range	% In	Mode	Dil	Shoulder	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
E. coli ATCC 25922	0.06-0.5	100	0.25	4	56.8%@ 0.12	3@0.25	2@0.12 6@0.25			Current CLSI range expanded June 2022 to include 0.06 based on tier 3 with >900 results from 5+ labs. Mode 0.12 with 69% shoulder @ 0.25
P. aeruginosa ATCC 27853	1-4	100	2	3	<30%	3@2	8@2			
K. pneumoniae ATCC 700603	0.06-0.5	99.2	0.12	4	<30%	3@0.12	8@0.12			Current CLSI range
K. pneumoniae ATCC BAA-1705	4 – 16	92.3	16	3	<30%	3@16	1@8 7@16			Current CLSI range 20 results out @ 32 (18 of these with Media C). All media lot modes at upper end.
K. pneumoniae ATCC BAA-2814	16-64	75.2	64	3	<30%	3@64	1@32 7@64			70 results out @ 128 (53 of these with Media C). All media lot modes at upper end.

Imipenem: Media C contributed to 18 of 20 out of range results with *K. pneumoniae* ATCC BAA-1705 (92.3% in range) and 53 of 70 for *K. pneumoniae* ATCC BAA-2814 (75.2% in range). >95% in range with Media lots A and B. Mode for all media lots are upper end. Note: Out of range high results have been observed with multiple drugs with QC integrity check strains suggesting variable expression. These QC ranges should be reassessed with Tier 3 (potentially as > instead of specific range).

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- Proposed QC Ranges

QC Strain	Range	% In	Mode	Dil	Shoulder %	Media Mode	Lab Mode	M23 Range	Range Finder	Comments
E. coli ATCC 25922	0.06/8 – 0.25/8	99.6	0.12/8	3	<30%	3@ 0.12/8	8@0.12	0.06/8 – 0.25/8 (3)	0.06/8 – 0.25/8 (3)	Imipenem alone 0.06-0.5
K. pneumoniae ATCC 700603	0.06/8 – 0.25/8	97.1	0.12/8	3	<30%	3@ 0.12/8	1@0.06 7@0.12	0.06/8 – 0.25/8 (3)	0.06/8 – 0.25/8 (3)	Imipenem alone 0.06-0.5
K. pneumoniae ATCC BAA-1705	0.06/8 – 0.25/8	98.5 (98.3)	0.12/8	3	50.6 (51.3) @0.06/8	3@ 0.12/8	3@0.06/8 5@0.12/8	0.06/8 – 0.25/8 (3)	0.06/8 – 0.25/8 (3)	Imipenem alone 4-8. 92.3% in current range. (see next slide) Routine QC
K. pneumoniae ATCC BAA-2814	0.06/8 – 0.25/8	96.8	0.12/8	3	<30%	3@ 0.12/8	2@0.06/8 5@0.12/8 1@0.25/8	0.06/8 – 0.25/8 (3)	0.06/8 - 0.25/8 (3)	Imipenem alone16-64 but only 75% in current range. (see next slide)
P. aeruginosa ATCC 27853	0.25/8 – 1/8	100	0.5/8	3	41% @ 0.25/8	3@0.5/8	2@0.25/8 6@0.5/8	0.25/8 – 1/8 (3)	0.25/8 – 1/8 (3)	Imipenem alone 1-4

- QCWG Discussion and Motion
  - Reassessed data for BAA-1705 after removing data associated with out of range data to confirm data sufficient for M23 and >95% performance. Revised data presented in (). No significant change.
  - No range for BAA-2814 after confirming >95% after removing data when control out of range for BAA-1705
  - Routine QC and QC integrity check strain *K. pneumoniae* BAA-1705
  - Should combination range be the same as imipenem alone and expand range to include 0.5 (like for IMR) since inhibitor has no activity. Approved based on data presented but if future Tier 3 signal out high, reassess and likely expand.
  - Could visit BAA-2814 QC range for Imipenem-XNW4107 current QC range issue for imipenem alone is resolved in future.
  - QCWG motion to approve the Imipenem-XNW4107 proposed QC ranges (except for *K. pneumoniae* ATCC BAA-2814). Pass: 11-1-1-1

A motion to approve the Imipenem-XNW4107 QC ranges for *E. coli* ATCC 25922 (0.06/8-0.25.8), *K. pneumoniae* ATCC 700603 (0.06/8-0.25/8), *K. pneumoniae* ATCC BAA-1705 (0.06/8-0.25/8), and *P. aeruginosa* ATCC 27853 (0.25/8-1/8) was made and seconded. Vote: 14 for, 0 against, 0 abstain, 0 absent (Pass)

CLSI TIER 3 MIC QC

- Ongoing Requests for Data

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recommended	Concern	Reported
K. pneumoniae BAA-1705	Imipenem/ relebactam	0.03/4-0.25/4	Request additoinal data.	Results at high end with one lab Dec 2021: Added 1 lab with limited 2021 data. Only 2% out high @ 0.5/4 µg/ml for all Tier 3 (n=1147 results). Jan 2022-Jan 2023: no new data	19-Jan
S. pneumoniae ATCC 49619	Levofloxacin	0.5-2	Request additional data or feedback as to whether Tier 3 criteria has been met to widen	Mode 0.5 USCAST data (86% of 1,520). Tier 3: 120 results, mode 0.5, 4% out at 0.25. Jan 2021-Jan 2023: no new data Tier 3: 120 results, mode 0.5, 4% out at 0.25. Dec 2021-June 2022: no new data	18-Jan
K. pneumoniae ATCC 700603	Pip/Tazo	8/4-32/4	Request feedback as to whether additional data is needed	Tier 2 data available. Data from 3 additional labs added June 2022. Data available from 6 labs total (N=735). Apart from two labs with less than 10 datapoints where one was bimodal at the low end of range and one had a mode at the low end of the range, data supports current range. Jan 2023: no new data	Jun-21
K. pneumoniae ATCC 700603	Amp/sulbactam	8/4-32/16	Request feedback as to whether additional data is needed	Data from 2 additional labs added June 2022. Data available from 6 labs total (N=1587). Data supports current range. Jan 2023: no new data	Jun-21
E. coli ATCC 25922	Colistin	0.25-2	Request additional data	Data from 2 additional labs added June 2022. Data available from 6 labs total (N=1946; 1274 from one lab with mode at low end of range and high frequency out of QC low; one other lab with mode at low end of range). Jan 2023: no new data	21-Jun

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recommended	Concern	Reported
P. aeruginosa ATCC 27853	Colistin	0.5-4	Request additional data	Data from 2 additional labs added June 2022. Data available from 6 labs total (N=2001; 1262 from one lab with mode at low end of range; 2 other labs with mode at low end of range). Jan 2023: no new data	21-Jun
E. faecalis ATCC 29212	Amikacin	64-256	Request additional data	CDC reported out low when testing gram-neg. panels, other strains in range. Dec 2021-Jan 2023: no new data	18-Jan
S. aureus ATCC 29213	Rifampin	0.004 to 0.016	Request additional data	One report of S. aureus out low Dec 2021-Jan 2023: no new data; data only from one lab	19-Dec
S. aureus ATCC 29213	Ciprofloxacin	0.12-0.5	Request additional data or feedback as to whether Tier 3 criteria has been met to widen	"bi-modal" MIC distribution noted from three studies. Consider revising range to 0.12-1. (Table 3-28). Refer to USCAST Quinolone report V1.2. New data added in June 2022 supports this. Jan 2023: no new data	18-Jan
S. aureus ATCC 29213	Exebacase	0.25-2	Request feedback. Tier 3 monitoring not needed?	According to sponsor, additional media data suggests a potential to narrow range; however there are developmental concerns that likely warrant removal from Tier 3 at this time. Jan 2023: no new data	22-Jun

- New Requests for Data

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recommended	Concern	Reported
K. pneumoniae ATCC 700603	Aztreonam	8-64	Request more data to determine if upper end of range should be extended or if a range of >16 should be applied.	New request. Concern is too many results at high end of range. Results from 3 labs available for analysis (N=1105). One lab had mode at the high end, the other and the original tier 2 at 32 with no significant shoulder.	22-Aug
E. coli ATCC 25922	Aztreonam/ avibactam	0.03/4-0.12/4	Request additional data & discuss use of stability data	Additional data June 2022 from 1 lab (multiple years) and Jan 2023 data from 1 lab. Tier 3 mode at 0.06/4, with 1% out high; 57% shoulder at 0.12/4 Jan 2023: new data from 1 lab	Jun-21
E. coli ATCC 25922	Aztreonam	0.06-0.25	Request more data to determine whether upper end of range should be extended to 0.5	New signal. Concern is too many results at high end of range. The distribution is nearly biomodal. Results from original Tier 2 and 2 labs available for analysis (N=885). One lab had modes at the high end, the other at 0.12 with a 50% shoulder and the original Tier 2 (from 1987) had a mode at the low end.	22-Dec
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.	22-Nov

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recommended	Concern	Reported
K. pneumoniae BAA-1705	Imipenem	4-16	New request for data	Signal from Tier 2 study showed a mode at 16 (70% of total results) and out of QC results at 32 (6.7%).	23-Jan
K. pneumoniae BAA-2814	Imipenem	16-64	New request for data	Signal from Tier 2 study showed a mode at 64 (67% of total results) and out of QC results at 128 (24.8%).	23-Jan

- New Data Presented (to be discussed in June 2023)
  - Aztreonam-avibactam for *E. coli* ATCC 25922
  - Aztreonam for *K. pneumoniae* ATCC 700603
  - Aztreonam for *E. coli* ATCC 25922

CLSI TIER 3 DISK DIFFUSION QC

- Requests for Data

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	Request data. Revise range? Harmonize reading instructions with EUCAST?	Fuzzy zone edges results in too small zones (also observed for S. aureus ATCC 29213).	Jan 2023: No additional data.	May-21
P. aeruginosa ATCC 27853	Cefiderocol 30 µg	22-31	Collect additional data, preferably from non-European labs. Archive, discuss further or collect more data on Hardy MH?	Major media differences observed in M23 study, which resulted in a 10 mm range. EUCAST QC range is set to 23-29 mm.	Jan 2023: Additional data added on Remel and Hardy MH added. * Comparative data for Hardy original agar and re-formulated agar. Hardy is ready to implement the change immediately as soon as they get the "go ahead" from the CLSI. * Data provided by Remel are within 23-28 mm, but data on remel from other labs are in the upper part of the range.	Jan-21
E. coli ATCC 25922	Minocycline 30 µg	19-25	Monitor. Collect additional data.	Values at top of range and above range from one lab.	Jan 2023: No additional data.	Jan-21
N. gonorrhoeae ATCC 49226	Spectinomycin	23-29	Request feedback/data	QC study out high	Observations in gentamicin QC study, especially with one lab and media	June-22

- Cefiderocol Update
  - Larger zone sizes with 2 media (Hardy and Remel), than others (BBL, Difco, Oxoid). Hardy “refined” formulation resulting in smaller zone sizes (pending release). No response from Remel.
  - Anecdotal report of potential VME associated with larger QC zone sizes. Frequency of occurrence unknown.
  - Discussed narrower range (some media high frequency out of range), add comment, investigate cause or leave as is.
  - Refer to MDSWG suggesting evaluation of multiple media manufacturers with clinical isolates. No new data for remaining organisms/antimicrobial agents.

ROUTINE/STREAMLINED QC

- Current State and Issues
  - Table 2s QC recommendations
    - Not clear/consistent
    - Many have off-scale results and provide minimal value for user QC (eg, *E. coli* ATCC 25922)
  - Table 4A-2 and 5A-2 for combination beta lactams
    - Large number of QC strains (8) if all antimicrobial agents are tested
    - Address confusion regarding strains not listed as “routine QC”. These strains do not assess potency of the beta lactamase inhibitor.
  - Other QC tables have similar but may be fewer issues
- Strategy to Streamline QC Recommendations
  - Focus on issues affecting **user** responsibilities for QC per M07/M02
  - User/laboratory:

- Proper storage (antibiotic deterioration)
- Proficiency of personnel (eg, reading, inoculum; adherence to procedures of incubation times/temperatures; interpreting results)

- Identify critical indicators based on user responsibilities and common failures with surveys
  - Sent to CLSI participants (AKA superusers)
  - Probability of risk for drug classes and drugs within class (high, medium, low)
  - What QC strain is indicator?
  - What are causes; what corrective actions are needed
  - Initial focus on deterioration, start gathering information on training/competency
- Use survey information to
  - Identify drug with highest risk deterioration OR list in order eg, Imipenem > Meropenem, Clavulanate > sulbactam, etc.
  - Identify QC strains that are best indicators and QC strains that do not detect deterioration

- Reference/Considerations for Survey
  - Consider who (user or manufacturer), what (QC strains) and when (lot/shipment, routine QC, training/CA, verification/validation, troubleshooting)
  - Created list of all antimicrobial agents and drug classes from glossaries.
  - Used the troubleshooting table to highlight responsibilities
  - Manufacturing issues should be detected when testing to approve/release lot or batch (whether manufactured in-house or commercially).
  - User/lab QC testing should include:
    - Shipping/stability: QC testing upon receipt or before use
    - Storage/stability: Routine QC testing
    - Technique: training and competency assessments (eg, preparation, set up, reading, endpoints, etc.)
    - Procedure: depends on other QA processes (eg, temperature, reading time, calibration of equipment, etc.)
  - Responsibility: materials (manufacturer), shipping/storage (lab), training/CA (lab)
- Proposed Table 2 Revisions
  - To be discussed in June 2023
  - Proposed added text 1: “Test one or more QC strains that are indicators of drug deterioration upon receipt of lot/shipment and routinely (weekly, daily) to ensure continued quality of the materials after shipment and storage.”
  - Proposed added text 2: “Test one or more QC strains that are indicators of drug deterioration upon receipt of lot/shipment to ensure quality of the materials after shipment. Select one QC strain that is an indicator of drug deterioration to test routinely (weekly, daily) to ensure continued quality of the materials after storage.”
- Next Steps
  - Conduct Clin Micro Survey on frequency of out-of-range QC, causes, corrective actions
  - Mock-up table (propose separate table/guidelines to minimize confusion)
  - Initial launch may start by removing those with no value (to detect deterioration) and continue to assess opportunities for further reduction in QC strains and/or frequency
  - Propose changes to Table 2 QC boxes
  - Share with CMS/CAP for feedback and potential support for inspector guidance
  - Suggest IQCP to justify streamlined QC
  - Conduct survey and assess disk diffusion (additional considerations for open packages, media/disk combinations)

	○ Recommendations for competency assessment/training **SC DISCUSSION (MAIN POINTS)** • Question about inoculums and if it is a tech reading issue or nephelometer calibrating issue. Will look into indicators and then provide guidance to the laboratory how to handle. • Question about deterioration. Will be applied to MIC first and then disk diffusion. **MISCELLANEOUS FUTURE DISCUSSIONS** • Beta lactam agents ○ Should they only be in "main" QC table (eg, 5A-1), combo table (5A-2) or both? ○ Should we list all QC ranges approved or select ones to include in M100? ○ How do we ensure there is clear understanding of what QC needs to be tested routinely for single beta lactam agents? • Other clarifications/improvements needed to M100 for QC?
5.	**ADJOURNMENT** Dr. Lewis thanked the participants for their attention. The meeting was adjourned at 5:00 PM Eastern (US) time.

2023 JANUARY AST MEETING SUMMARY MINUTES PLENARY 3: Tuesday, 23 January 2023 (In-person/Hybrid) 7:30 AM – 12:00 PM Eastern (US) Time	
#	**Description**
1.	**OPENING** Dr. Lewis opened the meeting at 7:30 AM Eastern (US) time.

2.	**JOINT CLSI-EUCAST WG REPORT (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. **RECORD KEEPING AND DATA STORAGE** • Excel spreadsheet (agenda book "CLSI EUCAST Joint WG Studies 12.12.33_D_JH) • Each new study is assigned a JWG (Joint WG) number consisting of "JWG-year-and next available study number (JWG-2022-1 to JWG-2022-x)" • Sheet 1 contains data stored by CLSI in SharePoint • Sheet 2 contains names of JWG members with their corresponding dates of membership • Sheet 3 contains an ongoing summary of disk content studies • Sheet 4 contains an ongoing summary of pre-QC studies • CLSI is looking into better ways to archive data. **M23 SUPPLEMENTS** • M23S, "Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria": This document describes the necessary technical steps for establishing the optimal disk content (potency) for single antimicrobial agents without the addition of enhancing or inhibiting substances. • M23S2, "Process to Submit Disk Content (Potency) Data for Joint CLSI-EUCAST Working Group Review and Approval": This document describes the process to submit disk content (potency) data to the joint CLSI-EUCAST working group for review and approval. **DISK CONTENT SELECTION IN PROGRESS**

WG Assigned Study #	Agent	Sponsor	Notes
JWG-2022-2	Contezolid	MicuRX	Phase 2 completed; additional studies in progress
JWG-2022-3	Imipenem-XNW4107 (fixed at 8 mg/L)	Evopoint Biosciences [a]	Phase 1 completed
JWG-2022-4	RG6006	Roche	Ongoing studies for BMD reference method
JWG-2022-5	Aztreonam-nacubactam (1:1) and Cefepime-nacubactam (1:1)	Meiji	Phase 1 planning
JWG-2022-9	Zoliflodacin	Nobelex	Phase 1 planning
JWG-2023-1	BWC0977	Evopoint Biosciences [a]	Phase 1 planning

[a] formerly Sinovent Pharmaceuticals

MHA AGAR EVALUATIONS IN PROGRESS

WG Assigned Study #	Agent	Sponsor	Notes
JWG-2022-6	Debio 1452	Debiopharm	Disk manufacturers report no problems with low content disks.
JWG-2022-7	Cefepime-enmetazobactam	AdvanZ	Projected Spring 2023
JWG-2022-8	Ceftazidime-avibactam	Pfizer	Projected Spring 2023

IMIPENEM-XNW4107 DISK CONTENT SELECTION – PHASE 1

- Background
  - XNW4107 is a novel ß-lactamase inhibitor being developed in combination with imipenem by Evopoint Biosciences (formerly Sinovent Pharmaceuticals) with activity against Class A, C, and many Class D ß-lactamases
  - January 2021: FDA approved for QIDP and fast track status for cUTI and HABP/VABP

- February 2022: 4 Phase 1 studies completed: FIH (NCT04482569), Pulmonary PK study (NCT04802863), Age/gender PK study (NCT04801043), Renal impairment PK study (NCT04787562)
- Active Phase 3 clinical trials for HABP/VABP (NCT05204563) and cUTI(NCT05204368)
- Ongoing drug development studies including CLSI M23 Tier 2 broth microdilution, disk development, and Tier 2 disk diffusion quality control studies

- Phase 1 Disk Development
  - Imipenem (10 µg) disk currently used by CLSI and EUCAST (Working group recommended testing commercial and laboratory prepared disks)
  - Working group recommend testing imipenem-XNW4107 disk concentrations of: 10/1, 10/2.5, 10/5, 10/10, 10/20, and 10/30 µg
  - Proposed testing 10 Enterobacterales, 10 *A. baumannii*, and 10 *P. aeruginosa* isolates in duplicate representing a range of imipenem and imipenem-XNW4107 MIC values covering S, I, and R in Phase 1
  - Goal: To advance 2-3 imipenem-XNW4107 disk concentrations to Phase 2 M23S (2020) testing with activity against Enterobacterales, *A. baumannii*, and *P. aeruginosa*
- Summary of optimal Phase 1 disk concentrations

			Optimal imipenem-XNW4107 disk potency (µg)					
Organism group	Analysis Performed	Replicate	10/1	10/2.5	10/5	10/10	10/20	10/30
A. baumannii	Zone diameter	A				X	X	X
A. baumannii	Zone diameter	B				X	X	X
Enterobacterales	Zone diameter	A			X	X	X	X
Enterobacterales	Zone diameter	B			X	X	X	X
P. aeruginosa	Zone diameter	A			X	X	X	X
P. aeruginosa	Zone diameter	B			X	X	X	X
A. baumannii	Consecutive potency	A		X		X	X	
A. baumannii	Consecutive potency	B						X
Enterobacterales	Consecutive potency	A			X	X		
Enterobacterales	Consecutive potency	B			X			
P. aeruginosa	Consecutive potency	A			X	X	X	
P. aeruginosa	Consecutive potency	B			X		X	X
A. baumannii	Greatest zone difference	A	X					
A. baumannii	Greatest zone difference	B	X	X		X		
Enterobacterales	Greatest zone difference	A				X		
Enterobacterales	Greatest zone difference	B	X	X		X		
P. aeruginosa	Greatest zone difference	A					X	
P. aeruginosa	Greatest zone difference	B					X	

Optimal imipenem-XNW4107 disk potencies are shaded in grey.

NEW ACCEPTIBILITY OF THE MUELLER-HINTON AGAR SOURCES DOCUMENT

- Title: “Procedure for Confirming the Acceptability of the Mueller-Hinton Agar Sources for Subsequent use in CLSI and/or EUCAST Studies to Establish Disk Diffusion QC Ranges”
- Aim
  - To confirm that the MHA sources selected are acceptable prior to performance of a full QC study to avoid problems when establishing QC ranges.
  - The testing procedure is designed to minimize factors (eg, inoculum, incubation, measuring zones) other than the MHA source that might affect the results.
- Study Protocol
  - QC strains and NWT strains/isolates
  - Disks from 2 manufacturers (same lots to be used in the QC study if possible) + 1 control agent
  - MHA from at least 4 manufacturers
  - Specifications as per ISO+TS+16782 (2016)
  - At least 2 of BBL, Hardy, Oxoid and Remel
  - Triplicate tests for each disk-agar combination
  - Calculate mean and median values, standard deviation and range for each disk-agar combination
  - Optimally, select MHA sources with mean or median zone diameter values within ±1 mm
- Updates Since Last Meeting
  - Minor clarifications based on discussions at CLSI meeting in June and subsequent review by the breakout group and the Joint WG.
  - A template in Excel for data registration to be provided with the SOP.
  - Two example tables as appendixes:
    - Example of acceptable results for three of four media sources
    - Example of results that need discussion with the CLSI-EUCAST Joint Working Group to decide how to proceed
- Plans Forward
  - Approved by Joint CLSI-EUCAST WG
  - Approval by CLSI (this meeting) and EUCAST (Steering Committee).
  - After approval, the document will be posted online and will be freely available together with the Excel template for result registration.
  - The Joint WG will continue to work to improve the procedure after reviewing real data produced according to the procedure.

SC DISCUSSION (MAIN POINTS)

- The procedure is a one-time protocol to make sure the Mueller Hinton agar used in the tier 2 QC study is adequate. It could possibly be used for other purposes, such as disk correlate studies.
- Question if the procedure needs to be followed with the same media lots as in the subsequent QC study. No, only with the same manufacturer of media.
- Changes in QC results over time and different experiences is monitored with tier 3 studies. The procedure could be used to investigate and troubleshoot tier 3 studies.
- Question if the procedure is a requirement or recommendation. The procedure is an encouraged protocol not a requirement. The intent is for the Joint WG to encourage all sponsors after the selection of disk potency to use this procedure before going into the full QC study. It will be up to the sponsor if they want to share the data with CLSI. A line will be added to the QC summary asking if a media study was conducted.

	• Opportunity for troubleshooting problematic zones (eg, fuzzy zones) using this procedure.
	A motion to approve the "Procedure for Confirming the Acceptability of the Mueller-Hinton Agar Sources for Subsequent use in CLSI and/or EUCAST Studies to Establish Disk Diffusion QC Ranges" as an encouraged (not required) procedure was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)
	JOINT WG - WHAT IS NEXT? • Continue with disk diffusion content selection and MHA (for QC) acceptability studies • Further work on harmonization of QC. Harmonization of analysis of QC data (including statistical analyses). • Disk diffusion and BMD reading guide harmonization

3. **METHODS DEVELOPMENT AND STANDARDIZATION WG (MDSWG) REPORT (B. ZIMMER AND D. HARDY)**

REFERENCE METHODS FOR AST

- Should disk diffusion remain a CLSI "reference" method?
- Value of Reference MIC
  - Current process is to use reference MIC for PK-PD assessments and reference for most FDA trials of new diagnostics (some exceptions)
  - Breakpoint setting process is MIC to disk
  - Disk:MIC correlate data have been published in CLSI documents inconsistently. Often "after the meeting" vote.
- CLSI Definitions
  - Reference method: a methodology that has exact and clear descriptions of the necessary conditions and procedures that provide sufficiently accurate and precise laboratory data for it to be used to assess the validity of other laboratory methods (MM19)
  - Reference method: an exactly defined technique that is used in association with an internationally agreed reference preparation to provide sufficiently precise and accurate data for assessing the validity of other methods (modified from ICSH) (H02)
  - Reference method: a 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 accuracy and precision of the method are commensurate with the method's use for assessing the accuracy of other methods for measuring the same property values or for assigning reference method values to reference materials (M52)
  - Comparator method: method against which a new system is evaluated; NOTE: Comparator methods may include reference methods or a previously verified US FDA-cleared commercial system (M52)
- Challenges with Disk Reference Method
  - One degree separated from the value used to set the breakpoint (ie, disk breakpoints are a correlate with acceptable errors)
  - Media differences noted throughout CLSI studies
    - Atypical S. *aureus* (some brands do not support growth)
    - *Burkholderia* study (some brands yield very different zones)
    - QC studies
  - Disk content differences noted throughout CLSI studies
    - Disk-broth elution method for ceftazidime-avibactam and aztreonam, issues noted with one brand of disk
    - QC studies
- Examples of Challenges with Disk Diffusion as a Reference Method
  - Many recent disk-to-MIC correlates are imperfect
  - Special "rules/exceptions" for disk diffusion and some drugs/organisms
  - Use as reference for validation studies in laboratories
  - Use as reference method for direct blood disk diffusion method
  - Use as reference method for development of machine-learning approaches to translate WGS data to susceptibility prediction
- Summary
  - Disk diffusion should not be a CLSI "reference standard" method
  - 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
- Does not serve as a good reference for device evaluations, or breakpoint implementations for clinical laboratories

- MDSWG Discussions
  - Disk diffusion as a qualitative test for S/I/R has worked well for many bug/drug combinations for many years.
  - Disk diffusion is a standardized method, but may not be a "reference method."
  - A vote was not taken (time issues)
  - Sentiment of group: Would suggest a plan for forward movement of the proposal which will include specific language changes and specific indications and clarifications for appropriate use of the disk test. Via ad hoc WG with MDSWG.

SC DISCUSSION (MAIN POINTS)

- Concern M100 and M02 are not consistent with disk diffusion.
- Intent is to remove the term reference method and keep MIC as the standard methods. It does not mean disk diffusion testing would be removed or discouraged.
- Concern with the quality of the disk diffusion results because of media variability and disk variability.
- Need to keep disk diffusion because of international users.
- The Antifungal Subcommittee needs to be included on this decision.
- In EUCAST documents, it is stated that disk diffusion is a standardized method and it is calibrated to reference MIC values. Provide guidance on how to implement disk diffusion in laboratories. EUCAST 's responsibility is to calibrate the disk diffusion test to MIC and then give advice on how the clinical laboratory should implement disk diffusion in the laboratories. Disk diffusion is not called a reference method.
- Many utilities for this method in the clinical laboratory (eg, new drug approval correlation studies, clinical diagnosis, comparator studies). FDA states it is a reference method in the sense that it is an accurate method for clinical use.
- Need a clear statement on the impact and the risk benefit of this change. Also, need a clear idea of what should and should not be done with disk diffusion.
- M52 states that a comparator method can be used. Also, with a new breakpoint verification, in which a breakpoint changed and the laboratory is trying to verify on a FDA cleared system, M52 currently states a reference method should be used. If changed to a comparator method, does that mean a FDA cleared system can be used to verify a new breakpoint on a different system? It may cause some problems.
- Disk diffusion and broth are not equal. Need to be clear that these are not equivalent methods and they cannot be substituted with each other 100% of the time.
- Sponsors use 2018 FDA guidance which states for sponsors to reference a standard method.
- Confusion between the terms reference method and standard method.
- Communication and a clear explanation will be need for users.
- Concern on downstream effects and not being able to report an MIC when verified with disk diffusion. Costly for laboratories to send out for verification.
- M100 does not state that disk diffusion is a commercial method.
- Suggestion to work with CMS because may impact them as well.
- Need to summarize the implications and recommended actions. Then work on word smithing.
- Helpful for M52 committee to coordinate on these efforts to include in the current M52 revision.
- Consensus is to move forward in the review of this topic in the MDSWG with the M52 committee.

ENTEROBACTERALES PIPERACILLIN-TAZOBACTAM DISK STUDY

- Variability on disk design
  - CLSI: 100 ug piperacillin/10 ug tazobactam
  - EUCAST: 30 ug piperacillin/6 ug tazobactam
- Background
  - CLSI 2021 revision of TZP breakpoints
  - Lack of contemporary data and disk-to-MIC correlates, historical data used for disk breakpoints
- Challenges
  - Challenge in disk diffusion correlates from old data.
  - OXA-1 prevalence increased after previous studies.
  - TZP has poor activity against isolates that harbor OXA-1 beta-lactamase
  - Present in ~30% of isolates in the US
  - TZP susceptibility testing is challenging for isolates with OXA-1 which tend to have MICs straddling the breakpoints 8-16 µg/ml
  - MERINO trial: Isolates co-harboring OXA-1 and ESBLs accounted for a major portion of strains that were not susceptible by BMD but susceptible by routinely used laboratory methods
- Study Aim
  - Evaluate if a different TZP disk potency could aid with accuracy issues
  - Generate contemporary disk-to-MIC correlation data to assess performance of TZP breakpoints
  - Determine disk potency that performs best for isolates with OXA-1
- Methodology
  - Pre-phase 1: Titrate piperacillin concentration
    - WT and NWT *E. coli*, *P. aeruginosa* strains (n=4)
  - Phase 1: Titrate tazobactam concentrations with a fixed piperacillin concentration
    - WT and NWT *E. coli*, *P. aeruginosa* strains (n=8)
  - Phase 2: Selected 3 concentrations of TZP disk to vs. reference broth microdilution
    - n=100 isolates of E. *coli* and K. *pneumoniae*
    - Some isolates excluded due to dropped disks
  - All disk diffusion tested across 3 brands of MHA
    - Remel, Hardy, BD
  - Reference BMD:
    - Two brands piperacillin
    - Two brands tazobactam
    - Two brands of CA-MHB
    - N=8 MICs per isolate
    - Mode used as “reference” MIC
- Conclusions
  - 20 µg/ 5 µg TZP disk yields improved separation between susceptible and not-susceptible isolates of *E. coli* and *K. pneumoniae* isolates tested
  - 30 µg/ 6 µg (current EUCAST disk) was the next best

- 100 µg/ 10 µg (current CLSI disk) does not separate S and NS isolates well, especially those harboring OXA-1
- Possible Future Directions
- Evaluate the 3 disk concentrations with an expanded set of isolates: include additional species of Enterobacterales
- Evaluate vs. *P. aeruginosa*
- Multicenter evaluation

- MDSWG Discussion
  - Is the 20/5 disk different enough from the current EUCAST 30/6 disk to warrant a totally new disk?
  - Collaboration with Joint CLSI-EUCAST Working Group disk development ad hoc WG?
  - No votes taken at MSDWG
  - Discussion included new process at FDA. Lots of communication.

SC DISCUSSION (MAIN POINTS)

- Consensus is to move forward with this project.
- Next steps are to work with the Joint WG to look at the studies and harmonize.
- Suggestion to have communication with FDA prior to bring forward to the Subcommittee.
- Suggestion to look at all organisms affected from a disk change.

CEFAZOLIN HIGH INOCULUM AD HOC WORKING GROUP UPDATE

- Background
  - Cefazolin clinical failures have been reported for deep-seated methicillin susceptible *Staphylococcus aureus* (MSSA) infections, particularly infective endocarditis
  - Cefazolin failure observed in isolates with inoculum effect (CzIE)
  - Phenotype NOT detected by routine susceptibility testing
    - Gold standard assay: BMD at standard inoculum (10^5 CFU/mL) and high inoculum (10^7 CFU/mL)
    - An increase in cefazolin MIC to ≥16 µg/mL with the high inoculum is considered positive for CIE
  - Availability of a rapid test for the CzIE phenotype could positivity impact treatment decisions for CzIE-positive MSSA in select clinically appropriate scenarios (ie, high inoculum infections such as endocarditis)
  - Prevalence of CzIE was previously not well-defined in N. America
  - A rapid method for CzIE detection was published in 2021, but is not practical for most clinical laboratories
- WG Objectives
  - PHASE 1: Assess the prevalence of CzIE phenotype in MSSA isolates in contemporary US strains
  - PHASE 2: Evaluate the revised rapid CzIE assay. Assess suitability for multi-center evaluation.
  - PHASE 3: Perform multi-center evaluation.
- Phase 2 Progress
  - Multiple different rapid methods have been assessed
  - WG felt that feasibility for clinical laboratories was most important
  - Methods 1: Rapid CzIE Nitrocefin Test Summary
    - The CzIE rapid test is sensitive and specific for MSSA with the CzIE
    - Test performance is brand dependent, likely due to differences in the specific formulations of BHI across suppliers

- Test performance between Type A enzymes did not seem to be affected by different suppliers of BHI
- The worst performance characteristics were seen for Type C enzymes

- Method 2: CzlE Disk Elution Method
  - Strengths:
    - No special reagents or media necessary
    - No specialized expertise required
  - Limitations:
    - Hands-on time
    - Still not "rapid" (requires 48 hours)
    - Does not work well for BlaZ Type C isolates

- Phase 3 Progress
  - Study protocol confirmed –Rapid CzlE Nitrocefin Method
  - Study sites confirmed –LAC, CHLA, UAH, DEA, CAB
  - Isolates selected
  - Required supplies need to be defined, acquired and distributed

SC DISCUSSION (MAIN POINTS)

- Suggestion to use a supplemental QC strain to confirm the media performance and selection of media.
- Suggestion that as these new platforms develop CLSI needs to be more involved to acquire data where there is no clinical data for other drugs.
- Question when a laboratory would be asked to perform. Clinical studies will show if this is useful and dictate how the test might be used.

DIRECT BLOOD DISK DIFFUSION AD HOC WORKING GROUP UPDATE

- 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)
- 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.

- 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 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
- Process
  - Main comparator of direct DD data is DD performed at site and, secondarily, DD performed at reference lab
  - MIC data included in presentation for background information
  - The following was the process for assessing zone cutoffs:
    - Applied zone cutoffs based on direct read (DISK) vs. standard site DD (std DD)
    - Examined those zone cutoffs vs. both REF DD and MIC
- *P. aeruginosa* Cefepime Disk Diffusion (16-18 hour read) Data
  - Current cutoffs

Current cutoffs (mm)		
S	I	R
≥18	15-17	≤14

  - Cefepime 16-18 hour vs Std DD at site

	Std DD			
16-18 hr	S	I	R	Grand Total
S	51			51
I	6	8	1	15
R		3	24	27
Grand Total	57	11	25	93

CA	83/93	89.3%
VME	0/25	0
ME	0/57	0
mE	10/93	10.75%

Category agreement at 89%, only minor errors

- Cefepime 16-18 hour vs REF DD

	REF DD			
16-18 hr	S	I	R	Grand Total
S	41			41
I	1			1
R			3	3
Grand Total	42		3	45

CA	44/45	97.8%
VME	0/3	0
ME	0/42	0
mE	1/45	2.2%

Category agreement at 97.8%

- Cefepime 16-18 hour vs MIC

	MIC			
16-18 hr	S	I	R	Grand Total
S	49	3		52
I	1	8	6	15
R			27	27
Grand Total	50	11	33	94

CA	84/94	89.4%
VME	0/33	0
ME	0/50	0
mE	10/94	10.6% (undercalling R)

- *P. aeruginosa* Cefepime Disk Diffusion (16-18 hour read) Prior Voting History
  - Proposed zone cutoffs

Proposed zone cutoffs (mm)		
S	I	R
≥18	14-17	≤13

  - AST SC voted against proposed cutoffs (4-5-1) in January 2022
- MDSWG Discussion and Vote

- Current zone cutoffs show performance that is close to acceptable vs. Std DD (CA=89.3%; mE10.75%) and REF DD (CA=97.8%). Performance vs. MIC (CA=89.4%; mE10.6%).
- Only minor errors; no VME or ME
- MDSWG motion to retain the current zone cutoffs and approve method for cefepime 16-18 hour direct read for *P. aeruginosa*.Pass 10-0-0-0
- Given 10.6% mE (predominantly under calling R) vs. MIC, intermediate readings should be confirmed by additional testing by a comparator method

SC DISCUSSION (MAIN POINTS)

- Suggestion to state comparator method versus reference method.
- Suggestion to remove comparator method.
- Similar to EUCAST AST method where the I category is the ATU for across all drug bug combinations. When the reading fall into the I or ATU, the EUCAST recommendation is that the result cannot be interpreted and to reincubate if too early. This method is only used to get an earlier AST result but sometimes it is unachievable. That does not stop the laboratory from using the traditional standard method.
- Percentage of isolates that fell into the intermediate category is 15-16%. This is a challenge set.
- Direct Blood Disk Diffusion AHWG is comfortable with the MDSWG voted on proposal.
- Suggestion to look at the correlation studies with the data.
- Concern that typically a comment is not added for 10% minor error rates. The blood culture source is a more serious infection. An intermediate result may question the variability of the testing and the dosage, so the comment would address the error of the testing and the need to do reflex testing instead.
- Wordsmithing on the intermediate comment will need to be completed.

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

EXEBACASE TESTING OF COAGULASE-NEGATIVE STAPHYLOCOCCI

- Background
  - Exebacase (CF-301) is a first-in-class, anti-staphylococcal lysin (Glossary I [Part 2], CLSI M100 32nd ed); to be reviewed by CLSI.
  - The exebacase MIC method for S. *aureus* broth microdilution (BMD) MIC was approved by CLSI Subcommittee for AST in 2017 and Tier 2 QC range approved and subsequently published in 2020 (CLSI M100 30th ed) prior to initiation of the Phase 2 trial (ClinicalTrials.gov Identifier:NCT03163446).
  - The media used for testing exebacase against S. *aureus* is cation-adjusted Mueller-Hinton broth (CAMHB) with horse serum (25% v/v) + 0.5 mM DL-dithiothreitol (CAMHB-HSD)1.
  - Following analyses of MIC data generated from extensive MIC testing at multiple independent laboratories to evaluate exebacase *in vitro* activity against S. aureus and evaluate test performance, proposed guidance for the determination of exebacase broth microdilution (BMD) MIC end points for S. *aureus* was approved by CLSI Subcommittee for AST in June 2022.
  - In addition, CAMHB-HSD was approved by CLSI as an acceptable media for BMD MIC testing of exebacase against beta-hemolytic streptococci (June 2022).

- Following extensive MIC testing at multiple independent laboratories to evaluate exebacase *in vitro* activity against staphylococci other than *S. aureus* (coagulase-negative staphylococcal species, CoNS) and evaluate test performance we propose CAMHB-HSD media is acceptable for BMD testing for CoNS if minor modifications in incubation conditions are employed.

- 2022 Surveillance Study
  - US bloodstream infection isolates from the 2020 SENTRY surveillance program tested by JMI laboratories
  - Exebacase MIC panels prepared using CAMHB-HSD, a CAMHB growth control (for oxacillin) and CAMHB-HSD growth control were included
  - When reading the CoNS isolates, it was noted that the CAMHB-HSD growth control was markedly decreased compared to growth in CAMHB when incubated in ambient atmosphere
  - Approximately 40% of CoNS tested exhibited poor growth in CAMHB-HSD in ambient atmosphere, in particular *S. epidermidis*
- Exebacase MIC Testing of CoNS
  - Aim: Improve growth of *S. epidermidis* in CAMHB-HSD
  - The following were investigated to overcome serum sensitivity of *S.* epidermidis observed in CAMHB-HSD incubated at 35^{oC} ± 2^{o}C in ambient air, as poor growth impaired the reading of MIC end points:
    - Use of heat inactivated serum: No improvement was observed.
    - Incubation conditions: 35^{oC} ± 2^{o}C in ambient air vs 5% CO_2 and 16-20 hours vs 20-24 hours
    - QC strain *S. aureus* ATCC 29213 tested in the same conditions
- Method Verification (MV) Isolates
  - In addition to recommended QC strains, a set of CoNS method verification isolates were selected and tested (N= 30) to assess the performance of MIC panels prepared by testing laboratories and assess MIC reproducibility and magnitude of MIC variation expected
  - The 30 CoNS Method Verification Isolates were tested in triplicate at LSI
- 3-Site Reproducibility Study of MV Isolates
  - Initial 3-site study using the SAME exebacase frozen MIC panel; each site tested 6 replicates (2 different lots of horse serum [HSD1, HSD2] x 3 days).
  - MIC was read at 100% inhibition following incubation in 5% CO_2 for 20-24 hours.
- 4-Site Reproducibility Study of *S. epidermidis* MV Isolates
  - Modal MIC distribution for *S. epidermidis* MV isolates (N=20)
  - MIC panels were prepared by each of the participating testing laboratories
  - Results are within 3-4 dilution range for 19/20 isolates; Strain 8 (VISE) is the exception
- 4-Site Reproducibility Study of Other Staphylococcal MV Isolates
  - MIC panels prepared by individual testing laboratories
  - 5 additional CoNS species tested (*S. capitis*, *S. caprae*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*)
  - Results are within 1-3 dilution range for 10/10 isolates
- Exebacase MIC distributions of clinically relevant CoNS isolates using CAMHB-HSD and incubation at 35^{o}C ± 2^{o}C, 5% CO_2 for 22 hours were shown for the following studies:
  - Evaluation of exebacase *in vitro* activity against US bloodstream infection CoNS isolates (N= 198) from the 2020 SENTRY Surveillance Program, testing performed by JMI Laboratories.
  - Evaluation of exebacase *in vitro* activity against *S. epidermidis* (N= 76) from consecutive patients with native valve and intra-cardiac devices, infective endocarditis diagnosed or treated at the Clinic Hospital of Barcelona, Spain during 2010-2020. Testing performed by the University Clinic Hospital Barcelona.

- Evaluation of exebacase *in vitro* activity against CoNS isolates (Total N=327) by ContraFect. Isolates were acquired from various specialist culture collections (136 isolates from ATCC, BEI, USDA, CCUG, infection source often not known) as well as clinical isolates (N=191 from various US and ex-US hospitals from various infection sources

- Comparison of Exebacase MIC Results: Ambient vs 5% CO_2 Incubation
  - 5% CO_2 incubation provides better growth in CAMHB-HSD, in particular for S. *epidermidis*
  - MIC distributions (S. *epidermidis* in particular) are shifted to the right
- Exebacase QC Summary
  - QC strain: S. *aureus* ATCC 29213
  - MIC distributions for S. *aureus* ATCC 29213 tested in 5% CO_2 are within the current CLSI range of 0.25-2 ug/mL
- Proposed Change to M100
  - For exebacase broth microdilution MIC testing of staphylococcus species other than S. *aureus*, CAMHB-HSD media is acceptable with the following modifications:

Testing Conditions	
Medium:	Broth dilution: CAMHB-HSD
Inoculum:	Colony Suspension, equivalent to a 0.5 McFarland Standard
Incubation:	35°C ± 2°C; **5% CO_2**
	Dilution methods: **20-24 hours**
MIC end points read as the lowest concentration that completely inhibits organism growth	

  - Routine QC recommendations: S. *aureus* ATCC 29213

SC DISCUSSION (MAIN POINTS)

- Concern it is premature to put the CO_2 or the extended incubation in the M100 prior to the revalidation of the QC.
- Contrafect is about to start a clinical trial. The focus of this drug has been about S. *aureus*. S. *epidermidis* will be studied. Need endorsement of the method to start the study.
- Suggestion to endorse the change in methodology for the CoNS and preliminary QC indication with the contingency of the QC reassessment prior to publication.
- Suggestion to publish and reconsider the QC after more data.
- Concern that this would most likely not pass with EUCAST because of the changed reference method. No data with agar dilution.
- Exebacase is not antibiotic it is a lysin. It will go through FDA as biologic and has a different set of rules. Exebacase is given in addition to another drug. FDA wants sponsor to work with CLSI to make sure a robust MIC method was performed.
- Question if testing was completed at 33-37°C and if the tolerance of the CO_2 concentration is known. Contrafect is investigating.
- QCWG will review the data to assess the QC performance and range with the method modifications to be presented in June 2023.

A motion to approve the exebacase broth microdilution MIC testing of *Staphylococcus* species other than *S. aureus* for CAMHB-HSD media with the 5% CO_2 and 20-24 hours incubation modifications with the contingency to confirm the acceptability of QC performance and range prior to publication was made and seconded. Vote: 11 for, 1 against, 0 abstain, 2 absent (Pass)

Against vote reasoning:

- Uncomfortable with applying the method to not an antimicrobial.

ROCHE RG6006 UPDATE

- Letter in agenda book from Andrej Trauner, Ph.D. and Claudia Zampaloni, Ph.D.; Roche Pharmaceuticals Division
- Based on all tests performed at EDL, Roche suggests that testing of RG6006 in standard CAMHB on panels sealed with an adhesive plastic film and reading of M/Cs at the dramatic decrease in growth should be further investigated as the reference MIC method. In addition to the above, EDL also recommended reading the panels at the earliest possible time -in the case of CLSI M100 that corresponds to 20h for *Acinetobacter*
- To elaborate on these findings, Roche will expand the scope of the planned Tier 1 broth microdilution AST assessment introduced in June 2022. Namely, Roche will complement the current approach focused on serum with another assessing commonly investigated variables in unsupplemented CAMHB. Building on the EDL recommendation particular attention will be paid to the impact of different read times, different reading guidelines (drastic decrease in growth) and the impact of sealing the plates with an adhesive plastic film.

CEFIDEROCOL TESTING VARIABILITY AND READING STUDIES

SELUX DIAGNOSTIC STUDIES

- Cefiderocol Broth Microdilution Testing: Data from Two Studies
    - QC strains
        - *P. aeruginosa* ATCC 27853 and *A. baumannii* NCTC 13301
        - 2 media manufacturers
        - 2 iron depletion times
        - 2 types of Chelex100 resin
    - Strains from CDC-FDA AR bank with Cefiderocol MICs available
        - *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*
        - Iron-depleted and non-depleted media
        - 2 iron depletion times
        - 2 types of Chelex100 resin
- Reference Broth Microdilution Test Conditions
    - All reference panels were produced following guidelines in CLSI M07
    - Iron-depletion was performed according to CLSI/Shionogi recommendation
    - Panels were produced and used immediately or frozen at -80C.
    - Cefiderocol Powder used: Med Chem Express Cat#:HY-17628/CS-0016-784
    - All panels read in CLSI M100-stated time window based on species
    - Shionogi MICs referenced are from June 2022 presentation from CLSI.
    - Summary of media conditions:

- BD BBL CAMHB:
  - 2 lots
  - Non-depleted, 2-hour depletion, 6-hour depletion
  - Chelex100 resins: 50-100 mesh (2-hour depletion only) and 200-400 mesh
- Difco CAMHB:
  - 1 lot
  - Non-depleted, 2-hour depletion, 6-hour depletion
  - Chelex100 resin: 200-400 mesh

- Note: not all media lots tested for all conditions.

- Panel Reading Guidelines
  - Read MIC at first well with substantial inhibition
  - Ignored trailing
- Conclusions
  - QC organism *P. aeruginosa* ATCC 27853 was within CLSI QC range for Cefiderocol in all iron-depleted media conditions
  - QC organism *P. aeruginosa* ATCC 27853 was within CLSI QC range for Cefiderocol in many, but not all non-iron-depleted media conditions
  - *A.* baumannii NCTC 13301 did not have consistent MICs in non-depleted or iron-depleted media.
  - AR-bank isolates tested typically had MICs within 2-3 doubling dilutions, but sometimes these spanned breakpoints.

SHIONOGI STUDIES

- Cefiderocol Broth Microdilution Reproducibility
  - Methodology
    - MIC determinations by broth microdilution for 36 isolates with MIC values "validated" with *in vivo* efficacy experiments using iron-depleted cation-adjusted Mueller-Hinton Broth (ID-CAMHB)
      - 4 different brands (1 lot for each brand) of (CA)MHB: BD BBL, BD Difco, Oxoid, and Merck
      - Perform testing over 3 days (three separate inoculum)
      - 10 replicates per isolate per media per day (same inoculum)
      - Total of 30 MIC determinations for each brand of (CA)MHB (120 total per strain)
    - Preparation of ID-CAMHB
      - Stir (CA)MHB with Chelex100 (analytical grade, 100–200 mesh, sodium form) for 6 hours
      - Filter and replenish medium with CaCl2(2.5 mg/L), MgCl2 (11.25 mg/mL) and ZnSO4 (0.65 mg/L)
      - Adjust pH to 7.2 -7.4 if needed
      - Confirm final iron concentration ≤0.03 mg/L
    - 5 *E. coli*, 7 *K. pneumoniae*, 12 *P. aeruginosa* and 12 *A. baumannii*
    - Historical cefiderocol MIC ranges from 0.25 -64 µg/mL
    - Bacterial inoculum (0.5 McFarland) controlled by nephelometer
    - Assessed reproducibility of MIC determinations (mode ±1 dilution) amongst and across media
  - Summary of Results
    - Good reproducibility (mode ±1-fold dilution)within single plates and across 3 days foreach ID-CAMHB
      - 34/36 isolates for Merck, 33/36 isolates for BBL and Oxoid, 32/36 isolates for Difco
      - Trailing was observed with *A. baumannii* for all media

- Variation in MIC values was observed between ID-CAMHB of different manufacturers
- MIC values in BBL and Difco medium were within ±1-fold dilution for 27 isolates
  - 1 *K. pneumoniae*, 3 *P. aeruginosa*, and 2 *A. baumannii* isolates showed ±2-fold dilution differences
- MIC values in BBL and Oxoid medium were within ±1-fold dilution for 19 isolates
  - 2 *E.* coli, 2 *K. pneumoniae*, 6 *P. aeruginosa*, and 1 *A.* baumannii isolates showed ±2-fold dilution differences
  - 3 isolates showed >2-fold dilution differences
- MIC values in BBL and Merck medium were within ±1-fold dilution for 17 isolates
  - 1 *E. coli*, 1 *K. pneumoniae*, 2 *P. aeruginosa*, and 3 *A. baumannii* isolates showed ±2-fold dilution differences
  - 6 isolates >2-fold dilution differences
- 13 isolates showed more than one-fold dilution difference in MIC compared to historical MIC; 6* of these showed >2-fold dilution difference (BD-BBL media comparison only)
- S/I/R category changed for isolate from intermediate to susceptible and one from susceptible to resistant
- *Two *K. pneumoniae* isolates (KP526 and KP544) also showed >2-fold dilution difference compared to historical MIC, but these were omitted as additional analysis showed different antibiograms and Β-lactamase content compared to historical data, indicating analysis of different isolates

- Conclusions
  - New procedure to generate ID-CAMHB resulted in reproducible MIC values for each ID-CAMHB
  - Some differences in MIC values were observed across the different ID-CAMHB
  - MIC values generated with ID-CAMHB from BD-BBL and Difco were the most reproducible and correlated the best with the *in vivo* pharmacology response
  - MIC values generated with Merck or Oxoid ID-CAMHB correlated the least with the *in vivo* pharmacology response
  - Some differences with historical MIC values might be due to the inoculum effect, loss of resistance markers upon isolate storage, different procedures of ID-CAMHB preparation
  - Enterobacterales and *P. aeruginosa* showed clear endpoints, while trailing was observed with some *A. baumannii* isolates for all media
  - Trailing complicates determination of clear MIC endpoint and improved reading guidance and example pictures are recommended to improve reproducibility
  - Aim to provide recommendations in June 2023 meeting

- Cefiderocol Disk Diffusion
  - Methodology
    - Zone diameter determination by disk diffusion
      - 2 different brands of disk: MAST/Hardy and Liofilchem
      - 2 different brands (1 lot for each brand) of Mueller-Hinton Agar: BD BBL and bioMerieux
      - Perform testing over 3 days (three separate inoculum)
      - 3 replicates per isolate per media (same inoculum)
      - Total of 18 readings for each disk (36 total per strain)
    - Inner and outer disk zones were determined
    - Bacterial inoculum (0.5 McFarland) controlled by nephelometer. Same inoculum was also used for broth microdilution.
    - Assessed reproducibility of zone diameters across disks and media
    - Assessed categorical agreement with broth microdilution

- Summary of Results
  - For Enterobacterales and *P. aeruginosa* reproducible results were obtained irrespective of the manufacturer of disks and agar medium used
    - Colonies within inhibition zone were not apparent and (outer) zone diameters could be easily determined
    - Colonies at edge of inhibition zone were observed
  - *A. baumannii* isolates with elevated MIC values frequently showed colonies in the inhibition zones. Phenomenon was not reproducible, so larger variation of inner inhibition zones were observed compared to outer inhibition zones
    - No clear correlation with trailing effect and appearance colonies in zone of inhibition
    - Good correlation between Hardy/Mast and Liofilchem disks
    - Good correlation between bioMerieux and BD agar
    - In general, more colonies appeared in the inhibition zone on BD agar compared to bioMerieux agar
    - In general, more variability in inner zone sizes on bioMerieux agar compared to BD agar
  - Good categorical agreement with broth microdilution MIC
- Conclusions
  - Inhibition zones could be easily determined for Enterobacterales and *P. aeruginosa* as few colonies appeared in the inhibition zones
    - Good categorical agreement with BBL and Difco modal MIC values
    - Good correlation with *in vivo* efficacy data
  - *A. baumannii* isolates with low cefiderocol MIC values showed reproducible zones of inhibition, but *A. baumannii* isolates with high cefiderocol MIC values showed variability in inhibition zones due to the appearance of colonies within the inhibition zone, which was poorly reproducible
    - While outer inhibition zones were much more reproducible, inner zones show better agreement with MIC values and *in vivo* efficacy data
    - Good categorical agreement with inner zone inhibition values and BBL and Difco modal MIC values
    - Correlation with inner zone inhibition values and *in vivo* efficacy data is weaker compared to MIC values, but for an isolate for which modal MIC could not be determined because of skipped wells, inhibition zones correlated well with *in vivo* efficacy (AB97)
  - Reading of inner zones is needed and provision of example pictures that demonstrate extend of colonies within zone of inhibition are recommended to improve determination of inhibition zone
  - Aim to provide suitable pictures in June 2023 meeting

- Additional QC Isolates to Assess ID-CAMHB
  - Methodology
    - Strains were selected from the following collections
      - Strains with *in vivo* efficacy data from in-house PK/PD studies (n=23)
      - Strains from SIDERO-WT (Year 1-3) studies which showed ≥16-fold difference between standard CAMHB and ID-CAMHB (n=32)
      - CDC AR bank isolates (n=52)
      - ATCC strains (n=30)
    - MIC determinations were performed in iron-depleted cation adjusted Mueller-Hinton Broth (ID-CAMHB) and CAMHB using 3 brands of media (with multiple lots): BD BBL (3 lots), BD Difco (3 lots), Oxoid (2 lots)

- 3 different inoculum (total 6 or 9 replicates per media source)
  - Strains with reproducible MIC differences between ID-CAMHB and CAMHB (n=6) were selected for follow-up testing in ID-CAMHB using 4 different brands of CAMHB: BD BBL, BD Difco, Oxoid, Merck (1 lot of each medium)
    - 10 replicates (same inoculum) per isolate per media
    - Repeat on 3 different tests (total 30 replicates per media; 10 replicates with 3 different inoculum)
  - Assess MIC variability in ID-CAMHB from different sources
- Conclusions
  - Identification of one appropriate QC isolate that can identify MIC differences between ID-CAMHB and CA_MHB across all different sources of media is complicated by the MIC spread across medium
  - Identification of such a QC isolate for a specific source of media is more easily achievable
  - Will continue to screen additional isolates

- Overall Summary and Conclusions
  - New procedures to generate ID-CAMHB resulted in reproducible MIC values for each ID-CAMHB
  - Different sources of MHB (to make ID-CAMHB) can lead to differences in MIC determinations, but with the standardization of the method most isolates showed MIC values within 2-fold dilutions across media
  - MIC values generated with ID-CAMHB from BD-BBL and Difco were the most reproducible and correlated the best with the *in vivo* pharmacology response
  - Enterobacterales and *P. aeruginosa* showed clear endpoints, while trailing was observed with some A. *baumannii* isolates for all media
  - Inner zones of inhibition need to be reported when using disk diffusion
  - Validation panel for cefiderocol testing across different media has been established
  - Identification of one appropriate QC isolate identifying MIC differences in ID-CAMHB and CA-MHB is complicated
- Next Steps
  - Additional studies using cefiderocol-resistant *A. baumannii*
  - Additional screening for QC isolates ID-CAMHB vs CAMHB
  - Confirmation of findings in other laboratories
  - Provide recommendations on reading guidance in June 2023 CLSI meeting

JMI STUDIES

- Problem
  - Susceptibility testing cefiderocol against *Acinetobacter* spp. isolates results in significant trailing, haziness, and regrowth surrounding MIC endpoints
  - Accordingly, it is difficult to consistently and objectively determine MIC endpoints
- Cefiderocol Reading Rules
  - The MIC should be read at the first drug well in which is significantly reduced relative to the growth observed in the growth control
  - If growth is significantly reduced but then shows regrowth, ignore the regrowth and call the MIC where growth was first reduced significantly
  - If there is a skip, read the MIC at and not above the skip
  - If there is haze present and no button, this should be ignored and not considered growth, assuming the control well has a solid button
- Methodology

- Tested 24 *Acinetobacter baumannii-calcoaceticus* species complex isolates from the 2020 SENTRY program
- 5 Cefiderocol-resistant, 4 Cefiderocol-susceptible, 5 Cefiderocol regrowth, 5 Cefiderocol hazy, and 5 Cefiderocol trailing
- Cefiderocol (64 to 0.004 mg/L) and piperacillin-tazobactam (256/4 to 1/4 mg/L)

- Conclusions
  - The cefiderocol results were generally reproducible (within +/- 1 dilution)
  - Isolates showing trailing are more challenging to read, so reproducibility may be a challenge
  - Media and panel manufacturing did not influence the growth pattern
  - As shown by MBC, cell viability indicators, and cell morphology, cefiderocol had bacteriostatic activity against 19 of 24 isolates tested
  - The trailing could be due to filamentous cells that are unable to divide

SC DISCUSSION (MAIN POINTS)

- Need to summarize all the data.
- Suggestion that all cefiderocol presentations are presented to one group at the same time to discuss. Possibly form an ad hoc working group under MDSWG.
- AR Bank is requesting that their *Acinetobacter* isolates be tested by Shionogi and to provide reading guidance. There were difficulties when testing in the CDC laboratory. CDC is working on additional cefiderocol studies.
- *Acinetobacter* and cefiderocol is a problem. There is not as much of a problem with the organisms but there are some challenges as well.
- Need to review the iron content and confirm the iron content.
- M02/M07 guides address cefiderocol reading. Important to include in these discussions.
- Suggestion that reading guidance needs to include other organisms besides *Acinetobacter*.
- Suggestion to review media from different manufacturers for disk.
- MDSWG will form an ad hoc working group under MDSWG to focus on the cefiderocol testing variability.

4.	**TEXT AND TABLES WG (TTWG) REPORT (A. BOBENCHIK)**
	
	M02/M07 AD HOC WORKING GROUP UPDATE
	• Timelines on Track
	  ○ Kickoff June 2021 –reviews split into common text, M02, M07
	  ○ 10 web conferences
	  ○ Writing assignments completed November 2022. One-voice edit November 2022. Committee Draft and Comment Table January 2023
	  ○ Most comments resolved at this meeting 1/21/23. Several deferred until after January AST meeting. Proposed draft for vote June 2023
	  ○ Still looking at instructions for preparing frozen reference panels to clarify
	  ○ Final Draft September 2023
	  ○ Document publication January 2024 with M100
	• Major Changes
	  ○ Aligned with M100 33rd ed on drug tiers (not groups) and selective reporting. Verbiage is same as M100 –(M100 examples were not brought over).
	  ○ Added equivalent agents section
	  ○ Updated Quick Reference Guides (QRG) –lots of new pictures
	  ○ Added MH-F and iron-depleted CAMHB. More standardized media preparation instructions
	  ○ Revised section on antimicrobial classes
	  ○ Added further colony count examples
	  ○ Will align with M100 34th ed on reading disk diffusion tests with reflected (not transmitted) light
	
	DOSAGE COMMENT REMOVAL (34TH EDITION)
	• Background
	  ○ Presented proposal of standardizing dosage comment language format (SC agreed)
	  ○ Presented proposal to SC for removal of dosage comments from Tables 2 in 34th edition (SC voted and approved 11-2-0-0)
	  ○ Topics for consideration when revision Appendix E

- **Dosages for different breakpoints**
  - Susceptible, SDD, intermediate
- **Species-specific info**
  - All staphylococci, *H. influenzae* only, etc
- **Indications or other comments**
  - For uUTI
  - Prediction comments
- **Route of administration**
- **Designations**
  - Urine only
- **Format**
  - Full dosage comment for each?
  - Example in Appendix intro and pertinent info in a table?
- **Intro & guidance for how to use this information**
- **Population, if applicable**
  - Adults
  - Peds

- Actions for Appendix E mockup in June 2023
  - Identified a few TTWG volunteers
  - Discuss key criteria for inclusion in table: what to keep, what to exclude, what needs modification
  - Engage with PharmD colleagues and other relevant colleagues for input
  - Explore options/capabilities within CLSI electronic platform
  - Draft mock up to present in June 2023

34TH EDITION CHANGES

- Breakpoint Additions Table to be revised to include direct disk in its own table
- Addition of Direct Disk Reporting Clarification: "Report only the interpretive category and not the measured zone size."
- Provide clarification of *Staphylococcus* spp. Oxacillin MIC and *mecA*/PBP2a comments in Table 3G. Possibly move to Tables 2C and Tables 4A-1 and 5A-1 QC tables.
- Consider all of Tables 3G content to determine if most of it is unnecessary due to redundancy with standard methods in Table 2C.
- Update terminology for "inconclusive" vs "indeterminate".

NEXT STEPS

- Coordinate interim TTWG virtual meeting(s) between now and June to finish agenda items
- Create mockups of Appendix E dosage comment content
- Create mockups of changes to Tables 3G mecA-mediated R Staph
- Create mockup of Table 2A-2 *Salmonella*, *Shigella*
- Demo of CLSI's newer electronic platform capabilities (Edaptive and Method Navigator)

5. **ADJOURNMENT**

Dr. Lewis thanked the participants for their attention. The meeting was adjourned at 11:45 AM Eastern (US) time.

PLENARY ATTENDEES

Plenary 1	Plenary 2	Plenary 3
Abdullah Kilic	Abdullah Kilic	Abdullah Kilic
Ajay Kumar Prajapati	Ajay Kumar Prajapati	Ajay Kumar Prajapati
Alex Greninger	Alex Greninger	Alex Greninger
Alexandra Bryson	Alexandra Bryson	Alexandra Bryson
Alhagie Dibbasey	Alhagie Dibbasey	Alhagie Dibbasey
Aliaa Fouad	Alice Gray	Alice Gray
Alice Gray	Alisa Serio	Alisa Serio
Alisa Serio	Alita Miller	Alita Miller
Alita Miller	Allie Malmberg	Allie Malmberg
Allie Malmberg	Allison Tsan	Allison Tsan
Allison Tsan	Amanda Sheets	Amanda Sheets
Amanda Sheets	Amanda Suchanek	Amanda Suchanek
Amanda Suchanek	Amelia S. Bhatnagar	Amelia S. Bhatnagar
Amelia S. Bhatnagar	Amira Bhalodi	Amira Bhalodi
Amira Bhalodi	Amity Roberts	Amity Roberts
Amity Roberts	Amy Gargis	Amy Gargis
Amy Gargis	Amy Mathers	Amy Mathers
Amy Mathers	ANALI MILAGROS SALAS FITZCARRALD	ANALI MILAGROS SALAS FITZCARRALD
ANALI MILAGROS SALAS FITZCARRALD	Andrea Ferrell	Andrea Ferrell
Andrea Ferrell	Andrea Feßler	Andrea Feßler
Andrea Feßler	Andrea Prinzi	Andrea Prinzi
Andrea Prinzi	Andrej Trauner	Andrej Trauner
Andrej Trauner	Andrew DeRyke	Andrew DeRyke
Andrew DeRyke	Andrew Fratoni	Andrew Fratoni
Andrew Fratoni	Andrew Fuhrmeister	Andrew Fuhrmeister
Andrew Fuhrmeister	Animesh Dhara	Animesh Dhara
Animesh Dhara	Anisha Misra	Anisha Misra
Anisha Misra	Anna Kallio	Anna Kallio
Anna Kallio	Anna Klavins	Anna Klavins
Anna Klavins	Anne Lamsa	Anne Lamsa
Anne Lamsa	Antonieta Jimenez	Antonieta Jimenez
Antonieta Jimenez	April Abbott	April Abbott

April Abbott
April Bobenchik
Arryn Craney
Åsa Karlsson
Ashley Beard
Audie Perniciaro
Audrey Schuetz
Ayesha Khan
Bahar Vafadar
Barb Gancarz
Barb Jones
Barbara Zimmer
Benjamin von Bredow
Besarta Mullalli
Beth Goldstein
Bill Brasso
Boudewijn DeJonge
Brandi Limbago
Camille Hamula
Carey-Ann Burnham
Carmila Manuel
Carrine Brown
Cassandra Parker
Cau Dinh Pham
Cecilia Carvalhaes
Chad Testa
Chalwe Sokoni
Charles Jakielaszek
Cheung Yee
Chie Ohno
Chris Longshaw
Chris Pillar
Christian Giske
Christina Chantell
April Bobenchik
Arryn Craney
Åsa Karlsson
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Christine Lam
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Christopher Haddock
Claire Burbick
Claudia Zampaloni
Collette Wehr
Crystal Minchew
Cynthia Knapp
Dale Schwab
Dana Dressel
Danielle Hilligoss
Daouda Touré
Darcie Carpenter
David Burgess
David Hilbert
David Lonsway
David Nicolau
David Paisey
Davina Campbell
Deborah Butler
Dee Shortridge
Denise Holliday
Derrek Brown
Diane Anastasiou
Diane Halimi
Divyaa Elangovan

Dmitri Debabov
Dmitri Iarikov

Dr Bhaskar Bhattacharya
Dr. Itzel Harriott, B.S.,Rph, MPA,MSP,MBA,PharmD,C.Ph.
Dr. Supriya Aher
Dwight Hardy
Dylan Staats

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Elaine Duncan

Elide Herrera
Elizabeth Berkow
Elizabeth Hirsch

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Eric Stern
Eric Wenzler
Erika Matuschek
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Jessica Zering
Jingzi Sherman
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Joseph Kuti
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Maria Burgos-Garay
Maria Karlsson
Mariana Castanheira
Marisa Winkler
Mark Fisher
Mark Redell
Mary Motyl
Maryann Brandt
Matt Mason
Matthew Wikler
Meenachi CT
Megan Hickey
Melanie Yarbrough
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Mervat Elanany
Michael Huband
Michael Satlin
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Mohamed Ellethy
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Samir Patel
Sandra McCurdy
Sandra Richter
Sara Blosser
Sarah Alsamarai
Sarah Hepler

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