What’s new in AST?

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VUMC

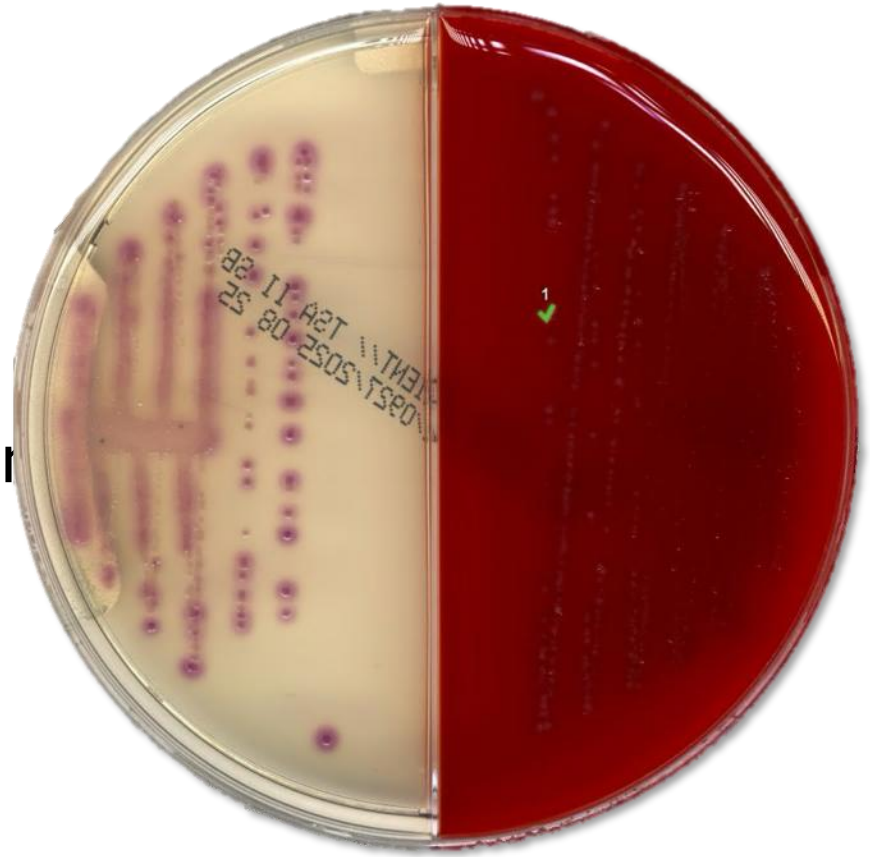
What we'll cover

- Strategies to deal with MDRO Gram negatives
- Strategies for streamlining AST QC
- Q&A

Part 1: MDR Gram negative bacteria

Case 1.

- 62 YO woman with urinary frequency and pain
- Culture: >100K *E. coli*



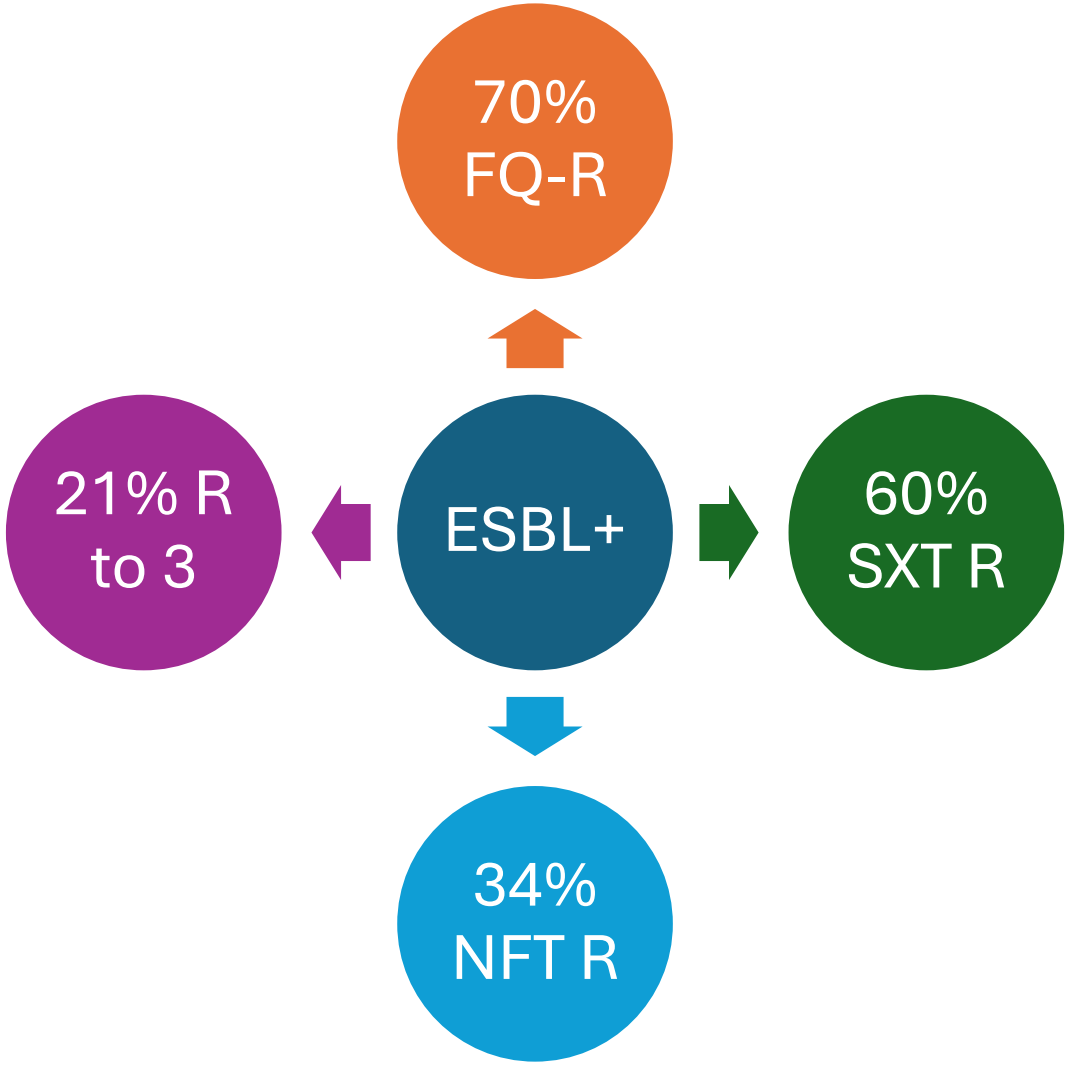
Antimicrobial	MIC (µg/mL)	Interpretation
Ampicillin	>32	R
Ceftriaxone	>32	R
Ertapenem	0.25	S
Ciprofloxacin	>4	R
Nitrofurantoin	>128	R
Trimethoprim-sulfa	>4	R

Call to lab from ID Physician:

"Could you start testing **fosfomycin** routinely for these ESBL UTI?

We need oral options!"

Treatment options: ESBL uncomplicated UTI



- Increasingly common: 15% outpatient uUTI, 25% inpatient uUTI[1]
- Very few **oral** options for ESBL isolates [2]
  - 21% are resistant to FQ, SXT, **and** NFT
  - Many patients have a SXT allergy
  - NFT has been avoided for elderly
  - FQ avoided unless last option
- **Fosfomycin** active vs. ESBL *E.coli*
  - Resistance documented but often only in critically ill patients

FQ, fluoroquinolone
SXT, trim-sulfa
NFT, nitrofurantoin

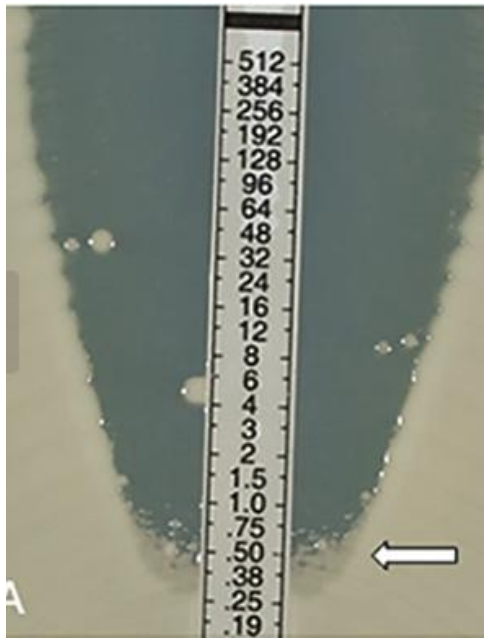
1. Fernandez et al. OFID 2023. 10, S2. 2. Dunne et al. BMC Infect Dis 22:194; 2. Dunne et al. CID. 2023 76:78
3. Puttagunta et al. 2025 OFID 12:Suppl 1

Fosfomycin; the story…

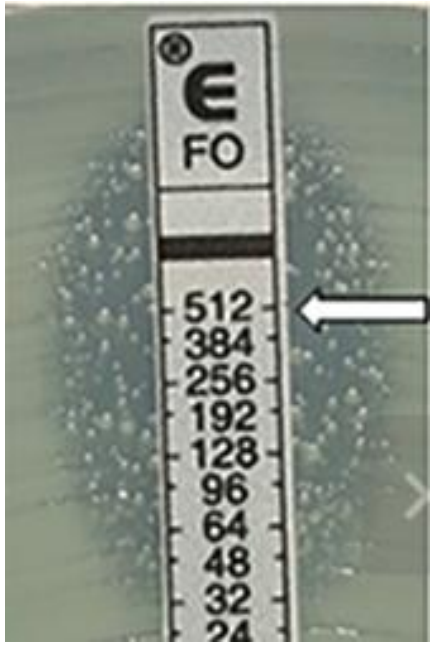
- Discovered in late 1960s
- Approved for lower urinary tract infection in 1996, brand “Monurol”
- Bactericidal, inactivates pyruvyl transferase (cell wall synthesis)
- In the USA:
  - Only available as an oral sachet
  - Only available as treatment for uncomplicated cystitis
  - Only for *Escherichia coli* and *Enterococcus faecalis*

Fosfomycin testing

- Breakpoints only for *E. coli* and *E. faecalis*
  - Supplement media with Glucose-6-Phosphate for testing[1]
- **Agar dilution (AD)** is reference method[1]
- New FDA-cleared Etest (FO) available, has 99% categorical agreement vs. AD [2]
- FDA-cleared Vitek2 available – data from Canada shows issues with detection of resistance[3]; FDA submission with new formulation shows 0 / 14 VME
  - Only available on some cards



Ignore microcolonies…



Unless they fill the ellipse!

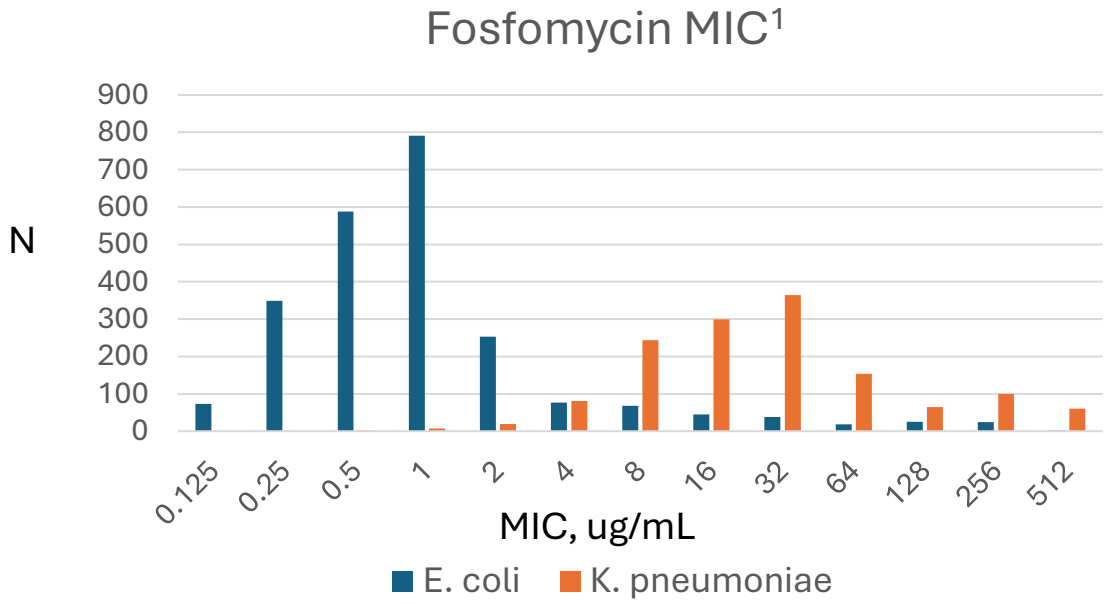
Disk diffusion:

Read inner, colony-free zone of inhibition!

1. CLSI 2025. M100
2. Goer et al. 2022 J Clin Microbiol 60:e00021-22
3. Karlowsky et al. 2020. J Clin Microbiology 58: 10

Fosfomycin testing: other Enterobacterales

- MIC distributions are very different from *E.coli*
- Oral fosfomycin breakpoints are ONLY for E. coli (CLSI and EUCAST)
  - EUCAST IV breakpoints are only for *E. coli* and infections originating in the urinary tract[2]
- Resistance to fosfomycin:
  - Fosfomycin cleavage by FosA
  - modification of *murA* target
  - mutation of fosfomycin transporter
- No relationship between MIC and outcome for K. pneumoniae[3]



Most *K. pneumoniae*, *K. aerogenes*, *P. aeruginosa* and *Enterobacter* have FosA

Testing should not be performed!

1. EUCAST MIC Distributions
2. EUCAST v 15.0 breakpoint tables, www.eucast.org
3. Kaye et al. 2019. CID 69:045

Fosfomycin: what are our options

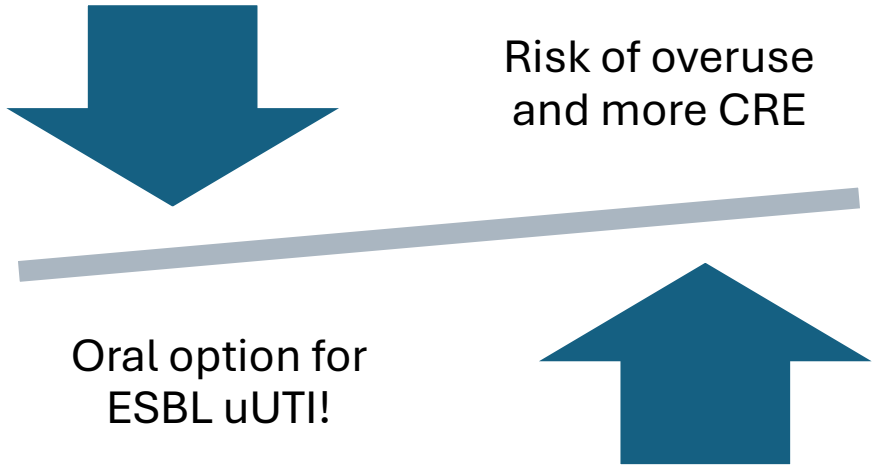
1. Routine test urine isolates… if card available (and verification ok)
2. Reflex testing by disk or Etest (either by request or routine for ESBL *E. coli*)
3. Consider generating a once-a-year fosfomycin antibiogram for *E. coli* (limited study)

Species	N	%S
E. coli (ESBL)	100	99

4. Educate clinicians on limitations of testing other species

What other oral options?

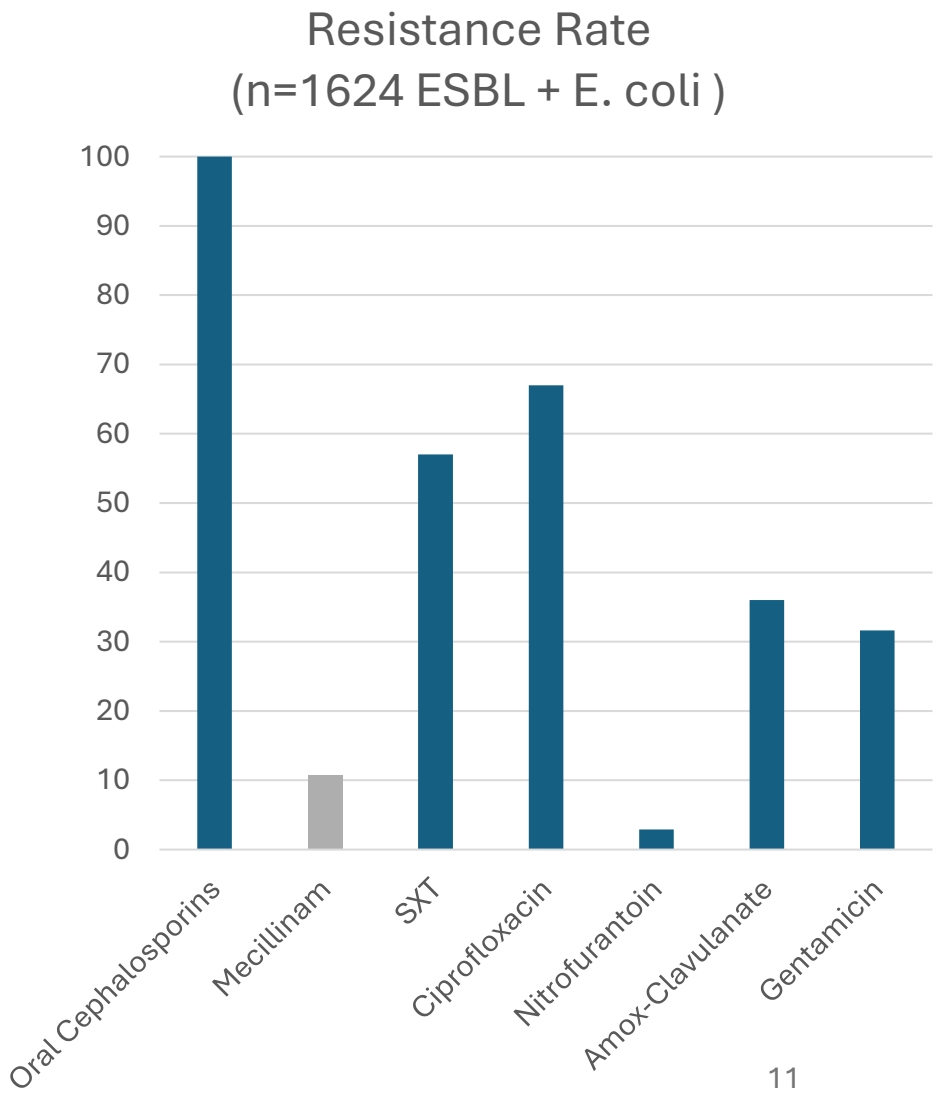
- ***Sulopenem:*** oral penem (2024)
  - Brand name: Orlynva
  - Similar to ertapenem in activity
  - IV and Oral formulations
  - FDA indication: uUTI
  - Failed to achieve primary endpoint vs. ertapenem[2]
  - Succeeded vs. ciprofloxacin in FQ-R infections and amoxicillin-clavulanate[3]
  - FDA-cleared: Liofilchem MIC strips



Talk to your ASP to see if sulopenem use is in discussion!

What other oral options?

- ***Pivmecillinam:*** oral beta-lactam (2024)
  - Brand name Pivya, prodrug of mecillinam
  - FDA indication: uUTI in women
  - *E. coli*, *P. mirabilis*, *S. saprophyticus* indications
    - Active against many ESBL and some carbapenemase producers!
    - No activity vs. P. aeruginosa, E. faecalis or S. aureus
  - Testing?
    - CLSI breakpoints recognized by FDA
    - No current FDA-cleared tests, most use empirical



Jansaker et al. Infect Drug resist. 2019. 12:1691
Hawkins et al. AAC. 2025. 69: e01824-24

What other oral options?

- ***Gepotidacin:*** oral triazaacenaphthylene (2025)
  - Brand name Blujepa
  - FDA indication: uUTI in women
  - Novel antibiotic class, targets DNA gyrase / topoisomerase IV in region unique from fluoroquinolones
  - *E. coli*, *K. pneumoniae*, *C. freundii*, *S. saprophyticus* and *E. faecalis*
  - Testing?
    - No CLSI breakpoints
    - Hardy Disk only FDA cleared test

Gepotidacin – Oral Products

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

Organism	% Susceptible
E. coli (n=3560)	99.9%
E. coli, ciprofloxacin R (n=899)	99.8%
E. coli, ESBL (n=616)	99.4%
S. saprophyticus (n=344)	100%

Arends et al. Antimicrob Agents Chemother 2023 67: e01525

What about ESBL testing?

IDSA Treatment Guidance

- ESBL-Producing Enterobacterales
  - Treatment recommendations guided by laboratory reporting presumed or confirmed ESBL

Tamma PD et al. 2022. https://www.idsociety.org/practice-guideline/amr-guidance/. Accessed 3/17/23

ESBL test realities

1. Most **sensitive** way to detect ESBL is a ceftriaxone MIC > 0.125 µg/mL or ceftazidime MIC >0.5 µg/mL
2. Historical "screening" criteria for ESBL is same as current intermediate ceftriaxone MIC breakpoint
3. ESBL test developed when only 217 ESBLs known; today >3000 described
4. Performance of CLSI reference ESBL test suboptimal
   - *E. coli*, PPV, 98% but NPV is 76%
   - *K. pneumoniae*, PPV, 82% and NPV is 95%
   - False-positive results for other species due to chromosomal enzymes (AmpC, OXY)
5. Commercial systems overall ESBLs
   - Vitek 2, specificity ~50%
   - Phoenix, specificity ~70%

Hadziyannis et al. 2000 DMID 36:113
Morrissey et al. 2014 J Med Microbiol. 63:556
El-Jade et al.2016 PLoS One 11: e0160203
Farber et al. JCM 46:3721

CLSI guidance on ESBL testing

(24) Following evaluation of PK/PD properties, limited clinical data, and MIC distributions, revised breakpoints for cephalosporins (cefazolin, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone) and aztreonam were first published in January 2010 (M100-S20) and are listed in this table. .... When using current breakpoints, routine ESBL testing is not necessary before reporting results. However, **in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders, laboratories may decide to perform phenotypic or genotypic testing for ESBLs, and the results may be used to guide therapeutic management or** for epidemiological or infection prevention purposes. **Limitations of phenotypic and genotypic methods must be considered (see Table 3A introductory text).**

M100 33rd ed. Table 2A. (p. 62).

Example
Presumed ESBL Reporting
***E. coli* - respiratory**

Antimicrobial	Result
Amoxicillin-clavulanate	**R**
Cefazolin	**R**
Ceftriaxone	**R**
Cefepime	**R**
Ciprofloxacin	**S**
Gentamicin	**S**
Ertapenem	**S**
Meropenem	**S**
~~**Piperacillin-tazobactam**~~	~~**R**~~
Trimeth-sulfa	**S**

Report Comment:

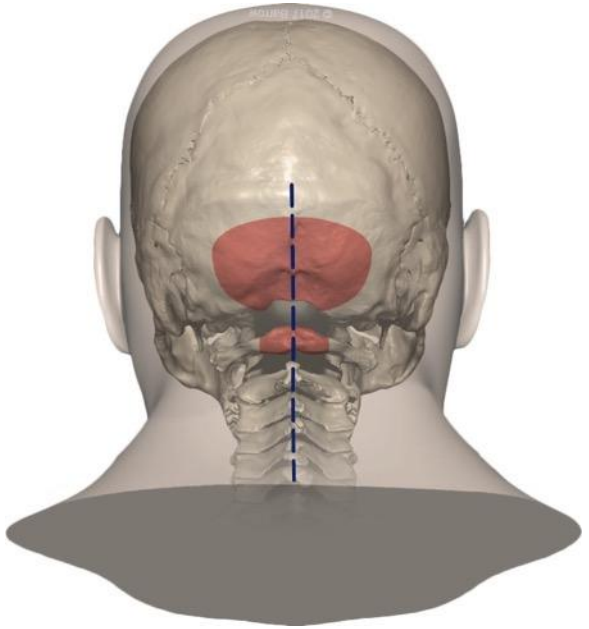
"Ceftriaxone-resistant *E. coli* are presumed to be ESBL producers. Place patient under contact precautions. Carbapenems are preferred therapy for ESBL-producers. ID consultation recommended."

Ceftriaxone/cefotaxime can serve as a proxy for ESBL producers to guide patient management, including infection prevention measures.

ID, Infectious Diseases

Case 2.

- 57 year old man
- Suboccipital craniectomy for 4th ventricular ependymoma
- Post-surgery, develops altered mental status and fever
- CSF: 60 WBC
- Gram stain = GNR



CSF Culture: *Enterobacter (Klebsiella) aerogenes*

Antimicrobial	MIC (µg/mL)	
Ampicillin	>16	R
Aztreonam	≤2	S
Cefepime	≤1	S
Ceftriaxone	≤1	S
Piperacillin-tazobactam	4	S

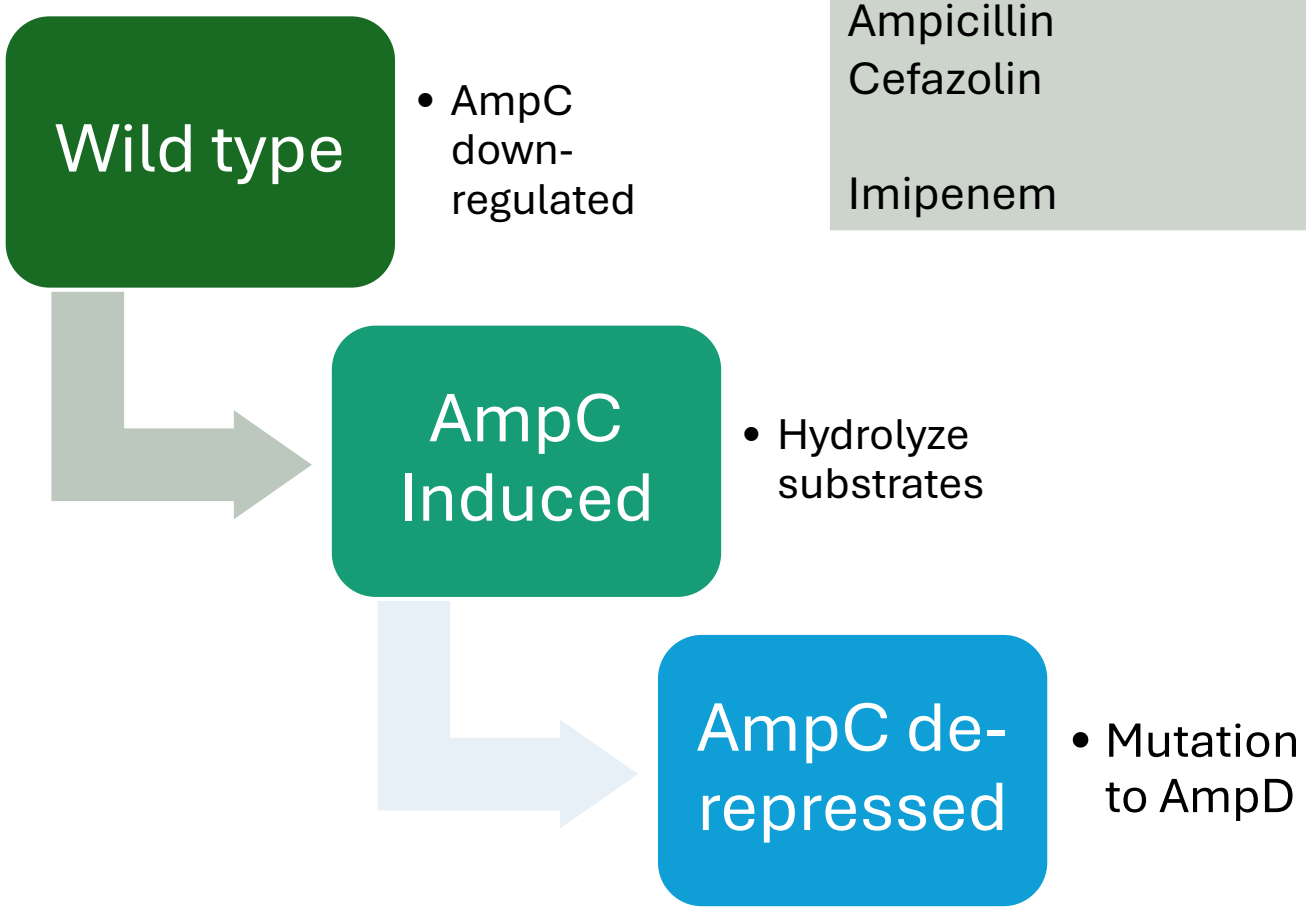
Image: Congress of Neurological Surgeons

Case 1, continued

- Patient started on cefepime, but changed to ceftriaxone due to seizures
- Patient continues to have high WBC in CSF
- Repeat isolates recovered, but AST not performed
  - Laboratory policy is to repeat AST only every 5 days
- Finally, day 6, AST performed: “R” to ceftriaxone

Antimicrobial	Isolate #1 Day 1	Isolate #2 Day 6
Ampicillin	>16, R	>16 R
Aztreonam	≤2, S	16, R
Cefepime	≤1, S	≤1, S
Ceftriaxone	**≤1, S**	**32, R**
Pip-tazo	**4, S**	**64, R**
Meropenem	S	S

AmpC Induction Overview



Inducer	Substrate
Ampicillin Cefazolin Imipenem	Ampicillin Cefazolin Ceftriaxone

Jacoby. CMR. 2009. 161-182

AmpC de-repressed mutants: Impact on AST results

Species	Piperacillin-Tazobactam	Ceftriaxone	Ceftazidime	Cefepime
E. cloacae	<5%	0%	0%	35%
E. aerogenes	10%	<5%	0%	98%
C. freundii	29%	0%	0%	80%
P. rettgeri	12%	5%	0%	81%
S. marcescens	55%	0%	75%	88%
Typically	**S or R**	**R**	**R**	**S**

0-25 %
25-50%
50-75%
>75%

Kohlmann, Bahr and Gatermann. 2018 JAC 73: 1530
Kohlmann, Bahr and Gatermann. 2019. CMI. 25:1158

Review of AmpC VUMC:
Enterobacter, *Citrobacter*, *Serratia* in Blood

N= 37 patients in Jan – July 2020
Initial culture results:
81% ceftriaxone – S
89% piperacillin-tazobactam - S

50% of patients treated with ceftriaxone (n=4) suspected of de-repression due to clinical decompensation

	Total	%
Patients with repeat testing	28	76%
Repeat + culture grew on repeat testing Median duration of bacteremia	8 36 h	29% 24-168 h
AST performed on repeat	3	37.5%
Number de-repressed AmpC	2	66.7%

Table 2

Enterobacter, *Klebsiella aerogenes*, *Citrobacter*, and *Serratia* may develop resistance during prolonged therapy with 3rd-generation cephalosporins as a result of de-repression of AmpC beta-lactamase. This derepression is most commonly seen with C. freundii, E. cloacae, K. aerogenes. Isolates that are initially susceptible may become resistant within a few days after initiation of therapy. Testing subsequent isolates may be warranted if clinically indicated. The approach to reporting AST results for these organisms should be determined in consultation with the ASP and other relevant institutional stakeholders.

H afnia alvei
E nterobacter cloacae
C itrobacter freundii
K lebsiella aerogenes
Y ErSinia enterocolitica

Table 1

Cefepime should be considered a Tier 1 agent for testing and/or reporting of *C. freundii, E. cloacae, H. alvei, K. aerogenes, M. morganii, Providencia* spp, *S. marcescens* and *Y. enterocolitica*

M100 35th edition

Opportunities for the lab

1. Provide guidance on frequency of repeating cultures
2. Repeat AST more often than every 5 days
3. “warning” re: use of ceftriaxone for AmpC organisms:
    1. Suppress ceftriaxone / ceftazidime results
    2. Provide a comment re: risk of AmpC de-repression

Which Enterobacterales have AmpC?

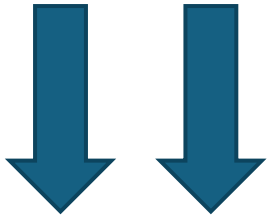
No – ceftriaxone works well	Yes – risk of de-repression
Citrobacter koseri Citrobacter amalonaticus group (C. amalonaticus, C. farmer, C. sedlaki) Escherichia spp. Klebsiella spp (excluding K. aerogenes) Proteus mirabilis Raoultella spp. Salmonella Shigella	All other Enterobacterales

Good clue:
If tested, cefoxitin is “R”

Additional resource:
CLSI Appendix B of M100

*note, Proteus vulgaris doesn’t have an AmpC but it has an enzyme that is similar to AmpC

CLSI Appendix B



Appendix B. (Continued)

B1. Enterobacterales

Antimicrobial Agent / Organism	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporin II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
Citrobacter freundii	R	R	R		R	R	R						
Citrobacter koseri, *Citrobacter amalonaticus* group[a]	R			R									
Enterobacter cloacae complex[b]	R	R	R		R	R							
Escherichia coli	There is no intrinsic resistance to β-lactams in this organism.												
Escherichia hermannii	R			R									
Hafnia alvei	R	R	R		R	R							
Klebsiella (*formerly Enterobacter*) *aerogenes*	R	R	R		R	R							
Klebsiella pneumoniae, *Klebsiella oxytoca*, *Klebsiella variicola*	R			R									

Example: AmpC Reporting

E. cloacae complex- tissue

Antimicrobial	Result
Ampicillin	R
Ampicillin-sulbactam	R
Cefazolin	R
Ceftriaxone	S
Cefepime	S
Ciprofloxacin	R
Gentamicin	S
Ertapenem	S
Meropenem	S
Piperacillin-tazobactam	S
Trimeth-sulfa	S

Option 1:
Add Report Comment:
"*E. cloacae* complex may develop resistance upon exposure to third-generation cephalosporins. ID consultation recommended."

Option 2:
Suppress third-generation cephalosporins or report as "R."

Note to Laboratory:
Isolates should be retested every few days to determine if resistance to third-generation cephalosporins is now being expressed.

Resistance Emerging on Therapy

Instructions for Use:

VI. Development of Resistance and Testing of Repeat Isolates

"Isolates that are initially susceptible may become intermediate or resistant after therapy is initiated …

Laboratory guidelines on when to perform susceptibility testing on repeat isolates should be determined after consultation with the medical staff."

- There are no definitive guidelines on how often to perform repeat AST.
- Depends on:
  - Organism
  - Patient
  - Institution preference
  - Laboratory capacity

CLSI M100-Ed35 Instructions for Use,

Example Policies – AST Repeat Testing

Hospital A	Hospital B	Hospital C	Hospital D
Repeat every 3 days	Repeat every 5 days	Repeat every day, sterile sources	Repeat every 7 days
Test more often if requested by ID physician	Test every day[1]: *S. aureus* *P. aeruginosa*	Repeat every 5 days, non-sterile sources	Test more often, if requested by ASP

[1] Giltner et al. 2014. JCM. 51:1324-6.

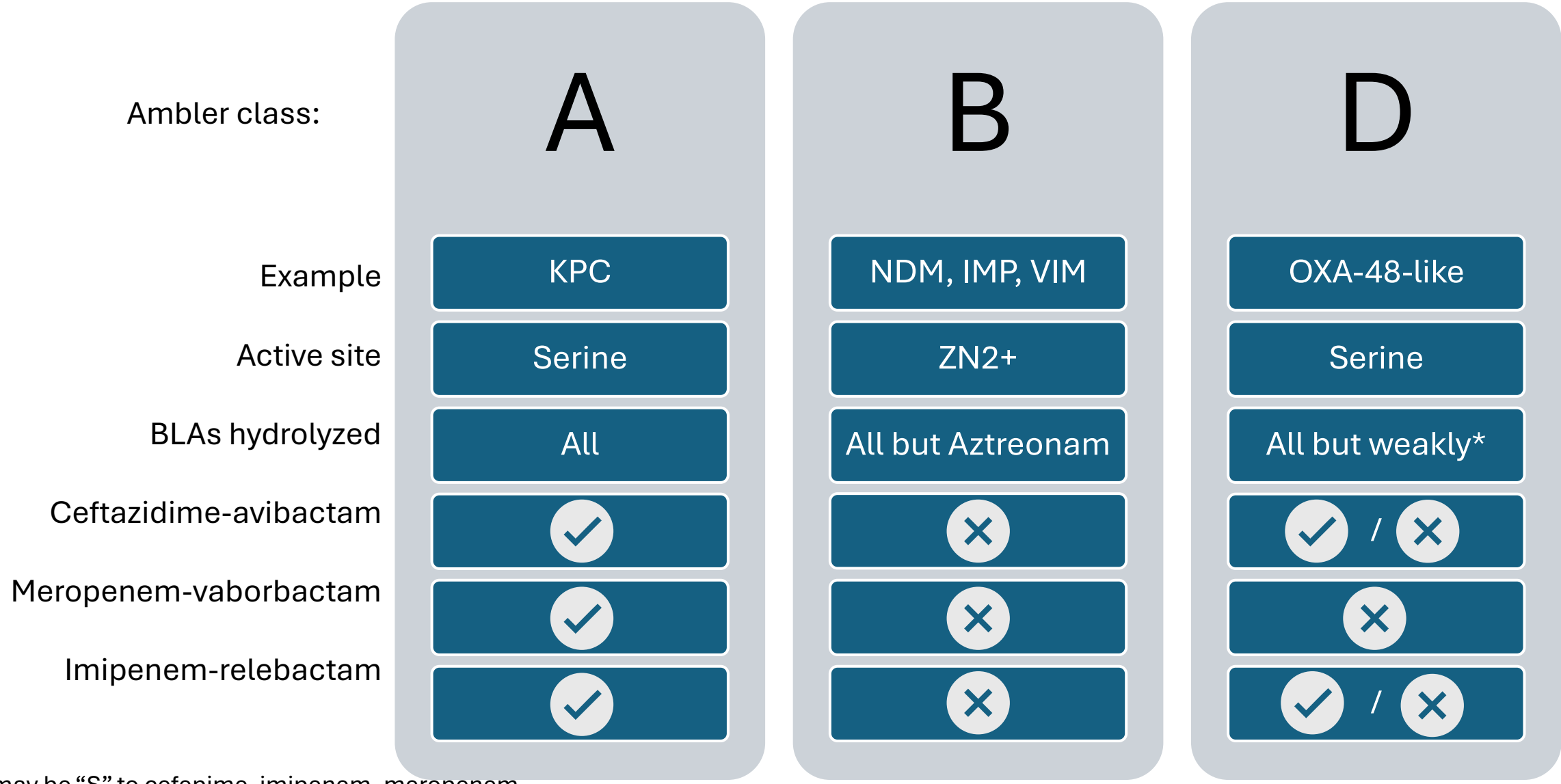
Case 3.

- 30-year-old with no previous medical history
- Sustained friction burns following flash diesel explosion at industrial plant
- 65% total body surface area burn wounds
- Multiple operations and allografts
- 2 weeks after admission:
  - *K. pneumoniae* in blood cultures

Molecular results (positive blood culture):
bla$_{VIM}$ carbapenemase gene detected

Antimicrobial	K. pneumoniae	
Amikacin	≤8 *	S
Cefazolin	>16	R
Cefepime	>16	R
Ceftazidime-avibactam	>8/4	R
Ceftriaxone	>32	R
Ciprofloxacin	>2	R
Ertapenem	>2	R
Gentamicin	>8	R
Levofloxacin	>4	R
Meropenem	>8	R
Meropenem-vaborbactam	>8	R
Piperacillin-tazobactam	>64/4	R
Tobramycin	>8	R
Trimeth-sulfamethoxazole	>2/38	R

Refresher – Carbapenemase Types



*may be “S” to cefepime, imipenem, meropenem

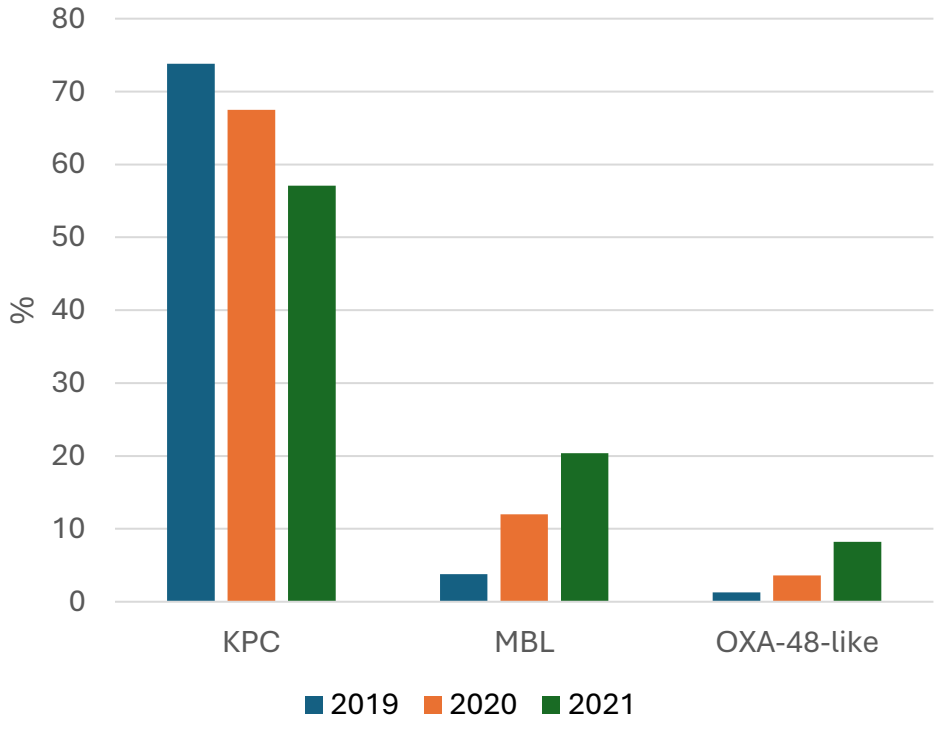
Carbapenemases and Enterobacterales – Again!

- Knowledge of specific carbapenemase type is needed to inform treatment
- RISK of not performing carbapenemase testing:
  - Assume isolate has a class A or D carbapenemase
  - May "miss" transmission events for patients with CRE

Carbapenemase Type	Optimal Treatment - Enterobacterales
Not done*	Ceftazidime-avibactam Imipenem-relebactam Meropenem-vaborbactam
Class A (KPC)	Ceftazidime-avibactam Imipenem-relebactam Meropenem-vaborbactam
Class B (NDM, IMP, VIM)	Ceftazidime-avibactam + aztreonam Cefiderocol
Class D (OXA-48-like)	Ceftazidime-avibactam

*IDSA guidance suggestions if no *specific* carbapenemase testing done
Tamma et al. CID 2023. doi:10.1093/cid/ciad428

Carbapenemase Type Among Carbapenem-resistant Enterobacterales (CRE), USA 2019-2021



Sader et al. OFID 10(2):ofad046

Table 2A-1 Enterobacterales Comment (25) – new in 2025

- "**Should**" perform carbapenemase testing of CRE
- Exception: ***Proteus, Providencia,* and *Morganella* spp.** that are only "R" to imipenem
- Possible exception: for ***E. cloacae* complex and *K. aerogenes*** isolates that are only "R" to ertapenem

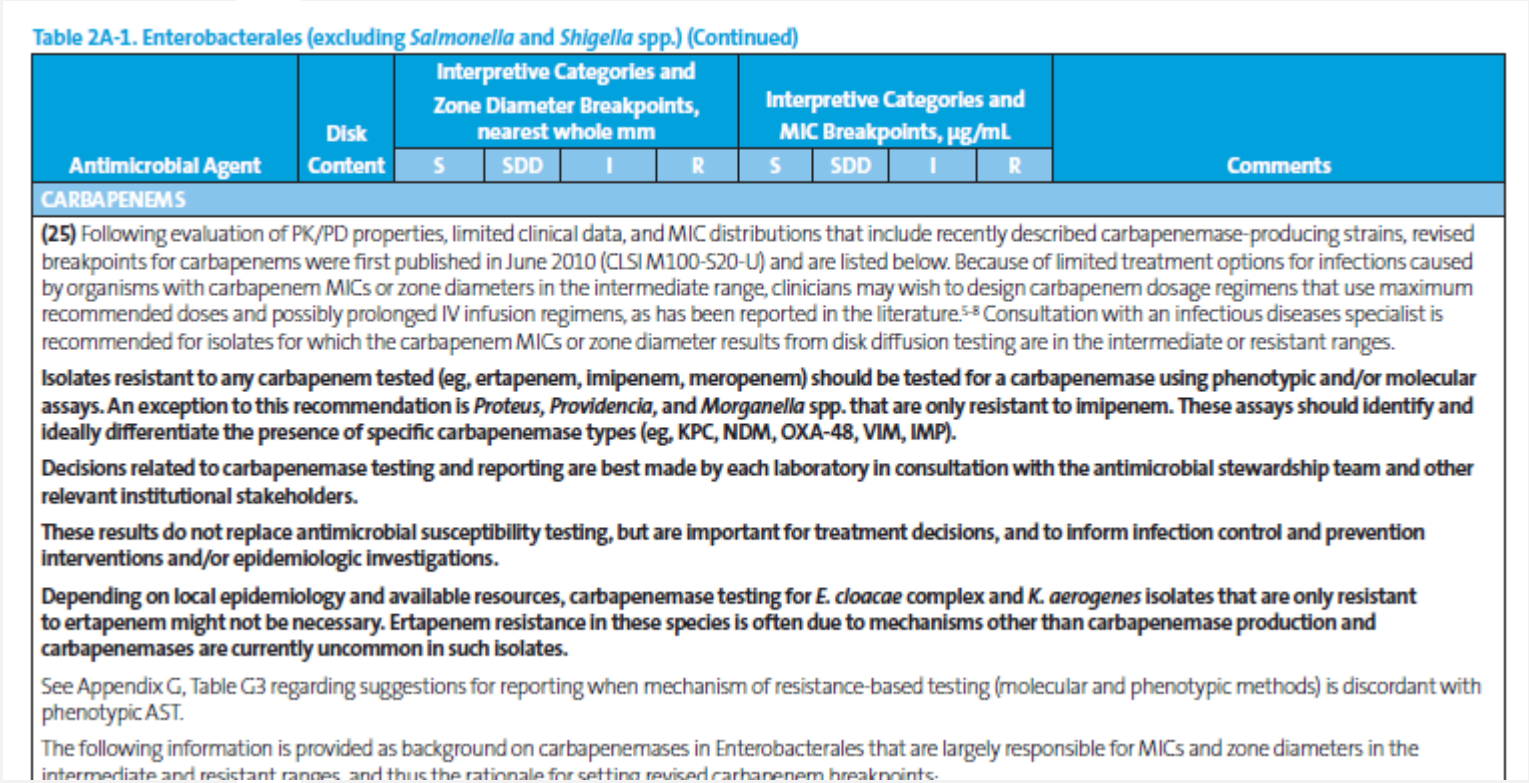


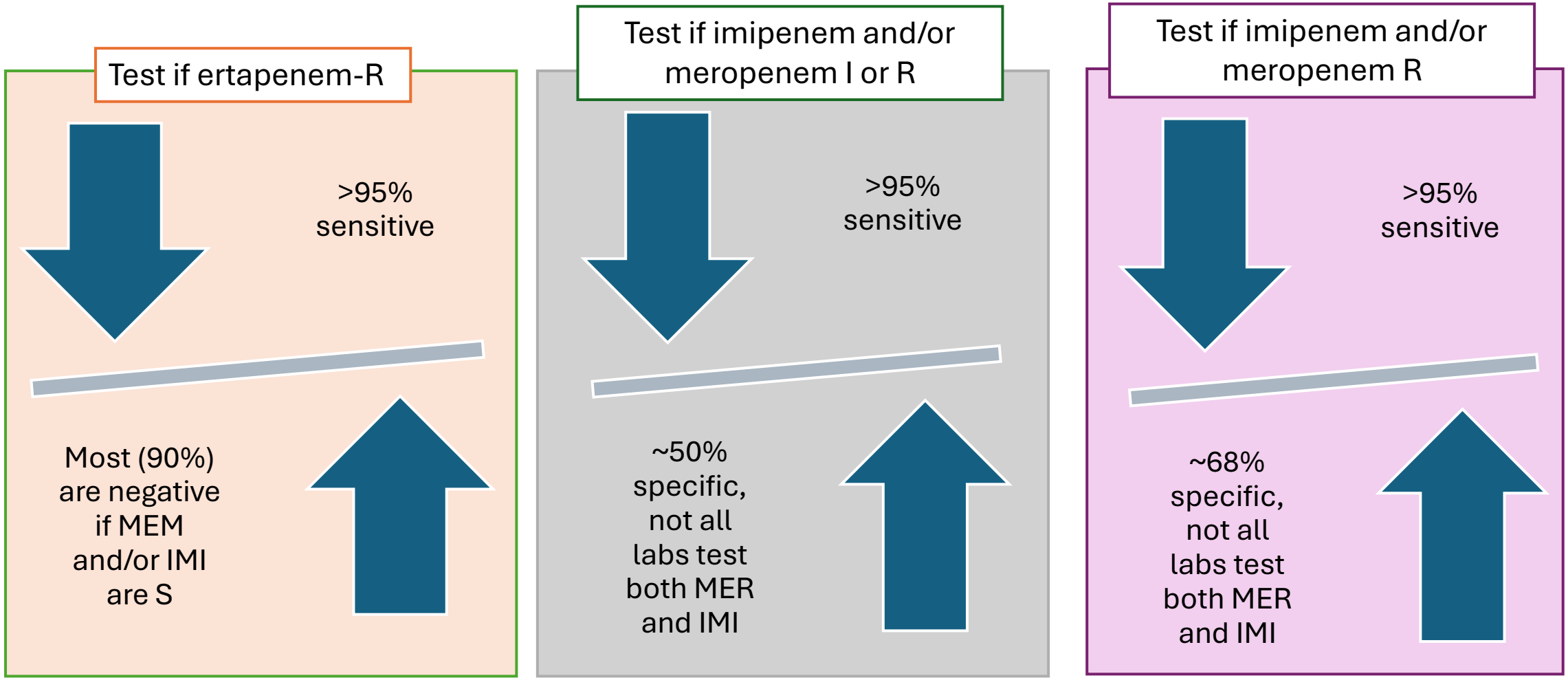
Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
CARBAPENEMS										
(25) Following evaluation of PK/PD properties, limited clinical data, and MIC distributions that include recently described carbapenemase-producing strains, revised breakpoints for carbapenems were first published in June 2010 (CLSI M100-S20-U) and are listed below. Because of limited treatment options for infections caused by organisms with carbapenem MICs or zone diameters in the intermediate range, clinicians may wish to design carbapenem dosage regimens that use maximum recommended doses and possibly prolonged IV infusion regimens, as has been reported in the literature.[5-8] Consultation with an infectious diseases specialist is recommended for isolates for which the carbapenem MICs or zone diameter results from disk diffusion testing are in the intermediate or resistant ranges.										
Isolates resistant to any carbapenem tested (eg, ertapenem, imipenem, meropenem) should be tested for a carbapenemase using phenotypic and/or molecular assays. An exception to this recommendation is *Proteus, Providencia,* and *Morganella* spp. that are only resistant to imipenem. These assays should identify and ideally differentiate the presence of specific carbapenemase types (eg, KPC, NDM, OXA-48, VIM, IMP).										
Decisions related to carbapenemase testing and reporting are best made by each laboratory in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders.										
These results do not replace antimicrobial susceptibility testing, but are important for treatment decisions, and to inform infection control and prevention interventions and/or epidemiologic investigations.										
Depending on local epidemiology and available resources, carbapenemase testing for *E. cloacae* complex and *K. aerogenes* isolates that are only resistant to ertapenem might not be necessary. Ertapenem resistance in these species is often due to mechanisms other than carbapenemase production and carbapenemases are currently uncommon in such isolates.										
See Appendix G, Table G3 regarding suggestions for reporting when mechanism of resistance-based testing (molecular and phenotypic methods) is discordant with phenotypic AST.										
The following information is provided as background on carbapenemases in Enterobacterales that are largely responsible for MICs and zone diameters in the intermediate and resistant ranges, and thus the rationale for setting revised carbapenem breakpoints:										

Goal – identify and differentiate KPC, NDM, OXA-48, VIM, IMP

CLSI M100-Ed35 Table 2A-1. (p. 64)

Carbapenemase Testing Protocols Based on Carbapenem Resistance Profile



Hard to balance over-testing (high lab cost and labor) with under-testing (miss carbapenemases)

Reference: CLSI January 2024 Agenda Book

Carbapenemase Testing for Carbapenem-Resistant Organisms (CRO)
A Primer for Clinical and Public Health Laboratories

Contents:

Google
CDPH CPO Primer

Table 1. Tests for Carbapenemases in Gram-Negative Bacteria

Method	Routinely performed on [1]			Enterobacterales	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*
	Isolates	Positive blood cultures	Rectal Swabs			
Phenotypic (for isolates)						
Modified Carbapenem Inactivation Method (mCIM) with or without EDTA Carbapenem Inactivation Method (eCIM)	yes	no	no	yes	yes (mCIM only)	no
CarbaNP [2]	yes	no	no	yes	yes	no
BioMerieux Rapidec® Carba NP	yes	no	no	yes	yes	no
BD Phoenix™ CPO Detect	yes	no	no	yes	yes	yes

AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews

REVIEW
Month YYYY Volume XX Issue XX e00054-22
https://doi.org/10.1128/cmr.00054-22

Laboratory detection of carbapenemases among Gram-negative organisms

Patricia J. Simner [1,2], Johann D. D. Pitout [3,4,5], Tanis C. Dingle [3,6]

Back to Case 3…. What are our treatment options?

- Cefiderocol: siderophore cephalosporin (2019)
  - Brand name: Fetroja
  - Active vs. 85% of MBLs[1]
  - CLSI and FDA breakpoints – these differ
  - Tests: Disk, ComASP
- Aztreonam-avibactam (2025)
  - Brand name: Emblaveo
  - Active vs. 98.4% of MBLs[2]
  - No (current) CLSI breakpoints, but FDA breakpoints →
  - Tests: Gradient diffusion, disk

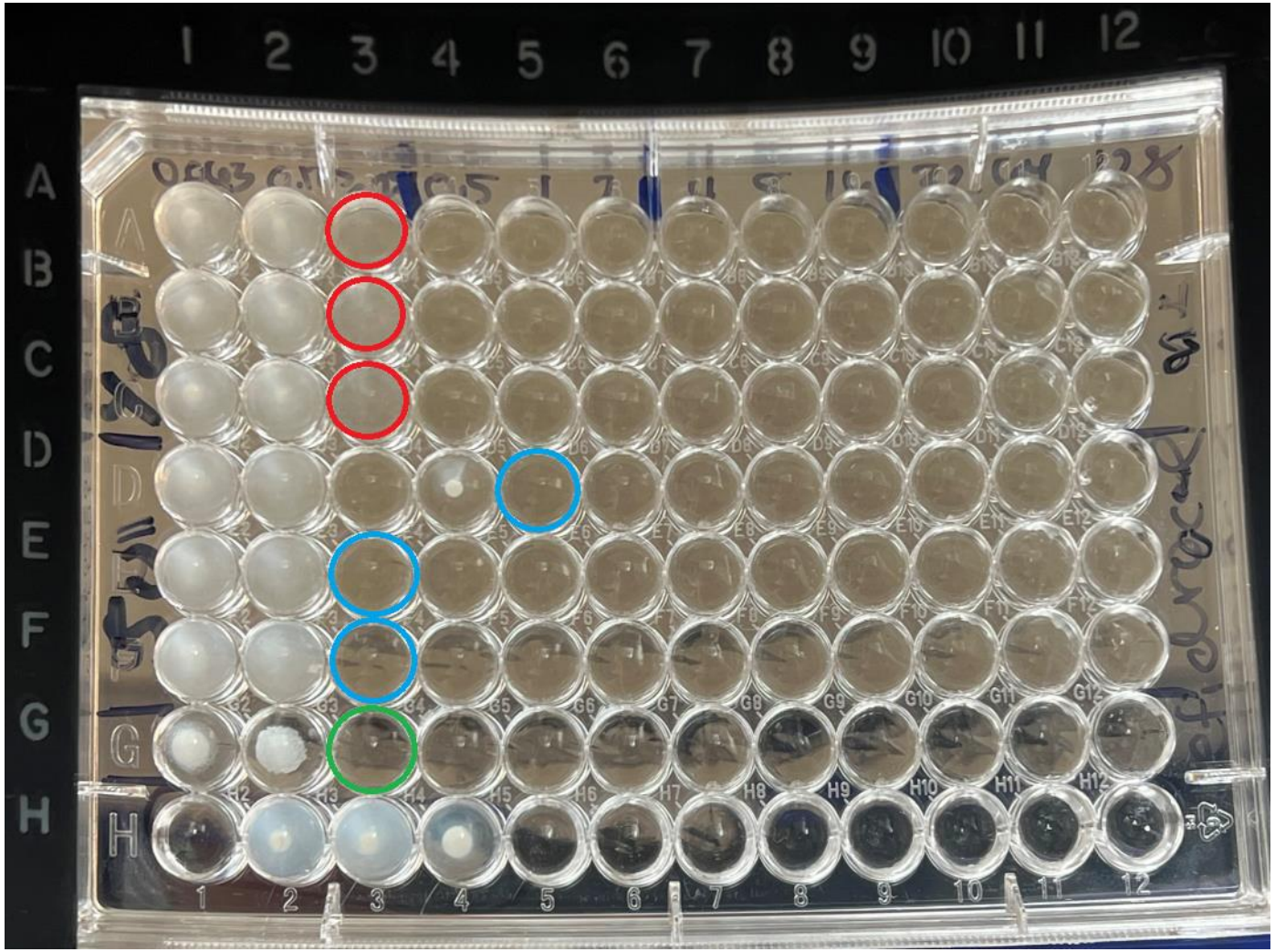
Aztreonam and Avibactam Injection

Exceptions or additions to the recognized standards

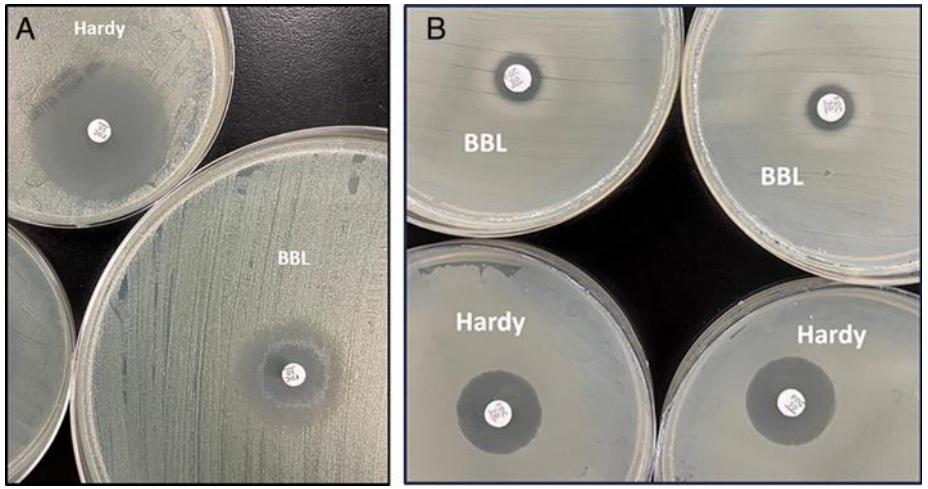
S = Susceptible; I = Intermediate; R = Resistant

	Minimum Inhibitory Concentrations (mcg/mL)			Disk Diffusion (zone diameters in mm)		
Pathogen	S	I	R	S	I	R
Enterobacterales	≤ 4/4	8/4	≥ 16/4	≥ 21	18 – 20	≤ 17

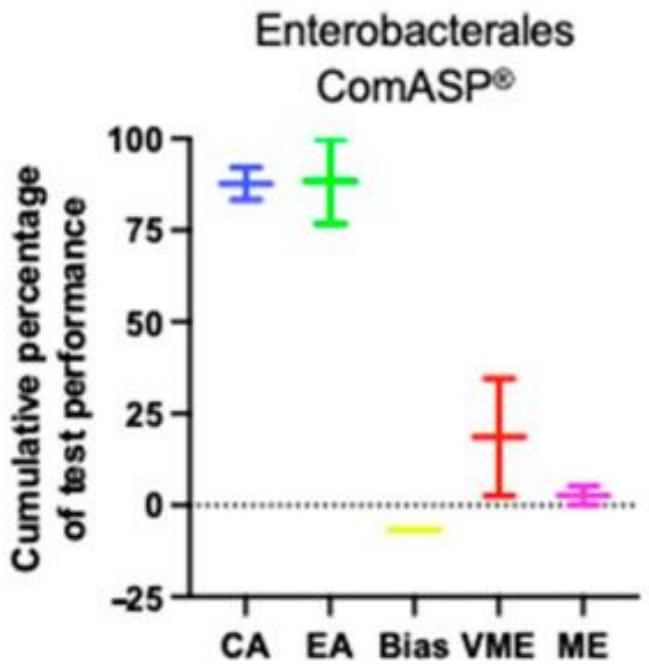
Cefiderocol testing… options?

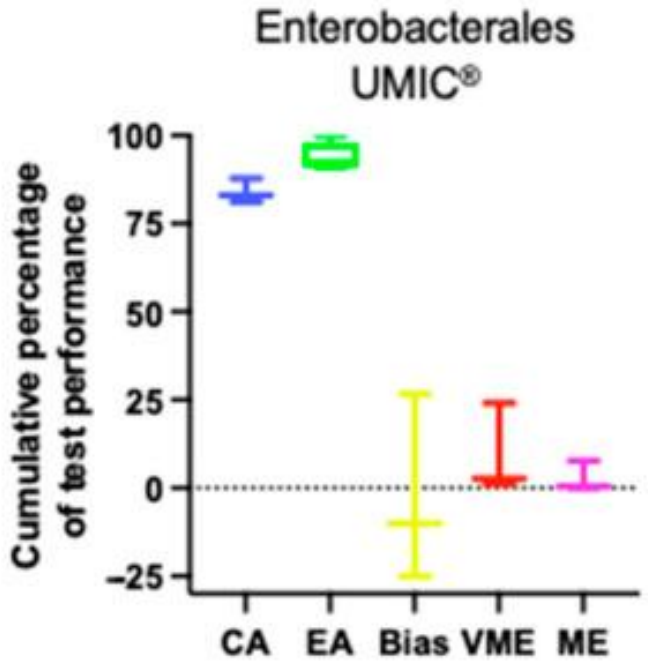


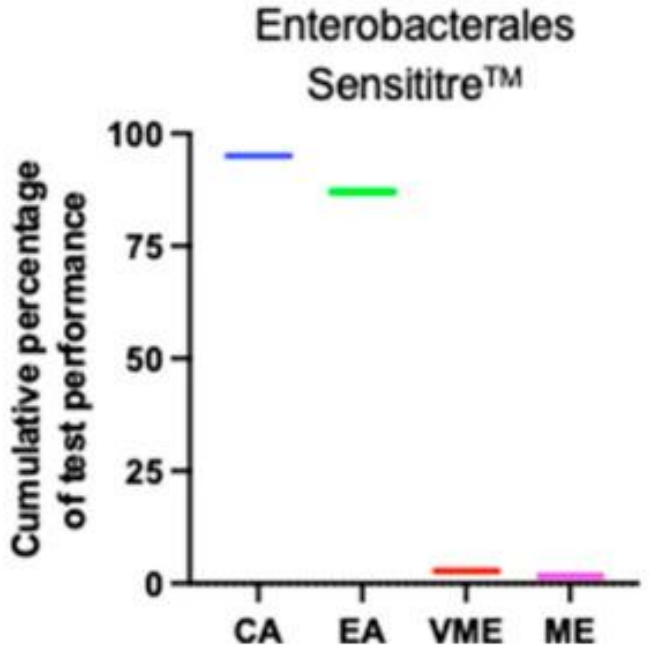
- Disk diffusion
  - Best with BBL agar
  - May miss resistance

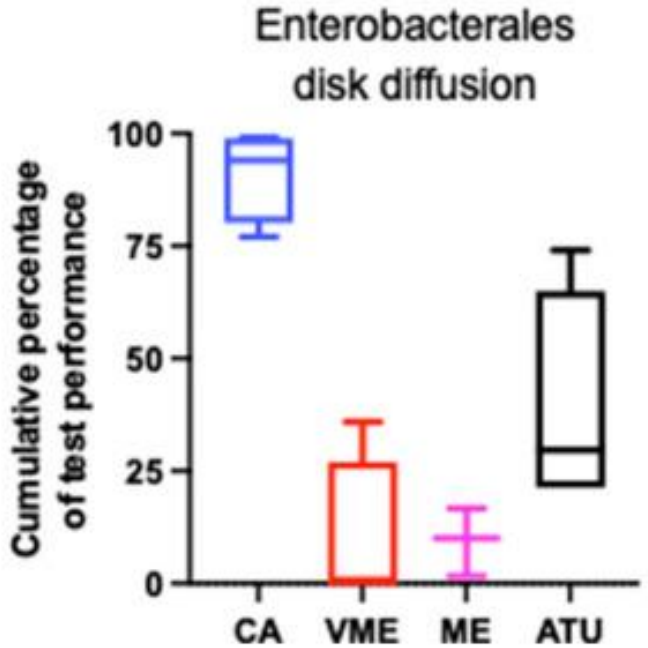


DeMarco et al. 2025. J Clin Microbiol. 63





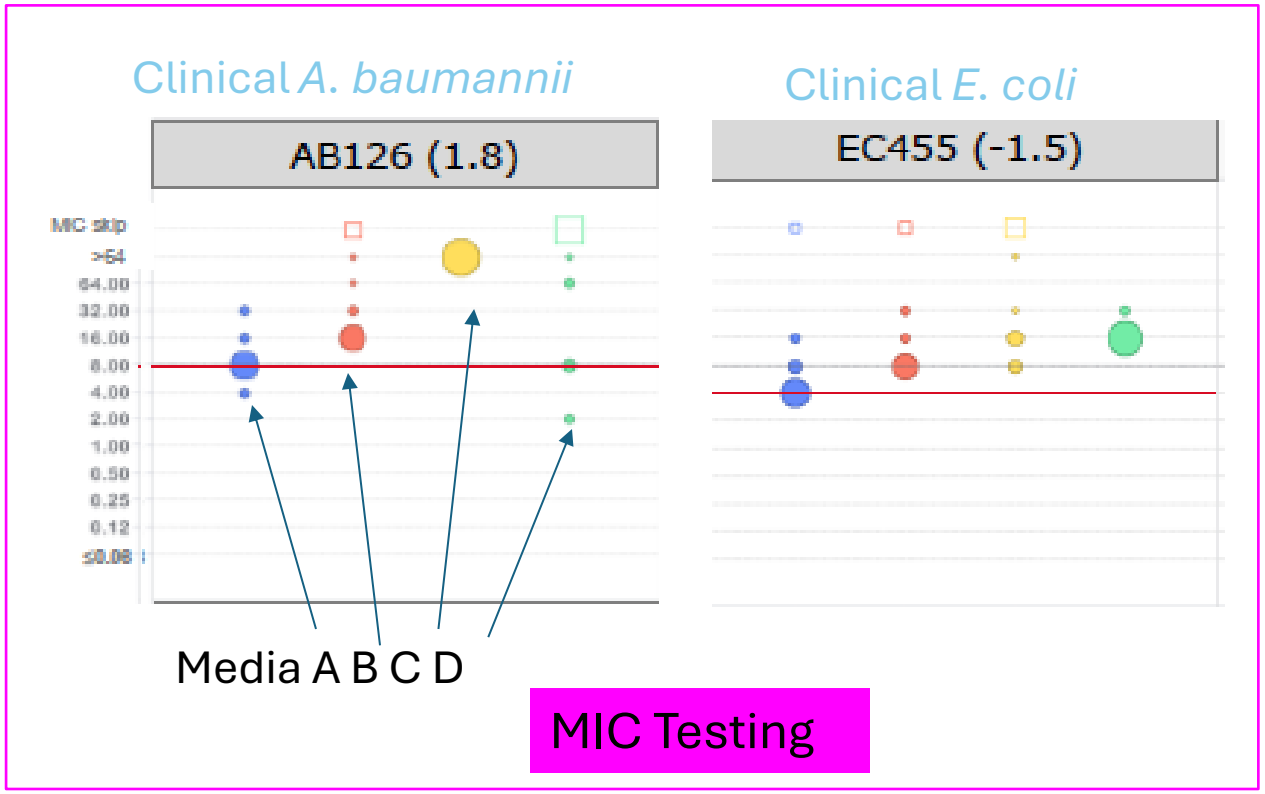




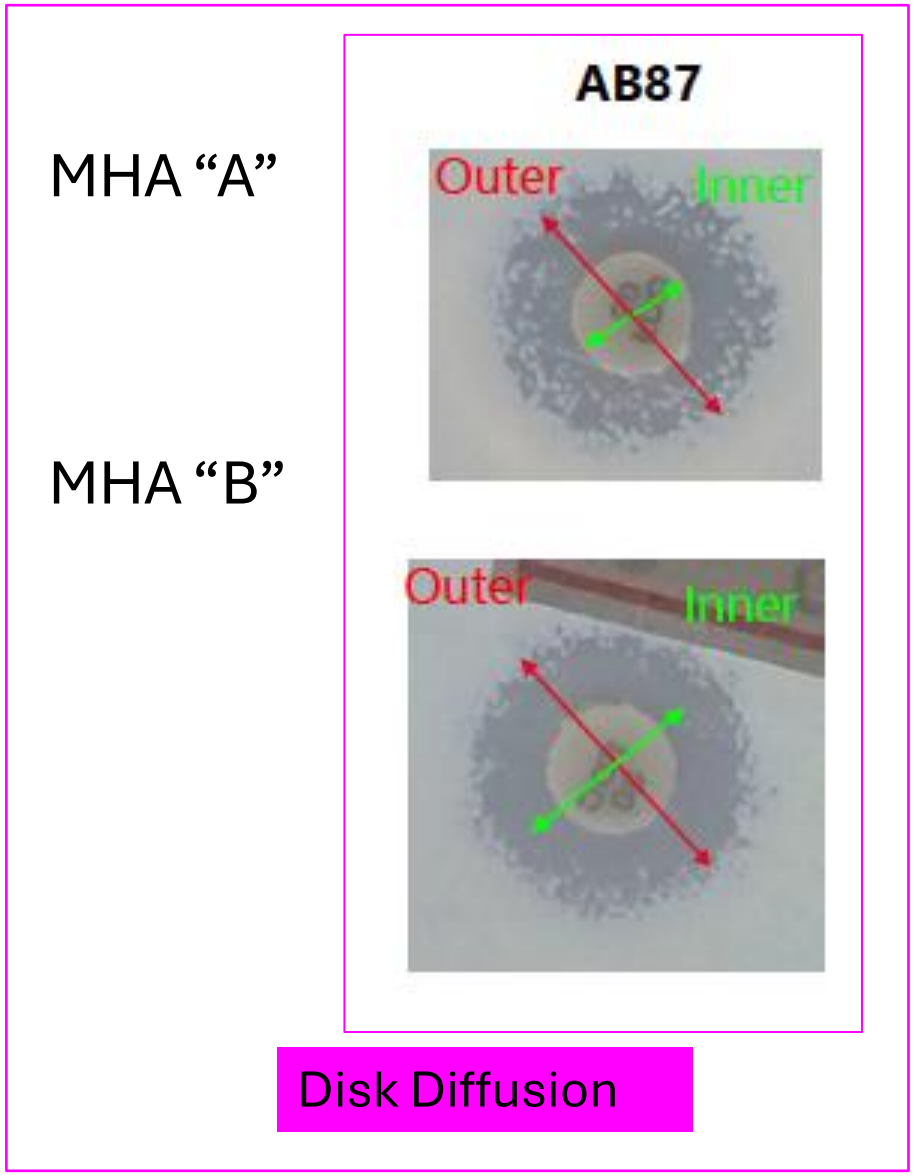
- Recommend disk diffusion for initial screen for S or R
  - Confirm with UMIC or reference BMD
- NOTE: studied with EUCAST breakpoints, which are different than CLSI

Stefani 2025. Antibiotics(Basel)14:760

Cefiderocol Reproducibility Concerns - Examples



Note: for many *A. baumannii* measuring "inner zone" correlates better with MICs



1 **Potential of Inaccurate Cefiderocol Susceptibility Results: A CLSI AST Subcommittee Advisory**

2 Patricia J. Simner[1,2], Elizabeth Palavecino[3], Michael J. Satlin[4], Amy J. Mathers[5], Melvin P. Weinstein[6],

3 James S. Lewis II[7], Romney Humphries[8], on behalf of the Clinical and Laboratory Standards Institute

4 Antimicrobial Susceptibility Testing Subcommittee

Issues:

1. Poor reproducibility of BMD and disk diffusion for *A. baumannii* isolates with MICs >2 µg/mL
2. Hard to read MIC and disk diffusion endpoints (trailing and inner colonies)
3. Small differences in inoculum = major differences in cefiderocol MICs

Resulted in warning language added to M100 33rd ed.
Work ongoing to fix these issues!

Cefiderocol

(37) The accuracy and reproducibility of cefiderocol testing results by disk diffusion and broth microdilution are markedly affected by iron concentration and inoculum preparation and may vary by disk and media manufacturer. Depending on the type of variance observed, false resistant or false susceptible results may occur. Testing subsequent isolates is encouraged. Discussion with prescribers and antimicrobial stewardship members regarding the potential for inaccuracies is recommended.

Applies to Enterobacterales, *P. aeruginosa, Acinetobacter,* and *S. maltophilia*

First added: M100 33rd ed. Tables 2A. (p. 64), 2B-1. (p. 76), 2B-2. (p. 81), 2B-4. (p. 87).

How to approach cefiderocol testing in your laboratory?

- Few testing options:
  - Disk diffusion, commercial MIC or MIC testing at a reference laboratory *
- Discuss pros/cons of testing with ASP partners:
  - Testing issues are known to lead to both false “S” and false “R”
  - Issues most pressing for *A. baumannii*
  - Careful evaluation of patient’s response to therapy with cefiderocol
  - Test subsequent isolates from the same patient routinely
- Resistance can emerge on therapy **

* Cefiderocol testing info: https://www.fetroja.com/microbiologist-diagnostic-toolkit#top

** Karakonstantis, S. et al. 2022. Antibiotics. 11:723.

Example: VUMC Approach

A. baumannii - burn wound

Antimicrobial	MIC (µg/mL)	
Amikacin	16	S
Amp-Sulb	>32	R
Cefepime	>32	R
Ceftazidime	>32	R
Ciprofloxacin	>4	R
Gentamicin	>16	R
Levofloxacin	>4	R
Meropenem	>8	R
Minocycline	>32	R
Piperacillin-tazobactam	>128	R
Trim-Sulfa	>4	R
Tobramycin	>16	R

Table 1D. *Acinetobacter* spp.

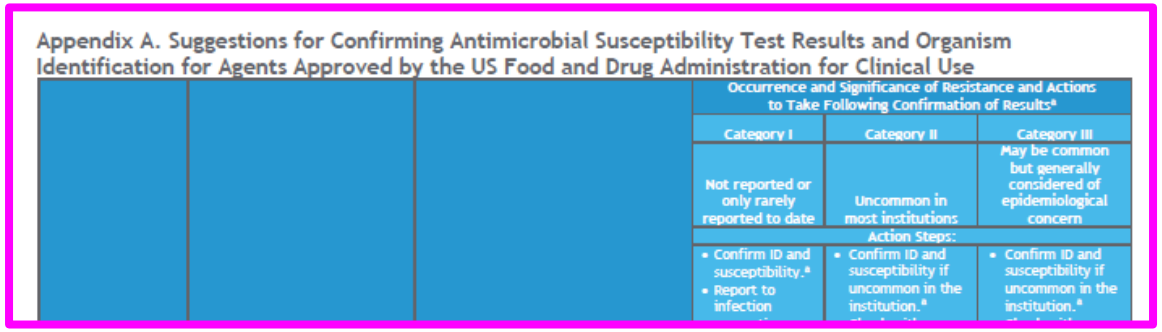
Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate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

Abbreviation: MDRO, multidrug-resistant organism.

M100 33rd ed. Table 1D. (p. 32)

- MDR *A. baumannii*
- Other test options – amp-sulbactam, colistin, cefiderocol
- Test cefiderocol by disk diffusion w/ director consultation
  - If zone very large (>28 mm) or no zone, feel confident reporting
  - If zone anywhere in between, or hard to read → send for reference MIC

Additions to M100 33rd ed. Appendix A*


Appendix A. Suggestions for Confirming Antimicrobial Susceptibility Test Results and Organism Identification for Agents Approved by the US Food and Drug Administration for Clinical Use

			Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
			Action Steps:		
			• Confirm ID and susceptibility.[a] • Report to infection	• Confirm ID and susceptibility if uncommon in the institution.[a]	• Confirm ID and susceptibility if uncommon in the institution.[a]

Organism Group	Antimicrobial Class	Antimicrobial Agents and Resistance Phenotypes Detected	Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Enterobacterales	Cefiderocol	I or R	X		
A. baumannii complex	Cefiderocol	I or R	X		
P. aeruginosa	Cefiderocol	I or R	X		
S. maltophilia	Cefiderocol	NS	X		

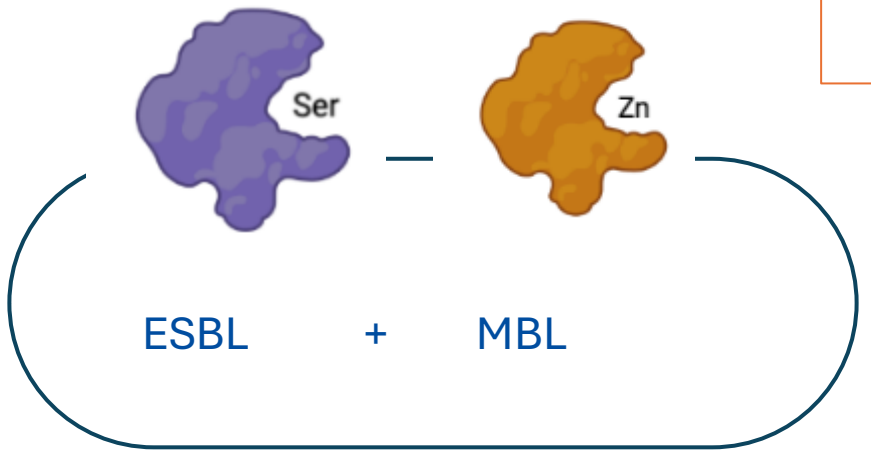
Cefiderocol resistance remains relatively rare – "R" results should be confirmed at reference laboratory that does MIC testing

M100 33rd ed. App A. (pp. 274-280).

New! Aztreonam + Avibactam for MBLs

ESBL:
Hydrolyze Aztreonam
Inhibited by avibactam

MBL:
Cannot hydrolyze aztreonam
NOT inhibited by avibactam



Together, avibactam protects aztreonam from ESBL = aztreonam activity

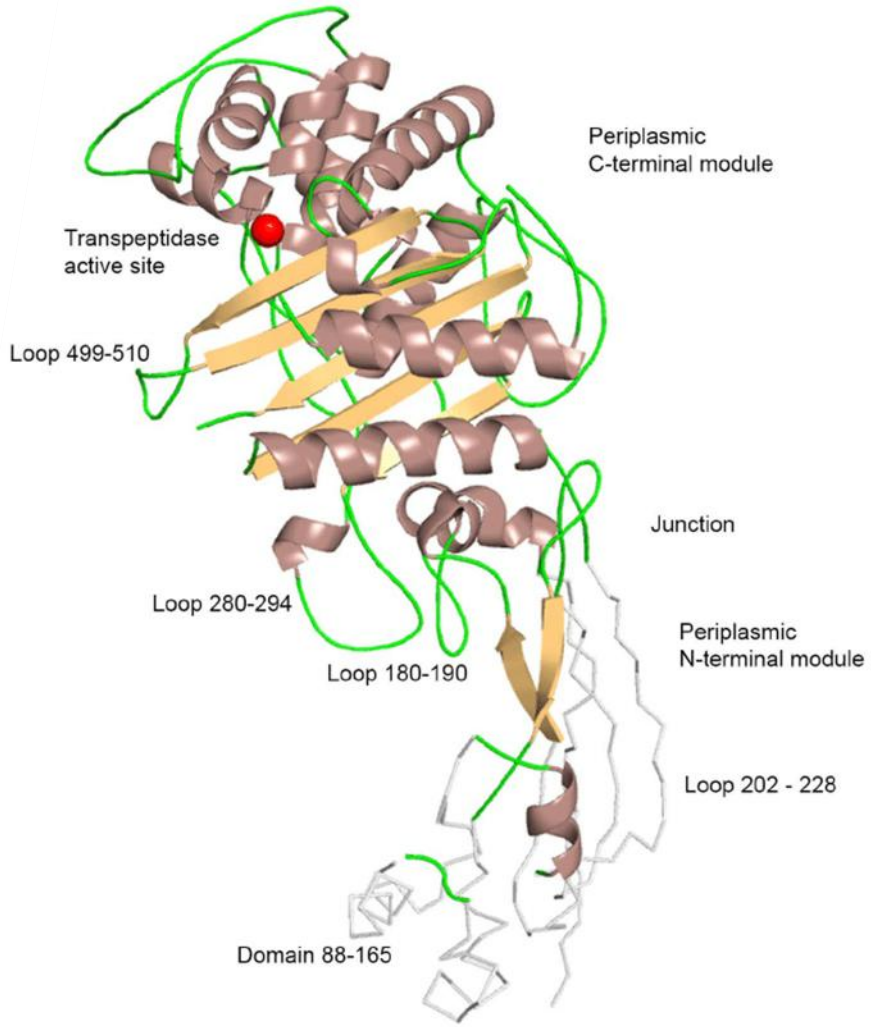
- Aztreonam-avibactam available in late 2024
- CLSI breakpoints to be published in 2026
- Recommended by IDSA for MBL-producers, including *S. maltophilia*

  EXCEPTION!! *A. baumannii* is intrinsically "R" to aztreonam

Tamma et al. 2022. CID. 75:187-212.
Marshall et al. 2917. AAC. 61(4).

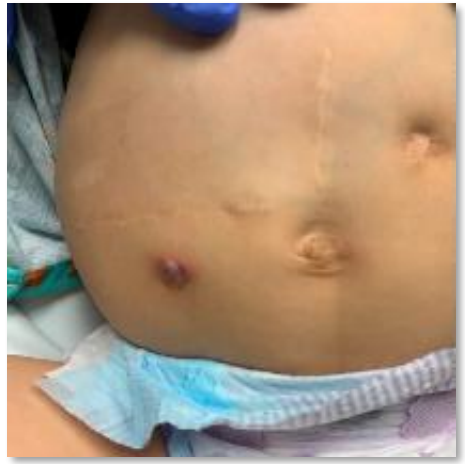
Do we need to test for ATM+AVI for susceptibility?

- Yes! Resistance is emerging.
- Aztreonam targets PBP3.
- YRN/K insertion at position 333 = ATM “R”
  - Also impacts cefiderocol, cefepime-taniborbactam
- PBP3 mutants prevalent globally
- CMY-42/other AmpC can also lead to resistance
- Isolates with NDM-5 more likely to be “R”



Mendes et al. 2021. JAC. 76:2833-8.
Sadek et al. 2020. AAC. 64:e01659.
Sader et al. 2022. Eur J Clin Microbiol Infect Dis. 41:477-87.
Rossolini. 2024. J Global Antimicrob Res 36:123

Case 4. New Cefepime Reporting Rule



Citrobacter freundii

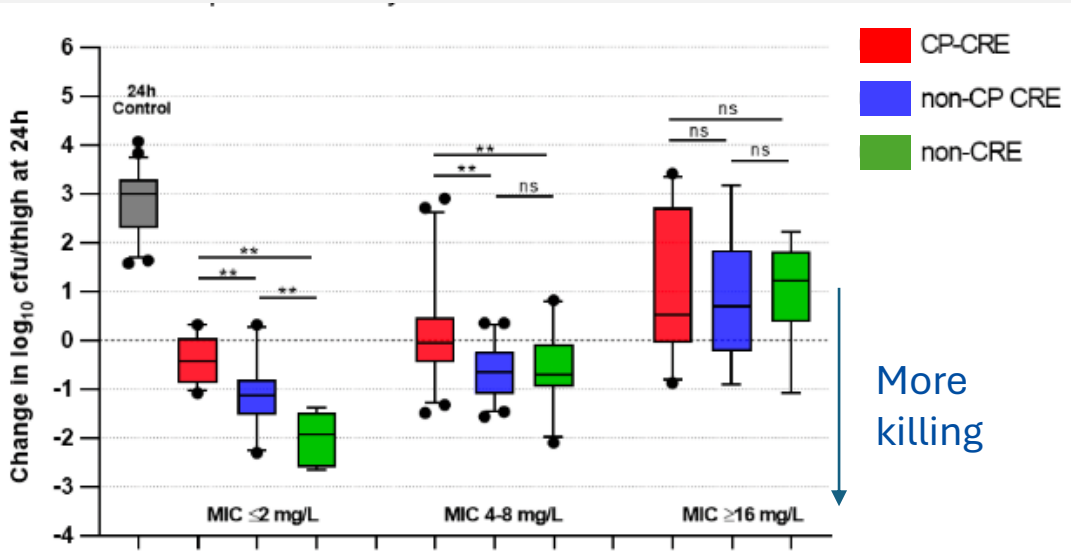
	MIC (µg/mL)	
Ampicillin	>16	R
Ceftriaxone	>32	R
Cefepime	4	SDD
Ertapenem	>4	R
Gentamicin	≤1	S
Meropenem	4	R
Trimeth-sulfa	≤0.5/9.5	S

NG-Test® Carba-5: KPC detected

Cefepime Treatment for KPC Producers Observations

- Barnes-Jewish Hospital
  - 149 KPC isolates, 14% S or SDD to cefepime
- Johns Hopkins Hospital
  - 209 KPC, 28% S or SDD to cefepime
- Mouse study
  - 2 g q 8h humanized dose of cefepime
  - Despite S or SDD MIC, do not achieve 1-2 log kill

Bacterial Killing with Cefepime, by MIC



Fissel et al. 2020. JCM. 58(9):e01271-20.

Cefepime and Enterobacterales
CLSI M100-Ed35

Table 2A-1. Enterobacterales

Comment (18):

> "Cefepime S/SDD results should be suppressed or edited and reported as resistant for isolates that demonstrate **carbapenemase production.**"

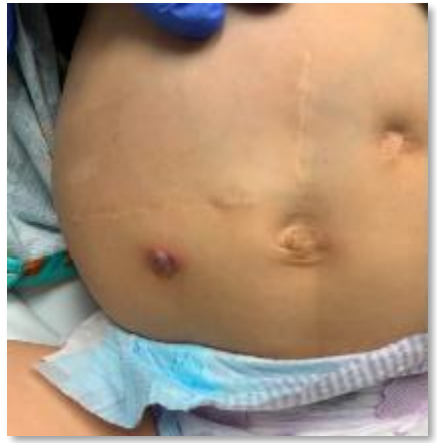
Appendix G. Using Molecular Assays for Resistance Detection

Table G3:

> For isolates S or SDD to cefepime but **positive for any carbapenemase by phenotypic or genotypic testing**: Cefepime should be suppressed or reported as R.
>
> **"NOTE:** Current evidence suggests cefepime therapy may not be effective against carbapenemase-producing strains. Most of these data are based on studies investigating KPC-producing CREs."

CLSI M100-Ed35 Table 2A-1; Table G3

Case 4. New Cefepime Reporting Rule



Citrobacter freundii (Final Report)

Antimicrobial	MIC (µg/mL)	
Ampicillin	>16	R
Ceftriaxone	>32	R
Cefepime	4	~~SDD~~ R
Ertapenem	>4	R
Gentamicin	≤1	S
Meropenem	4	R
Trimeth-sulfa	≤0.5/9.5	S

NG-Test® Carba-5: KPC detected

Case 5.
New Meropenem-Vaborbactam Reporting Rule

Escherichia coli

Antimicrobial	MIC (µg/mL)	
Ceftriaxone	>32	R
Ceftazidime-avibactam	≤0.5/4	S
Ciprofloxacin	2	R
Ertapenem	>2	R
Fosfomycin		S
Gentamicin	8	R
Meropenem	4	R
Meropenem-vaborbactam	4	S
Nitrofurantoin	128	R
Trimeth-sulfa	>2/38	R

Challenge of Combination Agents vs OXA-48

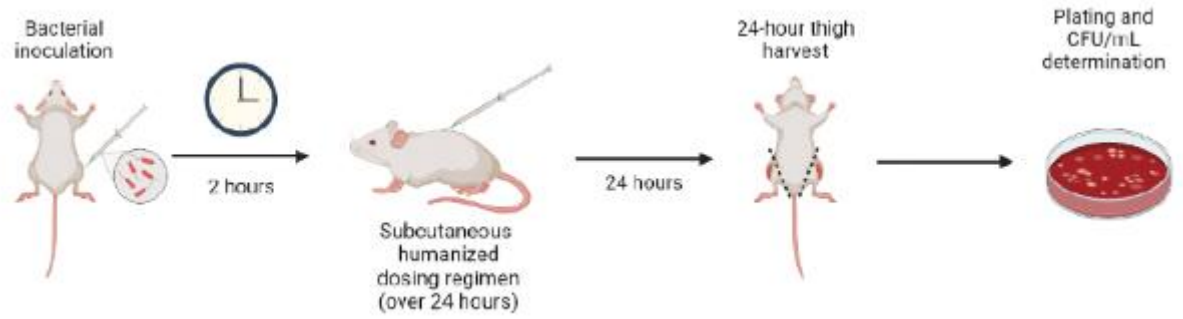
OXA-48-like enzymes

- Poor activity vs cephalosporins (isolates usually ”R” due to coproduction of ESBL)
- Relatively low MICs to carbapenems (vs KPC)
- Inhibited by avibactam, relebactam, but NOT by vaborbactam
- Challenge with combination agent breakpoints:

Antimicrobial	Susceptible MIC (µg/mL)	Dose
Ceftazidime	≤4	1 g IV q 8h
Ceftazidime-avibactam	≤8/4	2.5 g IV q 8h (pneumonia) or 1.5 g IV q 8h (other infection)
Imipenem	≤1	500 mg IV q 6h or 1 g q 8h
Imipenem-relebactam	≤1/4	1.25 g IV q 6h
Meropenem	≤1	1 g IV q 8h
Meropenem-vaborbactam	≤4/8	4 g IV q 8h, 3 h infusion

Ceftazidime-avibactam and Meropenem-vaborbactam “S” breakpoints are higher than ceftazidime or meropenem alone due to dosing.

Impact of Meropenem-Vaborbactam Breakpoint vs OXA-48 Producers



Antimicrobial	Susceptible Breakpoint MIC (µg/mL)	% Bactericidal Activity if "S"
Ceftazidime-avibactam	≤8/4	96-100%
Imipenem-relebactam	≤1/4	100%
Meropenem-vaborbactam	≤4/8	9-50%

January 2023 CLSI AST Subcommittee Agenda Book

Case 5.
Meropenem-Vaborbactam Reporting Rule

- (13) Enterobacterales that harbor OXA-48-like enzymes may test susceptible to meropenem-vaborbactam, but may not respond to meropenem-vaborbactam *in vivo*. If an OXA-48-like gene or enzyme is detected, suppress meropenem-vaborbactam or report as resistant.

CLSI M100-Ed35 Table 2A-1.

Escherichia coli (Final Report)

Antimicrobial	MIC (µg/mL)	
Ceftriaxone	>32	R
Ceftazidime-avibactam	≤0.5/4	S
Ciprofloxacin	2	R
Ertapenem	>2	R
Fosfomycin		S
Gentamicin	8	R
Meropenem	4	R
Meropenem-vaborbactam	~~4~~	~~S~~ R
Nitrofurantoin	128	R
Trimethoprim-sulfamethoxazole	>2/38	R

Summary: Considerations for Addressing β-lactam Resistance Mechanisms in 2025

"R" Mechanism	Why might you test?	Considerations?
ESBL	• Infection control precautions • Guide appropriate therapy (eg, avoid piperacillin-tazobactam)	• *E. coli, K. pneumoniae, K. oxytoca, P. mirabilis* only • Some false negatives & false positives • Use 3rd gen cephalosporin as surrogate
Cabapenemase	• Infection control precautions • Public health follow up • Guide appropriate therapy (eg, encourage newer β-lactam combination agents)	• Know local epidemiology • Delayed results with some phenotypic tests (eg, mCIM) • Molecular and lateral flow test "kits" limited targets
AmpC	(No standardized test available)	• Provide cautionary comments on laboratory reports for 3rd generation cephalosporins if "S"

CLSI recommendation is to not edit "S" to "R" if ESBL

Case 6

- 37 Year old Kidney Transplant Recipient
- Presents with pneumonia, septic shock
- BAL: Many *Acinetobacter baumannii*
- Blood: *Acinetobacter baumannii*
  - OXA-23/-48 detected by ePlex

Antimicrobial	MIC (µg/mL)	
Ampicillin/sulbactam	**8**	**S**
Ceftriaxone	>64	R
Cefepime	64	R
Ciprofloxacin	>4	R
Gentamicin	>16	R
Meropenem	>16	R
Minocycline	≤4	S
Tobramycin	>16	R
Trimethoprim-Sulfamethoxazole	>32	R

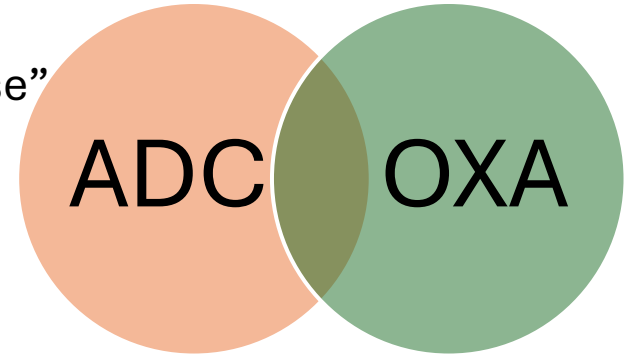
Call to lab from ID Fellow to Micro Fellow:

"Is it weird that **ampicillin/sulbactam is "S"** but **meropenem is "R"**?
Would it be better to use sulbactam-durlobactam?"

Acinetobacter baumannii: all about beta-lactamases

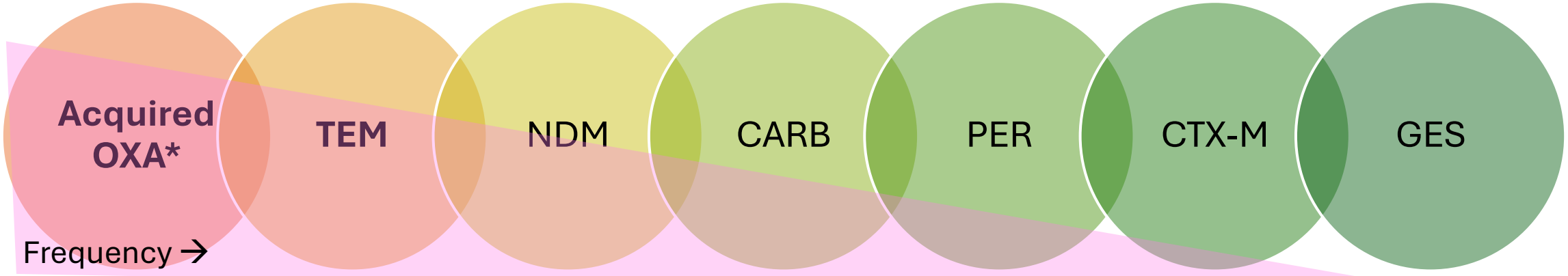
Intrinsic

- ”Acinetoacter-derived cephalosporinase”
- Class C beta-lactamase
- Non-inducible
- Usually expressed at low level
- Penicillin, cephalosporin resistance



- OXA-51 intrinsic to A. baumannii
- Class D beta-lactamase
- Low-level carbapenem resistance
- Many, many mutants with variable carbapenemase activity

Acquired

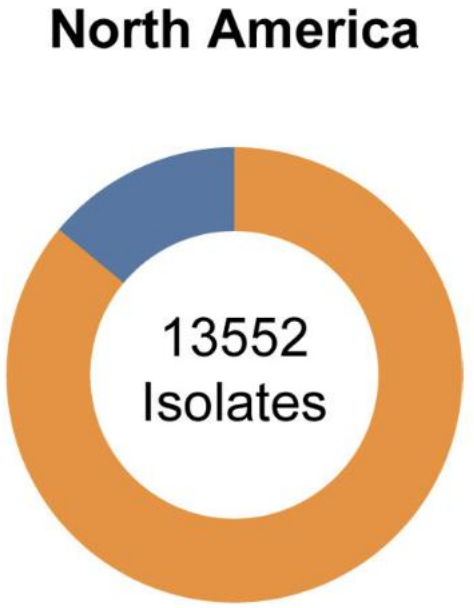


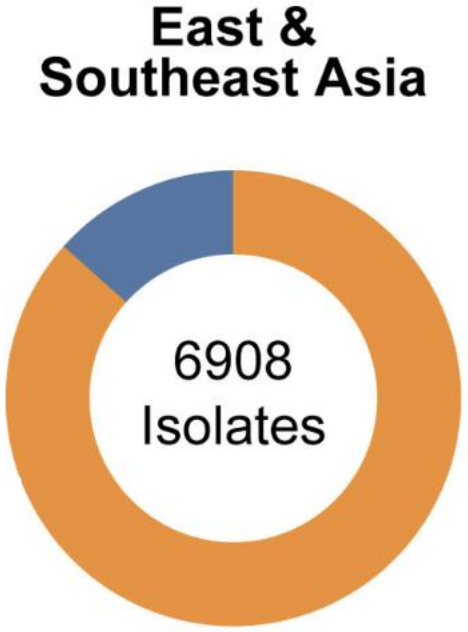
*note, most common carbapenemase, OXA-23 is from A. radioresistans

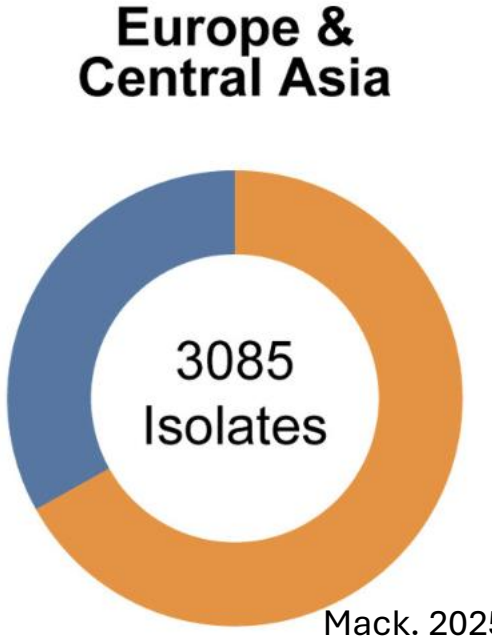
Mack. 2025 AAC 69(3)
Evans 2014. CMR. 27:241

Carbapenem resistance

- Globally, carbapenem resistance in *A. baumanni*
  - OXA-23 family (64%)
  - OXA-24 family (14%)
  - Overexpression of intrinsic OXA-51



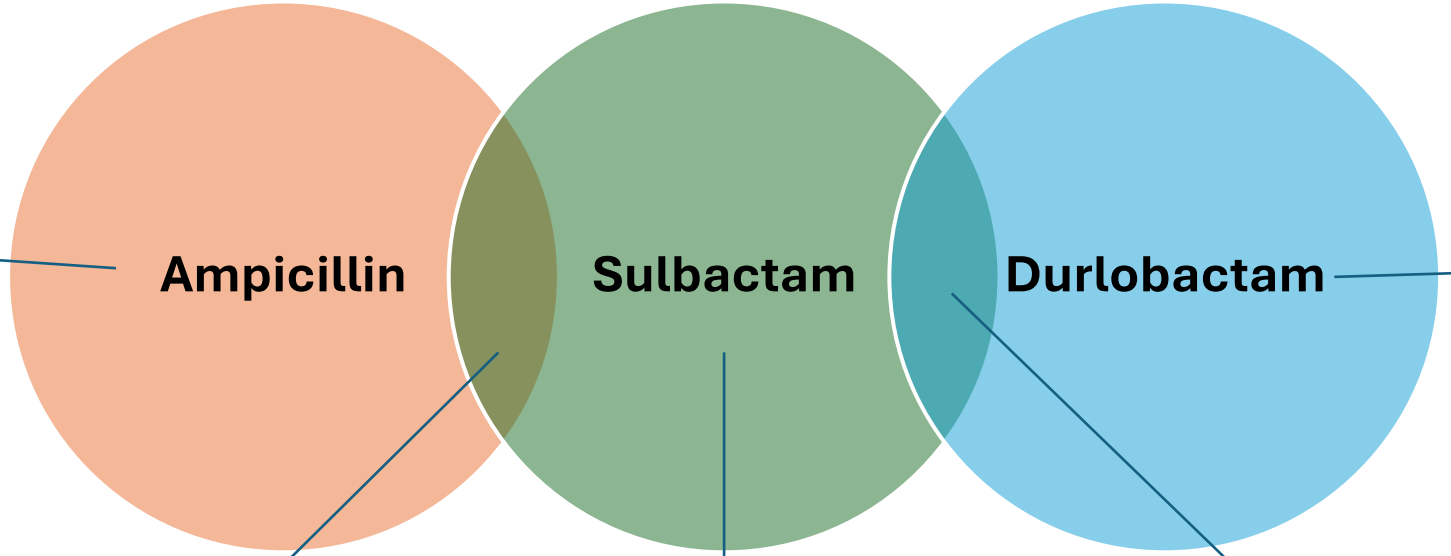




Carbapenemase Absent Present

Mack. 2025 AAC 69(3)

So – what's the story with Ampicillin, Sulbactam and Durlobactam



Narrow—spectrum beta-lactam hydrolyzed by ADC

No activity vs. *A. baumannii*

Non-beta-lactam inhibitor of class A, C and D BLA

No activity alone vs. *A. baumannii*

Activity alone vs. *A. baumannii*

Ampicillin-**sulbactam** for A. baumannii, as sulbactam alone is not commercially available

Resistance: beta-lactamases (TEM, NDM)

Sulbactam-durlobactam used if amp-sulbactam "R"

Resistance: PBP3 mutation, NDM

BLA, β-lactamase

Penwell et al. 2015. AAC 59:1680.

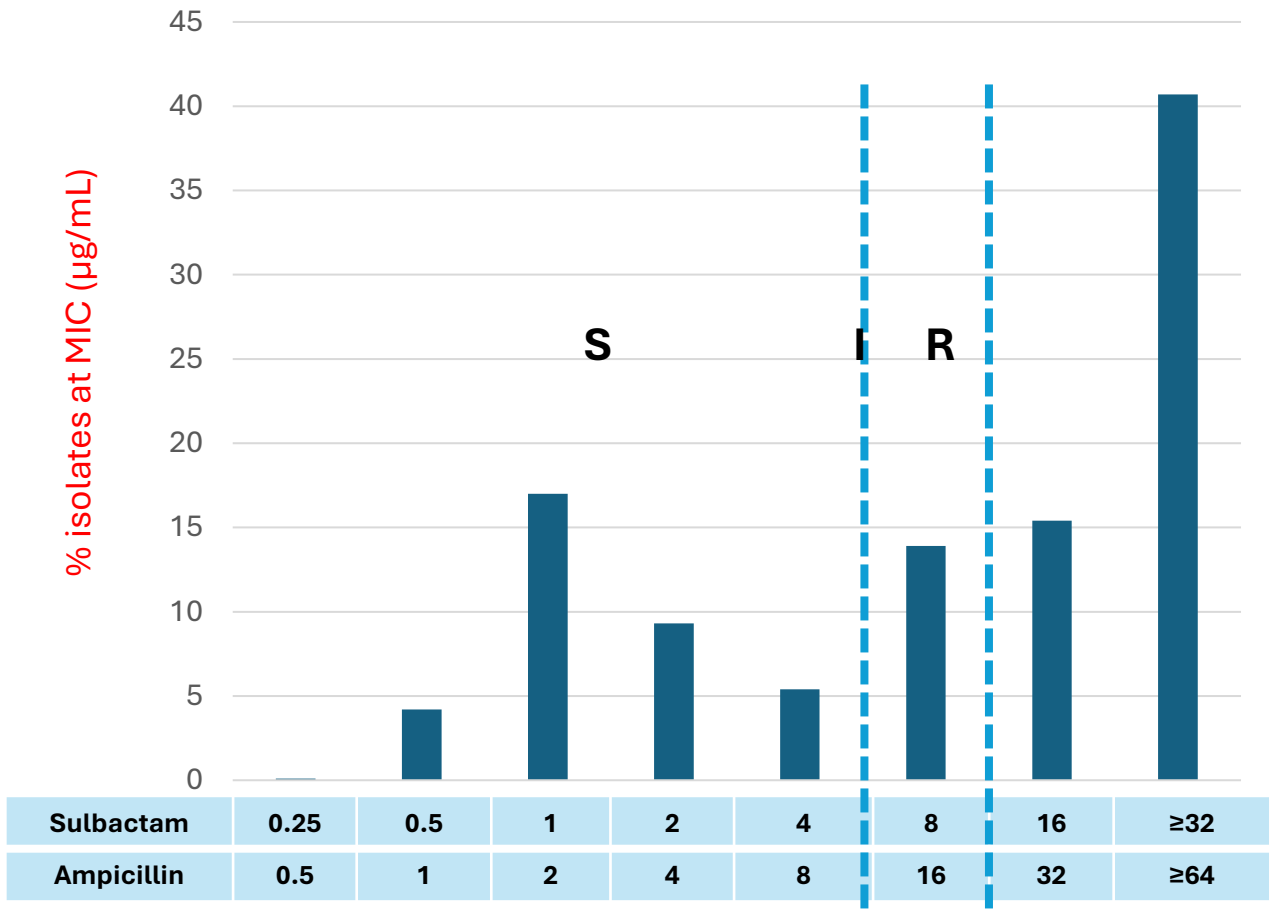
Acinetobacter spp. Breakpoints in CLSI M100

- **Originally** "all" bacteria interpreted with same breakpoints
- **Mid-2000s:** genus-specific breakpoints published
- **2014:** Breakpoints for imipenem, meropenem, doripenem updated
- *2019*: Added breakpoints for cefiderocol
- **2024:** Added breakpoints sulbactam-durlobactam
- 2025: Reviewed ampicillin-sulbactam

Do we believe ampicillin-sulbactam breakpoints are correct?

What about other breakpoints for *Acinetobacter* spp.

Ampicillin-Sulbactam for *A. baumannii*



Sulbactam	0.25	0.5	1	2	4	8	16	≥32
Ampicillin	0.5	1	2	4	8	16	32	≥64

- ECV for sulbactam, 4 µg/mL (equivalent to 8/4 µg/mL ampicillin-sulbactam)
- PK/PD variable, but high dose extended infusion needed to achieve targets [3 g (2 g ampicillin + 1 g sulbactam) given IV q 6 hour, over ≥3h)]
- Clinical outcomes show reasonable response with MIC 8/4 µg/mL
- Change: added dose for ampicillin-sulbactam susceptible in M100 35th ed (Table 2 dosages, page 175)

ECV, epidemiological cutoff value

Treatment options…. CRAB

Ampicillin Sulbactam	Sulbactam durlobactam	Minocycline	Cefiderocol	Polymyxins
• Used at high dose, IDSA treatment of choice if "S" • breakpoints reviewed for 2025 by CLSI	• Used with a carbapenem • IDSA treatment of choice	• PK unfavorable • CLSI 2025 breakpoints are for stasis	• Unclear data: many studies show excess mortality • FDA & CLSI breakpoints differ	• High toxicity • Poor outcomes
16% “S”	98% “S”	34% “S”	97% “S”	92% “I”

Testing challenges:

- Cefiderocol testing is exceedingly hard for A. baumannii. Best to perform reference BMD
- No FDA-cleared tests for polymyxins (labs shouldn’t test anyway)

Susceptibility from JMI MVP program, USA isolates

Case 7.

- 52 YO presents via air ambulance from Cabo, Mexico with new diagnosis of ALL for induction chemotherapy
  - Patient had PICC line placed in Mexico
- Develops fever on hospital day 1 (before induction)
- Blood cultures: Gram-negative Rods
- Molecular ID: no identification
- Final ID: *B. cepacia*
- AST performed using gradient diffusion

***Burkholderia cepacia* complex**

	MIC (µg/mL)	
Ceftazidime	4	?
Levofloxacin	0.5	?
Trimethoprim-sulfamethoxazole	0.5	?

M100 35th Edition, Table 2B-3

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

Testing Conditions	
Medium:	Broth dilution: CAMHB
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	35°C ± 2°C; ambient air; 20–24 hours

QC Recommendations

Refer to the following:
- **Table 5A-1 that lists acceptable QC ranges**
- **Appendix I to develop a QC plan**

General Comments

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

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

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

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

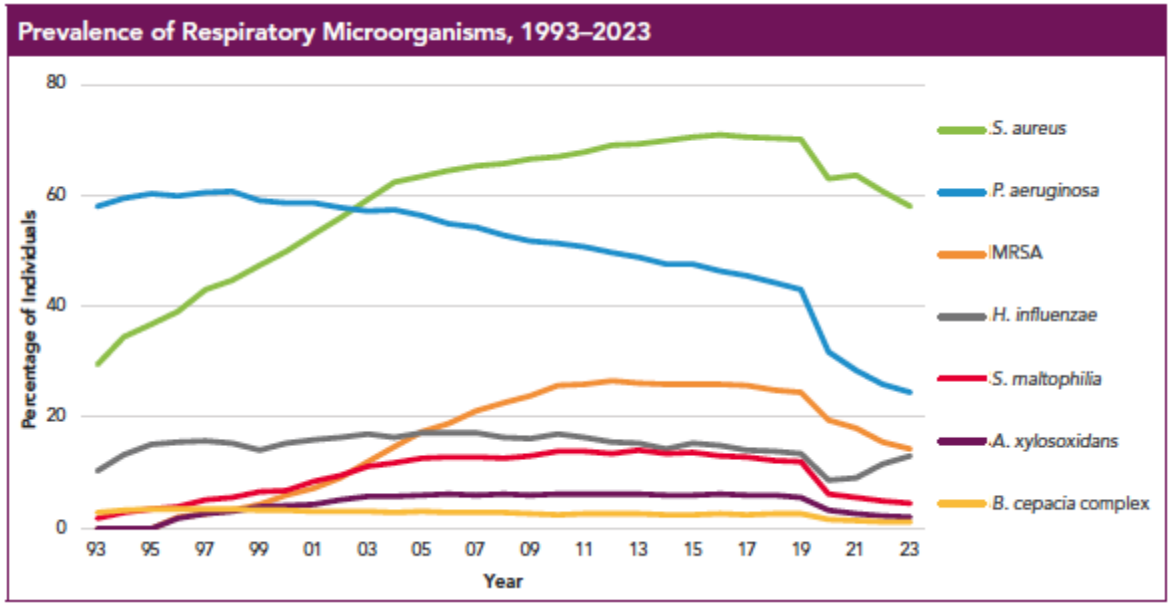
NOTE: Information in boldface type is new or modified since the previous edition.

- No more breakpoints!
- Reference broth microdilution (frozen panels) are the only reproducible method

Infections caused by BCC

- Traditionally in CF patients
- Sporadic cause of disease in patients with serious underlying disease
  - Chronic granulomatous disease
  - Often from contaminated environmental sources
  - Nosocomial infections due to oral, ophthalmic, infusion solutions

Chakhtoura et al. CID 65: 1327-34.

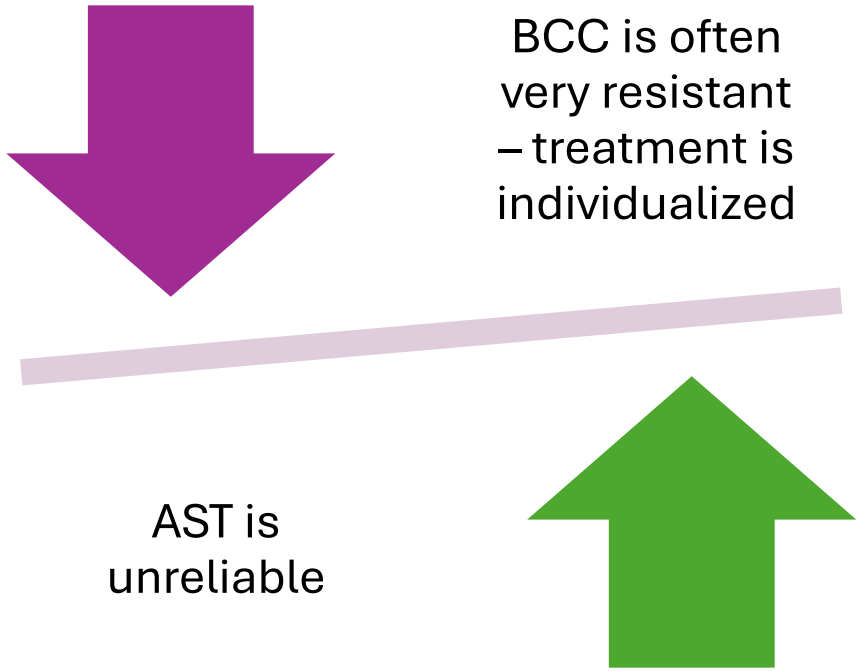


Reduced incidence of bacterial pathogens in CF:

- Fewer cultures performed
- Better therapies for CF
- Better early eradication for patients with CF

CFF Patient Registry Annual Report 2023

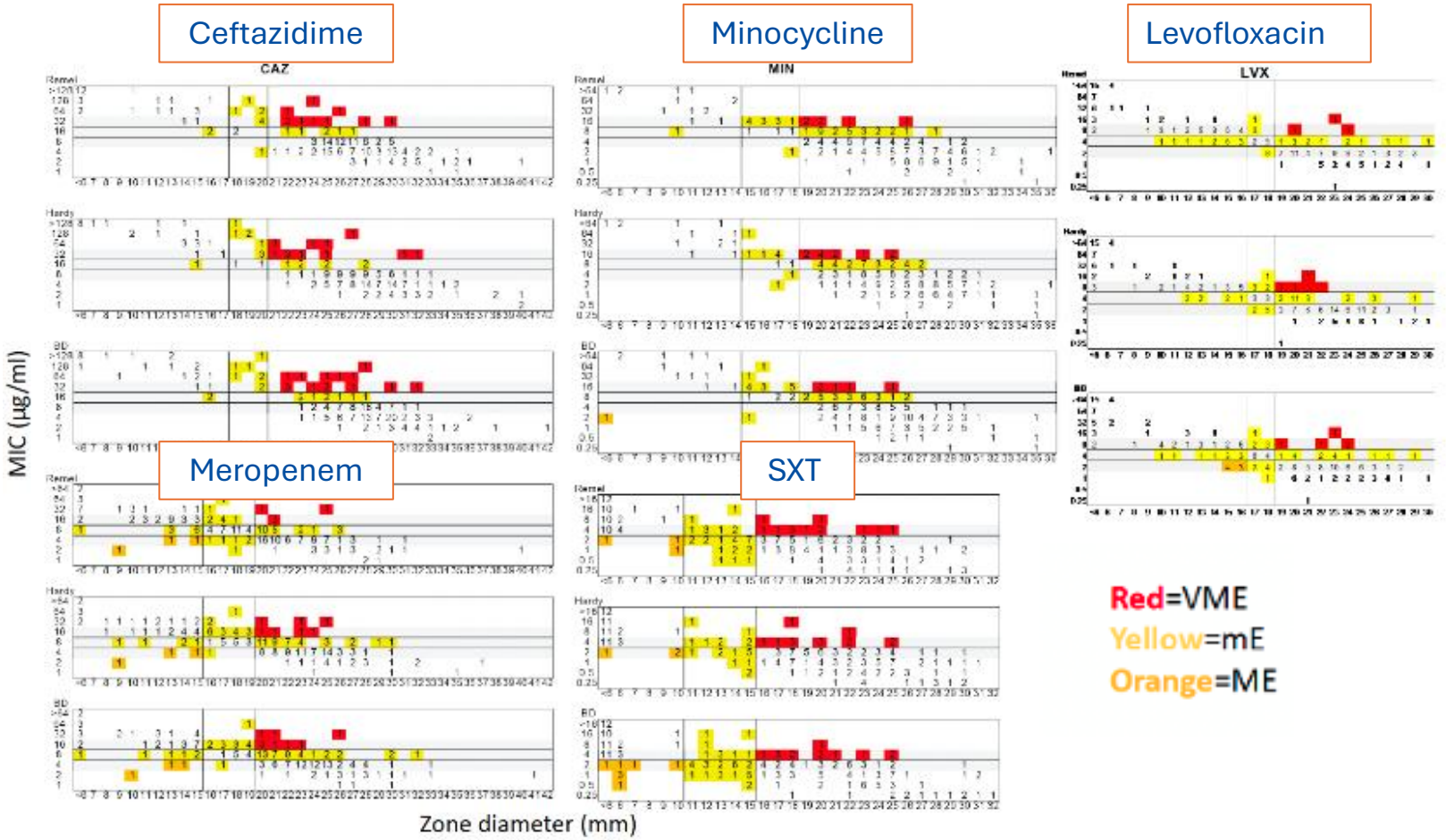
Treatment of *Burkholderia cepacia* complex (BCC)



- UpToDate (treatment of CF patients):
- "We attempt to treat *B. cepacia* complex species when present, guided by AST. …The problem of choosing an antibiotic for *B. cepacia* complex species is compounded by poor performance of AST."
- EUCAST BCC Rationale document[1]
- "There is no relationship between MIC and clinical outcome" Potentially due to mismatch between in vivo and in vitro expression of resistance.
- AMR in CF International Working Group[2]
- "There is little evidence that AST predicts the clinical outcome of CF treatment, suggesting careful consideration of current AST use by CF community."
- ASM Practice guidelines on CF microbiology[3]
- "All method demonstrate poor reproducibility, which may explain poor correlation of AST and clinical outcomes in CF."

https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/BCC_susceptibility_testing_130719.pdf
Somayaji et al. 2019. J Cyst Fibros. 18:236-43
Saiman et al. 2025. Clin Micro Rev. In press

B. cepacia
Study of Disk Diffusion vs BMD MICs



- Testing done by 2 labs
- Multiple brands of MHA, CAMHB
- Both CF and non-CF isolates
- Tested:
  - Ceftazidime
  - Levofloxacin
  - Meropenem
  - Minocycline
  - Trimethoprim-sulfamethoxazole (SXT)
- errors
- sts failed to meet CLSI standards per CLSI M23

Burkhoderia cepacia complex: CLSI work

- Ongoing studies show:
  - Disk diffusion breakpoints do not agree with reference BMD (removed in 2024)[1]
  - Agar dilution and broth microdilution MICs do not agree[2]
  - Commercial test performance is unknown or poor[2,3]

Antimicrobial	Agar Dilution	Etest
Ceftazidime	73%	71%
Levofloxacin	96%	90%
Meropenem	86%	83%
Minocycline	88%	84%
SXT	68%	55%

- Data from literature, other societies show *no correlation* between in vitro MIC and clinical outcomes[4, 5]

1. CLSI M100 34th Edition
2. Jorth et al. 2025 JCM doi.org/10.1128/jcm.01480-24
3. Wootton et al. 2020. Clin Microbiol Infect S1198-743X(20)30708-4
4. Somayaji et al. 2019. J Cyst Fibros. 18:236-43
5. Saiman et al. 2024. Clin Microbiol Review 37: e0021521

Appendix B: Intrinsic resistance: BCC

Intrinsic Resistance	Usually "R"*	No intrinsic resistance
• Ampicillin, Piperacillin, Ticarcillin • Ampicillin-sulbactam • Amoxicillin-clavulanate • Ertapenem • Polymyxin B, Colistin • Fosfomycin	• Piperacillin-tazobactam • Cefotaxime • Ceftriaxone • Cefepime • Aztreonam • Imipenem • Aminoglycosides • Trimethoprim	• Ceftazidime • Levofloxacin • Meropenem • Tetracyclines • Tigecycline • Trimethoprim-sulfamethoxazole • Chloramphenicol

*these antimicrobials do not meet definition of intrinsic resistance as wild-type, environmental isolates do not harbor resistance mechanisms. However, most clinical isolates are "R"

Appendix F: Epidemiological cutoff values for BCC

Note! These are going away in 2026

Antimicrobial agent	Interpretive category and MIC, µg/mL		MIC typically achievable, µg/mL*
	WT	NWT	
Ceftazidime	≤16	≥32	≤8
Levofloxacin	≤8	≥16	≤2
Meropenem	≤16	≥32	≤4
Minocycline	≤8	≥16	≤4
SXT	≤2	≥4	≤2

PK-PD estimates suggest WT MIC is not treatable

"Caution: ECVs should not be used as clinical breakpoints"

Caveats to BCC ECVs:

1. Not species specific
2. >50% of data for minocycline and SXT was from 1 laboratory

Note! These are under review and will not be published for M100 S36 (2026)

CLSI M100 35th Edition. Appendix F.

Back to Case…

› 52 YO with ALL and *B. cepacia* bacteremia

Ongoing work at CLSI to address:
- non-CF patient isolates
- Updated ECV
- Evaluation of lyophilized BMD

Option 1:

Source: Blood
ID: *Burkholderia cepacia* complex

"AST not routinely performed due to lack of accurate test methods."

Option 2:

Source: Blood
ID: *Burkholderia cepacia* complex

	MIC (µg/mL)	
Ceftazidime	4	-
Levofloxacin	0.5	-
Trimethoprim-sulfamethoxazole	0.5	-

"MIC testing performed by XX reference laboratory using BMD. Correlation of MIC values with clinical outcomes is not known. Consultation with ID is recommended."

Option 3:

Source: Blood
ID: *Burkholderia cepacia* complex

	MIC (µg/mL)	
Ceftazidime	4	WT
Levofloxacin	0.5	WT
Trimethoprim-sulfamethoxazole	0.5	WT

"MIC testing performed by XX reference laboratory using BMD. Correlation of MIC values with clinical outcomes is not known. Isolate displays wild-type (WT) results, indicating no acquired or mutational resistance mechanisms. Consultation with ID is recommended."

Probably not an option in 2026

Part 2: QC

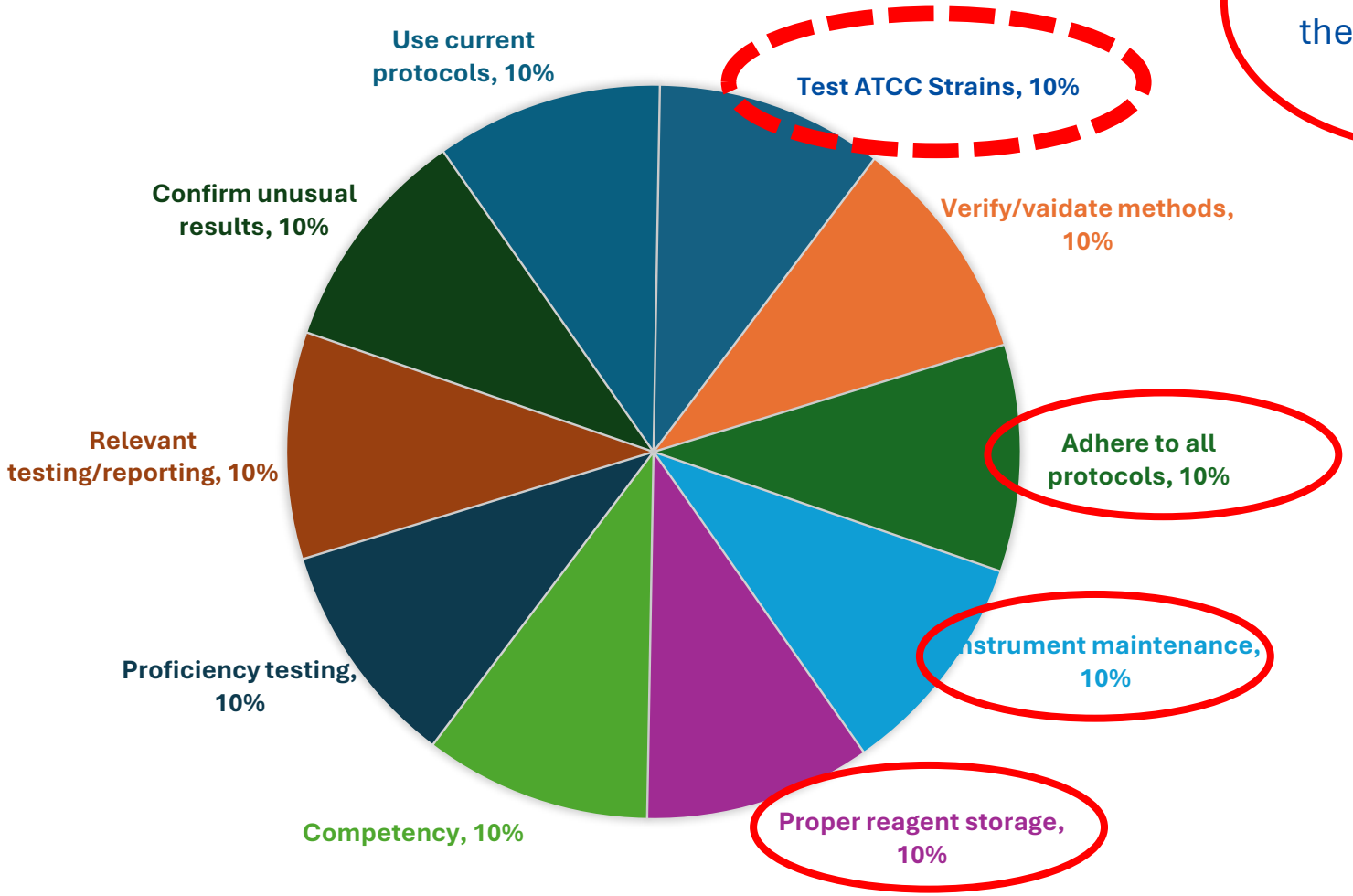
Achieving Quality AST Results

Testing QC strains modestly "controls" these parameters

QC strains are in control…
Will you report these results without question?

S. aureus

Antimicrobial	Result
Ciprofloxacin	S
Clindamycin	S
Daptomycin	S
Erythromycin	S
Oxacillin	S
Trimeth-sulfa	S
Vancomycin	**R**



When testing ATCC QC strains, what types of errors or out of range results might we encounter?

Random or Identifiable Errors

- Can be easily explained
- Most correct on repeat testing with the same or a new QC strain
- Can be a result of chance and not a system failure
- Are very unlikely to affect patient results

Examples:
- No growth of the QC strain
- Mixed culture used for QC
- Incorrect QC strain tested.

Most frequent errors

System Errors

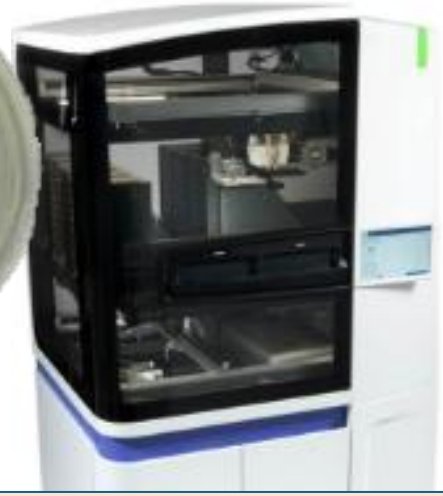
- Due to a malfunction of an instrument
- Due to defective media and/or reagents
- Do not correct with repeat testing with the same or a new QC strain
- Can affect patient results

Examples:
- Manufacturing issue with media and/or reagents (eg, incorrect concentration of drug, incorrect contents of media)
- Issues with optical system
- Blocked reagent line
- Degradation of drug or media in the test system.

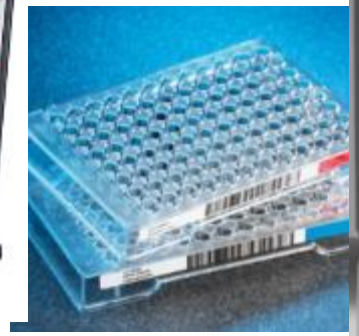
Very Uncommon

CLSI M100 Ed35. Appendix I. p. 369.





Must follow manufacturer's Instructions for Use (IFU) for testing, including QC



Background to CLSI 2025 Change

- AST QC - CMS requires daily testing using "appropriate control organism(s)" to check the procedure
  - CLIA § 493.1261
- 2016 CMS introduced IQCP option
  - Alternative QC option for all laboratory testing including AST QC testing
- CLSI provided a plan to support converting from daily to weekly QC
  - Since 2016 requires IQCP
- IQCP can support less frequent than weekly QC

- ***Why change now?***
  - Nearly 10 years experience with IQCP
  - Routine QC testing rarely identifies an AST **system problem** that would contribute to patient report errors
  - Manufacturers of commercial AST systems perform extensive QC before release of media/reagent/equipment
  - Laboratories have protocols beyond testing QC strains to minimize patient report errors
  - Laboratories are increasingly focusing on how to best use limited resources.

CMS, Centers for Medicare and Medicaid Services;
IQCP, Individualized Quality Control Plan

Tables 2 – Modified QC Recommendations Box

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

Testing Conditions		QC Recommendations
Medium:	Disk diffusion: MHA Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix H, **section H1**)[1] Agar dilution: MHA	**Refer to the following:** • **Tables 4A-1, 4A-2, 5A-1, and 5A-2 that list acceptable QC ranges applicable for each method** • **Appendix I to develop a QC plan**
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [4])	When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.
Incubation:	35°C ± 2°C; ambient air Disk diffusion: 16–18 hours Dilution methods: 16–20 hours	

Refer to Tables 3A, 3B, 3C, 3D, 3E, 3F-1, and 3F-2 for additional testing, reporting, and QC for Enterobacterales.

M100 35th ed

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

Testing Conditions		Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)
Medium:	Disk diffusion: MHA Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix H)[1] Agar dilution: MHA	*Escherichia coli* ATCC® 25922 *Pseudomonas aeruginosa* ATCC® 27853 (for carbapenems)
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [4])	Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.
Incubation:	35°C ± 2°C; ambient air Disk diffusion: 16–18 hours Dilution methods: 16–20 hours	When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

M100 34th ed

CLSI M100 Ed35. All Tables 2. pp. 56-146.

Appendix I. Selection of Quality Control Strains and Quality Control Testing Frequency

New!

Appendix I. Selection of Quality Control Strains and Quality Control Testing Frequency

Abbreviations for Appendix I

AST	antimicrobial susceptibility testing
ATCC®	American Type Culture Collection
CMS	Centers for Medicare & Medicaid Services
IQCP	individualized quality control plan
MIC	minimal inhibitory concentration
NaCl	sodium chloride
pH	negative logarithm of hydrogen ion concentration
QA	quality assurance
QC	quality control

I1 Regulatory Requirements for Selection of Quality Control Strains and Quality Control Testing Frequency

The Centers for Medicare & Medicaid Services (CMS) requires laboratories in the United States to perform appropriate QC testing for antimicrobial susceptibility testing (AST) with each lot/batch or shipment of media and antimicrobial agent(s) before, or concurrent with initial use.[1] Thereafter, QC must be performed with each day of testing (subsequently referred to as "daily" QC testing). The specific QC strains required for daily QC testing are not specified by CMS. Other regulatory agencies may have alternative QC requirements.

I2 Development of an Individualized Quality Control Plan

A laboratory in the United States must develop an individualized quality control plan (IQCP) if it wishes to deviate from CMS's daily AST QC requirement. If an IQCP is acceptable to the laboratory's director and accreditation requirements, an IQCP can be designed to reduce AST QC frequency and to determine which QC strains to test.

When developing an IQCP, the laboratory should select QC strains to detect both system and identifiable errors. The IQCP should include data from the laboratory to support less frequent (eg, weekly, monthly) than the CMS-required daily QC testing.

The IQCP considers both QA processes (eg, equipment maintenance, laboratory procedures, personnel competency assessment) and QC (QC plans for media and reagents). The examples in CLSI M100 focus on QC plans.

Appendix I

- I1 **Regulatory Requirements** for Selection of Quality Control Strains and Quality Control Testing Frequency
- I2 Development of an **Individualized Quality Control Plan**
- I3 Resources for **Development of an Individualized Quality Control Plan** for Antimicrobial Susceptibility Testing
- I4 Type of **Quality Control Errors**
- I5 **Selection of Quality Control Strains** to Quality Control Antimicrobial Agents and Specific Media Component
- I6 **Quality Control Plans**
- I7 Indicators to **Detect Antimicrobial Susceptibility Testing System Problems**
- I8 **Example** Quality Control Plans: User's Laboratory

CLSI M100 Ed35. Appendix I. p. 368.

Modified "Routine" QC Guidelines in CLSI M100 Ed35 *…What to do??*

Review modified CLSI guidelines and your current QC plan

Continue daily/weekly QC

(based on current lab protocols)

IQCP Templates

Google **ASM AST IQCP**

AMERICAN SOCIETY FOR MICROBIOLOGY | CLINICAL AND LABORATORY STANDARDS INSTITUTE | COLLEGE of AMERICAN PATHOLOGISTS

Prepare / Modify IQCP

- **Frequency of testing**
  - Weekly? Biweekly? Monthly?, Other (e.g., rotate instruments)?
- **Selection of QC Strains**
  - Batch/lot/shipment
  - "Routine"

Importance of a Quality Control Plan

Review

- Previous QC records, patient test results, physician complaints
- Product recalls and literature especially if this is a new product for the laboratory
- Manufacturer's IFU

Tabulate errors / complains

- Data analysis

Assess risk

- How do these errors affect patient AST test results
- Frequency, harm, and risk level

Plan

- Risk factor, possible error, and how could the error be reduced

QC Functions – Manufacturer vs. User

Manufacturer QC

User Shipment QC

User Routine QC

Confirm quality of media and/or reagents……..

for a **newly** manufactured lot/batch

following **receipt** of a new lot/batch

throughout their shelf life

New Lot/New Shipment

Same Lot/New Shipment

Table I1. Example QC Strain Selection for MIC Methods When Testing Nonfastidious Gram-Negative Organisms

Table I3: Example QC Strain Selection for MIC Methods When Testing Nonfastidious Gram-Negative Organisms

Antimicrobial Agents	Manufacturer Lot QC[a]	In User's Laboratory		
		New Lot, New Shipment QC	Same Lot, New Shipment QC	Routine QC
Ampicillin Cefazolin	*E. coli* ATCC®[b] 25922	*E. coli* ATCC® 25922	*E. coli* ATCC® 25922	*E. coli* ATCC® 25922
Cefepime Cefiderocol Ceftriaxone Ciprofloxacin Gentamicin Imipenem[c] Tetracycline Tigecycline Tobramycin	• *E. coli* ATCC® 25922 • *P. aeruginosa* ATCC® 27853[c]	*P. aeruginosa* ATCC® 27853[c]	*P. aeruginosa* ATCC® 27853[c]	*P. aeruginosa* ATCC® 27853[c]
Trimethoprim-sulfamethoxazole	• *E. coli* ATCC® 25922 • *E. faecalis* ATCC® 29212	• *E. coli* ATCC® 25922 • *E. faecalis* ATCC® 29212	*E. coli* ATCC® 25922	*E. coli* ATCC® 25922
Amoxicillin-clavulanate[c,d] Piperacillin-tazobactam[d]	*E.coli* ATCC® 35218[c] or *K. pneumoniae* ATCC® 700603[c]	*E.coli* ATCC® 35218[c] or *K. pneumoniae* ATCC® 700603[c]	*E.coli* ATCC® 35218[c] or *K. pneumoniae* ATCC® 700603[c]	*E.coli* ATCC® 35218[c] or *K. pneumoniae* ATCC® 700603[c]
Ceftazidime-avibactam[d] Ceftolozane-tazobactam[d]	*K. pneumoniae* ATCC® 700603	*K. pneumoniae* ATCC® 700603	*K. pneumoniae* ATCC® 700603	*K. pneumoniae* ATCC® 700603
Imipenem-relebactam[c,d] Meropenem-vaborbactam[d]	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.

New!

CLSI M100 Ed35. Appendix I. p. 374.

Selecting QC Strains

Minimum Requirement

- 1 QC stain for each:
  - antimicrobial
  - resistance mechanism test, if present (i.e. ESBL)

Resources

- Manufacture IFU
- M100 Tables 4 / 5
- M100 Appendix C
- M100 Appendix I

Considerations

- QC similar to the organism tested (ie GNR for a GNR)
- QC strain that is on-scale for the dilutions on the panel

AST Methods

Disk Diffusion

Gradient Diffusion

Commercial Panels

Frequency of QC Testing (if not Daily) Supporting Data

Historical records

- 15-replicate (3x5-day) plan or 20-30 day plan
- Ongoing weekly QC

Consider:

- Any previous AST system failures in your lab/beyond?
- How long drug / method tested in your lab/beyond?
- Other IQCP risk assessment parameters

Define frequency in IQCP

- Weekly, Bi-weekly, Monthly, Rotate instruments

Up to the laboratory director –
strain selection and frequency!
Retain documentation supporting IQCP.

Example 1: Automated Commercial MIC Test System Panel *Current Plan*

Current Plan

QC Strain	Purpose
E. coli ATCC® 25922	Entire panel
P. aeruginosa ATCC® 27853	Entire panel
E. coli ATCC® 35218	Beta-lactamase inhibitor combinations
K. pneumoniae ATCC® 70060	ESBL test
K. pneumoniae ATCC® BAA-1705	Meropenem-vaborbactam

Frequency	Total QC Tests
New lot/Shipment	100 (6 errors)
Weekly	520 (15 errors)
Total	620 (21 errors)

Risk Assessment

QC Strain	# of errors	Type
E. coli ATCC® 25922	11 (9%)	Random (reset by turbidity)
P. aeruginosa ATCC® 27853	8 (6%)	Random (reset by turbidity)
E. coli ATCC® 35218	2 (2%)	Random (isolate issue)
K. pneumoniae ATCC® 70060	0	
K. pneumoniae ATCC® BAA-1705	0	

0 system errors found = low risk to patient

Example 1: Automated Commercial MIC Test System Panel *Revised Plan*

Strain Selection

QC Strain	Purpose
E. coli ATCC® 25922	Entire panel
P. aeruginosa ATCC® 27853	Entire panel
E. coli ATCC® 35218	Beta-lactamase inhibitor combinations
K. pneumoniae ATCC® 70060	ESBL test
~~*K. pneumoniae* ATCC® BAA-1705~~	~~Meropenem-vaborbactam~~

Meropenem-vaborbactam not reported, not on hospital formulary

Frequency Adjustment

Frequency Options	Total QC Tests
New lot/Shipment	10 / year
Weekly	52 / year
New lot/Shipment	10 / year
Bimonthly	24 / year
New lot/Shipment	10 / year
Monthly	12 / year

Risk

Example 2: Commercial Gradient Diffusion Strip

Current Plan

QC Strain	Purpose
S. pneumoniae ATCC® 49619	Penicillin G

4 Years of Historical QC Data

Frequency	Total QC Tests
Day of testing	174 (1 random error)

- Average 4 QC tests a month
- Within last year increased to 6 QC tests a month

Revised Plan

QC Strain	Purpose
S. pneumoniae ATCC® 49619	Penicillin G

QC Tests per IQCP

Frequency	Total QC Tests
New Lot/ Shipment	2 / year
Weekly	4 / month

- No 3x5 plan or 20/30 day QC data available
- Used 4 years of historical QC data

0 system errors found =
low risk to patient

Example 3: Commercial MIC Test System Panel with a New Drug / New Panel

Manufacturer IFU Recommended Strains

QC Strain	Purpose
E. coli ATCC® 25922	Entire panel
P. aeruginosa ATCC® 27853	Entire panel
E. coli ATCC® 35218	Beta-lactamase inhibitor combinations
K. pneumoniae ATCC® 70060	ESBL test
***K. pneumoniae* ATCC® BAA-2814**	**Imipenem-relebactam**

Laboratory had an IQCP for the old panel

Frequency
New lot / shipment
Bimonthly

- New panel with new drug **imipenem-relebactam**
- No historical data for this drug and K. *pneumoniae* ATCC® BAA-2814
- **Remaining drugs and dilutions on panel have not changed**
- Laboratory opted to a 3x5 plan for *K. pneumoniae* ATCC® BAA-2814 to incorporate this strain into its existing IQCP
  - Weekly for 1 year
  - Then move to bimonthly

QC Range Adjustments and Other Minor QC Changes

Modified QC Range

Antimicrobial	DD (mm)
***E. coli* ATCC® 25922**	
Minocycline	20-26

CLSI M100 Ed35 Table 4A-1. p. 228.

QC Table Footnote:

"Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations."

CLSI M100 Ed35 Tables 4A-1 p. 230; 5A-1 p. 254.

Aztreonam Plus Ceftazidime-avibactam Broth Disk Elution – Alternative "R" QC Strains

QC Strain	Organism Characteristics	Expected Results
E. coli AR Bank #0348 **Alternative strains:** *E. coli* AR Bank #0434 or *E. coli* AR Bank #0450	Not susceptible to any antimicrobial agents evaluated	ATM: Growth – not susceptible CZA: Growth – not susceptible ATM + CZA: Growth – not susceptible

CLSI M100 Ed35 Table 3D. pp. 184.

Summary

- QC should be informed by *your* risk assessment
- No requirement to change!
- Opportunity to streamline

Part 3: Q&A