2025 Updates to CLSI M100

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The presenter states no conflict of interest and has no financial relationship to disclose relevant to the content of this presentation.

OUTLINE

I. Quick discussion(s) relative to major revisions

II. Objectives of webinar

Describe significant changes relevant to pre-existing antimicrobial susceptibility breakpoints…

Describe significant changes relevant to antimicrobial susceptibility testing methodology…

Identify (new) organism/antimicrobial combinations for which susceptibility breakpoints now exist…

as outlined in the CLSI M100-Ed35 document.

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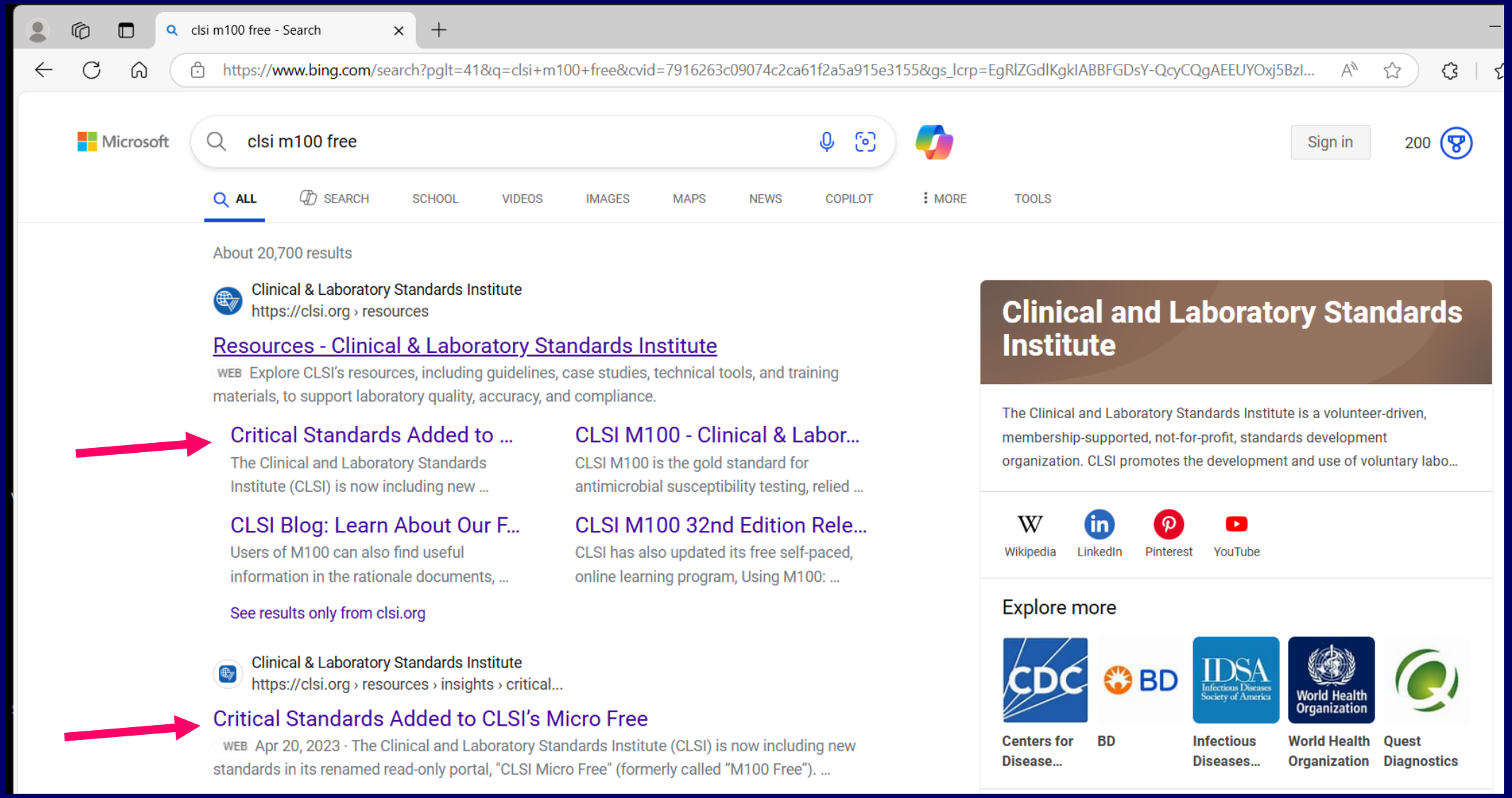

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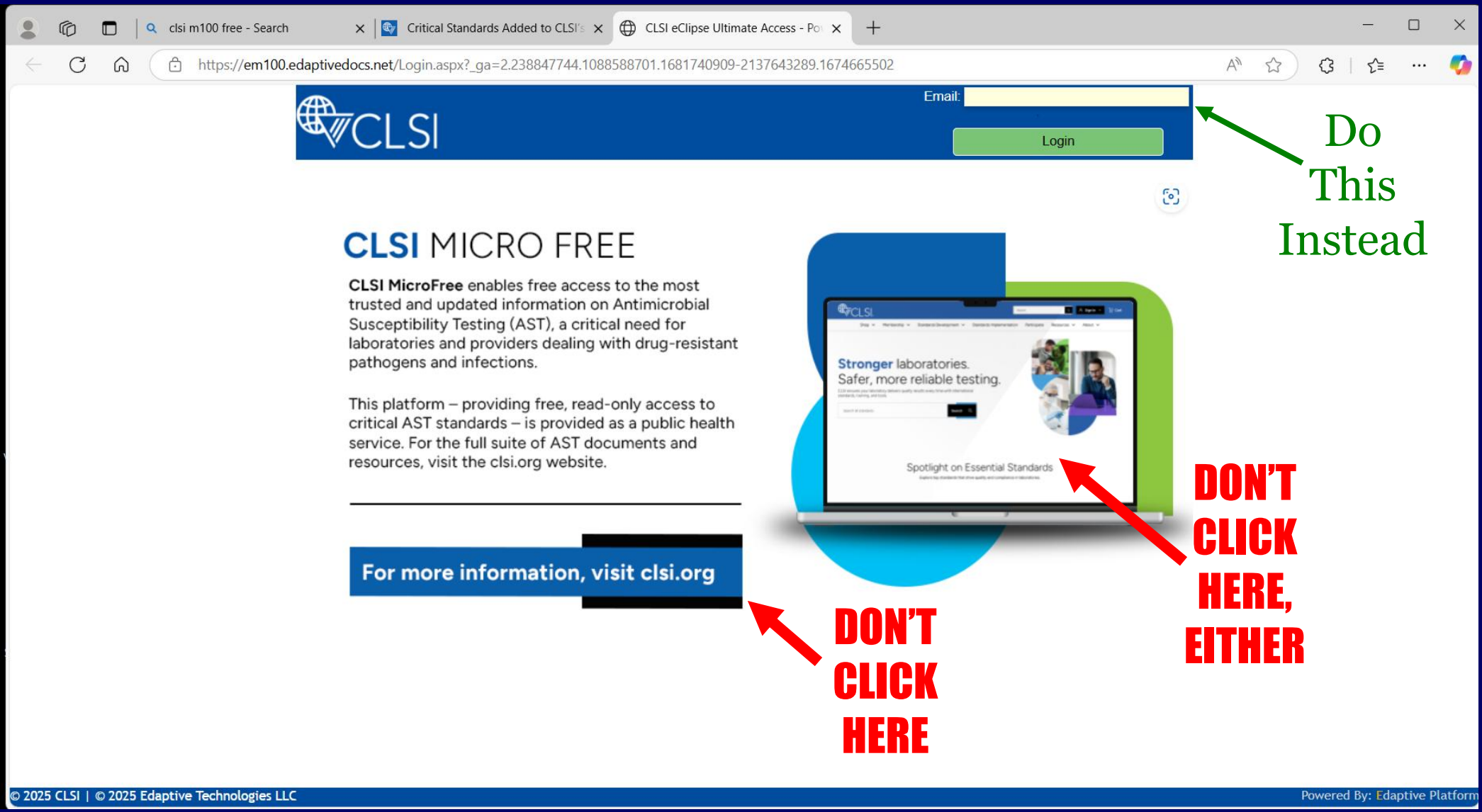

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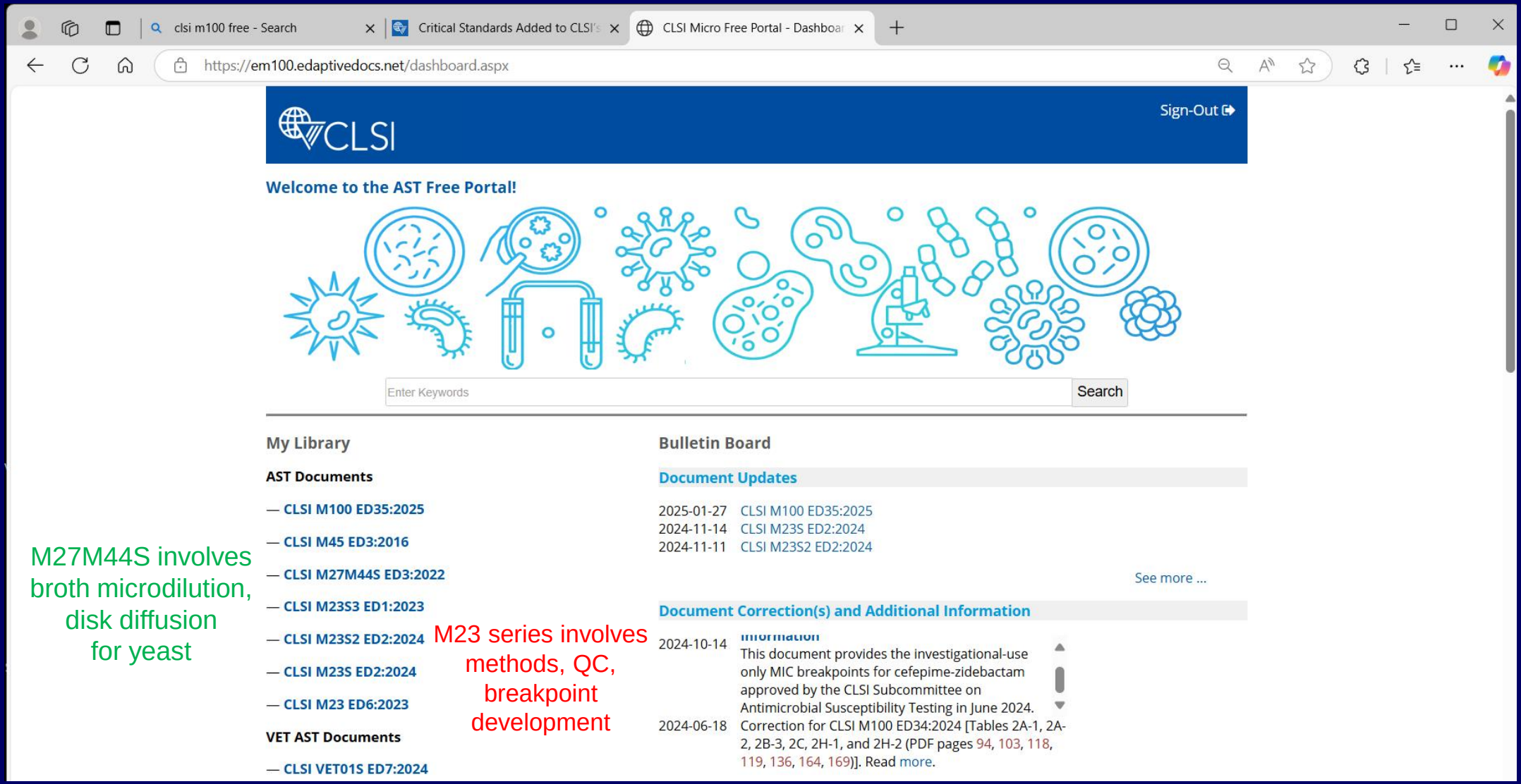
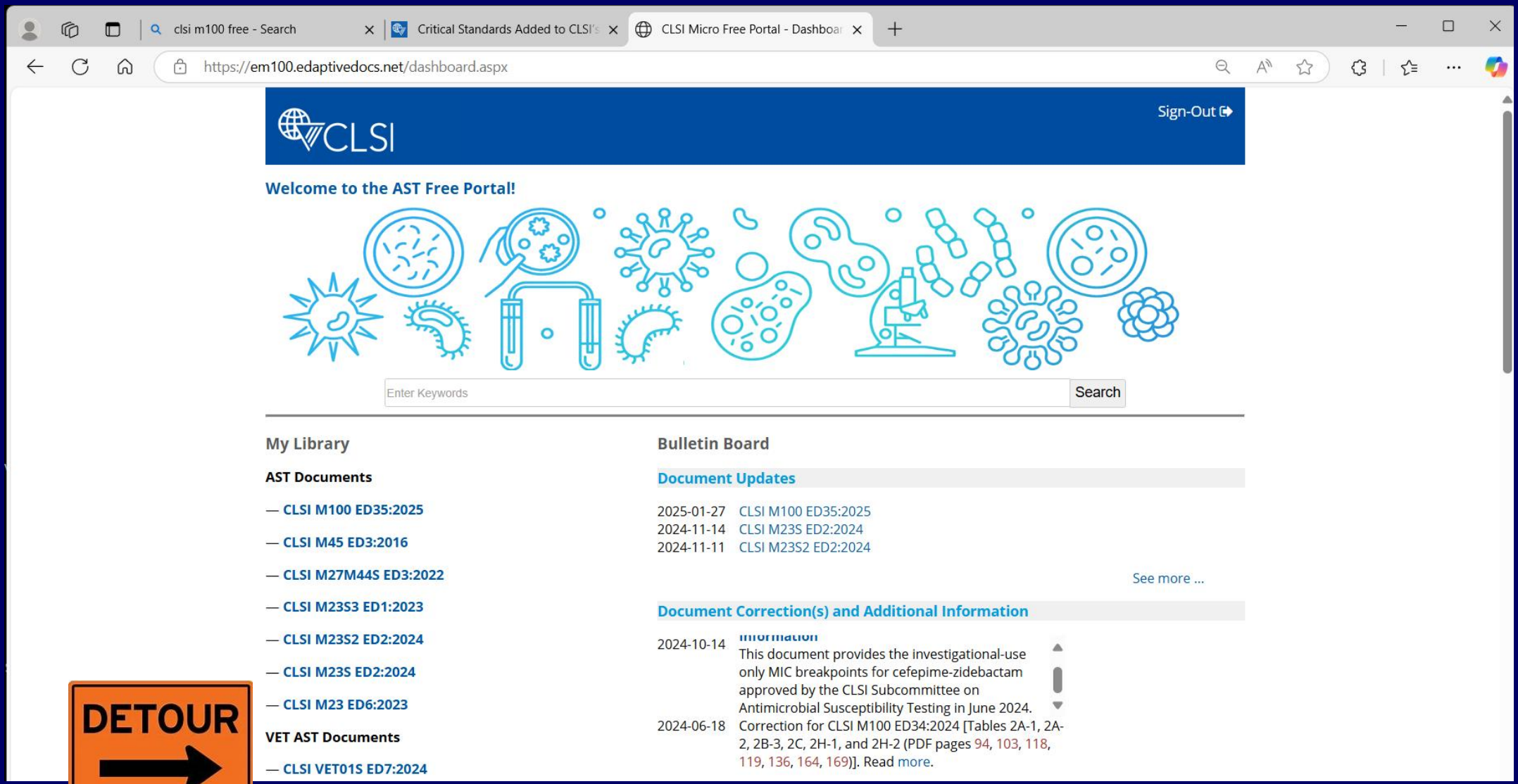


M27M44S involves broth microdilution, disk diffusion for yeast

M23 series involves methods, QC, breakpoint development

QUICK DETOUR I



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- Table 2D. Zone Diameter and MIC Breakpoints for *Enterococcus* spp.
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- Table 2F. Zone Diameter and MIC Breakpoints for *Bordetella bronchiseptica*
- Table 2G. Zone Diameter and MIC Breakpoints for *Mannheimia haemolytica*
- Table 2H. Zone Diameter and MIC Breakpoints for *Pasteurella multocida*
- Table 2J. Zone Diameter and MIC Breakpoints for *Histophilus somni*

Related Information

CLSI VET01S-ED7:2024 Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 7th Edition

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CLSI VET01S includes updated tables for the Clinical and Laboratory Standards Institute veterinary antimicrobial susceptibility testing standard VET01.

A CLSI supplement for global application.

CLSI VET01S-Ed7

January 2024

Replaces CLSI VET01S-Ed6

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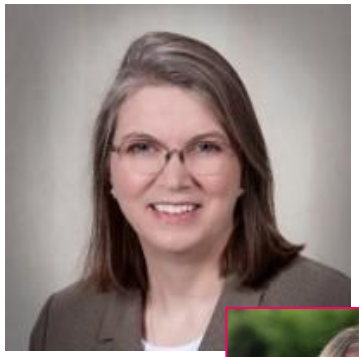

PEOPLE

QUINOLONES AND FLUOROQUINOLONES (Please refer to Glossary I.)									
Ciprofloxacin Levofloxacin	5 µg 5 µg	≥ 26 ≥ 21	– –	22–25^ 17–20^	≤ 21 ≤ 16	≤ 0.25 ≤ 0.5	– –	0.5^ 1^	≥ 1 ≥ 2
Cinoxacin* (U)[a]	100 µg	≥ 19	–	15–18^	≤ 14	≤ 16	–	32^	≥ 64
Enoxacin* (U)[a]	10 µg	≥ 18	–	15–17^	≤ 14	≤ 2	–	4^	≥ 8
Gatifloxacin*	5 µg	≥ 18	–	15–17^	≤ 14	≤ 2	–	4^	≥ 8
Gemifloxacin*	5 µg	≥ 20	–	16–19	≤ 15	≤ 0.25	–	0.5	≥ 1
Grepafloxacin*	5 µg	≥ 18	–	15–17	≤ 14	≤ 1	–	2	≥ 4
Lomefloxacin*	10 µg	≥ 22	–	19–21^	≤ 18	≤ 2	–	4^	≥ 8
Nalidixic acid* (U)[a]	30 µg	≥ 19	–	14–18	≤ 13	≤ 16	–	–	≥ 32
Norfloxacin* (U)[a]	10 µg	≥ 17	–	13–16	≤ 12	≤ 4	–	8	≥ 16
Ofloxacin*	5 µg	≥ 16	–	13–15^	≤ 12	≤ 2	–	4^	≥ 8
Fleroxacin (Inv.)	5 µg	≥ 19	–	16–18^	≤ 15	≤ 2	–	4^	≥ 8

* “Other” agents that are not included in Table 1 but have established clinical breakpoints

(U) urine

[a] report only on isolates from urinary tract

inv., investigational agent

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ANIMALS

Test/ Report Group	Body Site	Antimicrobial Agent	Antimicrobial Agent Class or Subclass	Organism	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			
						S	I	R	S	SDD	I	R
Dogs (Continued)												
A	SST, **ur**	Difloxacin	Fluoro-quinolones[c]	Enterobacterales	10 µg	≥ 21	18–20	≤ 17	≤ 0.5	–	1-2	≥ 4
A	Resp, SST, **ur**	Enrofloxacin	Fluoroquinolones[c]	Enterobacterales	–	–	–	–	**≤ 0.06**	**0.12-0.25**	–	**≥ 0.5**
B	SST	Levofloxacin	Fluoroquinolones[c]	Enterobacterales	5 µg	≥ 21	17–20	≤ 16	≤ 0.5	–	1	≥ 2
A	SST, **ur**	Marbofloxacin	Fluoroquinolones[c]	Enterobacterales	–	–	–	–	**≤ 0.12**	**0.25**	–	**≥ 0.5**
A	SST, **ur**	Orbifloxacin	Fluoroquinolones[c]	Enterobacterales	10 µg	≥ 23	18–22	≤ 17	≤ 1	–	2–4	≥ 8
A	Skin, **ur**	Pradofloxacin	Fluoroquinolones[c]	*E. coli*	5 µg	≥ 24	20–23	≤ 19	≤ 0.25	–	0.5–1	≥ 2

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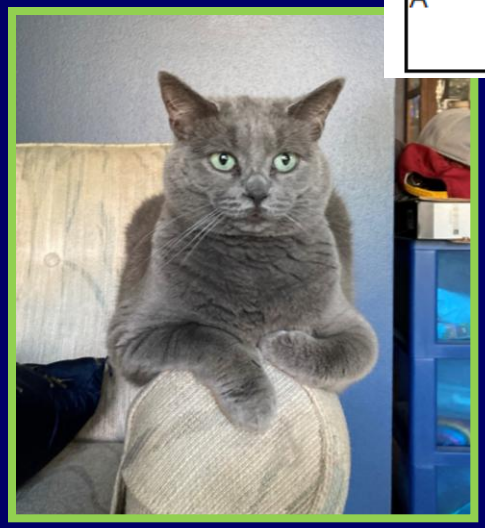
DIE KATZE

Test/ Report Group	Body Site	Antimicrobial Agent	Antimicrobial Agent Class or Subclass	Organism	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			
						S	I	R	S	SDD	I	R
Cats												
A	SST	Enrofloxacin	Fluoroquinolones[C]	Enterobacterales	5 µg	≥ 23	17–22	≤ 16	≤ 0.5	–	1-2	≥ 4
A	SST	Marbofloxacin	Fluoroquinolones[C]	Enterobacterales	5 µg	≥ 20	15-19	≤ 14	≤ 1	–	2	≥ 4
A	SST	Orbifloxacin	Fluoroquinolones[C]	Enterobacterales	10 µg	≥ 23	18-22	≤ 17	≤ 1	–	2-4	≥ 8
A	Resp, skin	Pradofloxacin	Fluoroquinolones[C]	*E. coli*	5 µg	≥ 24	20–23	≤ 19	≤ 0.25	–	0.5–1	≥ 2



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OTHER FRIENDS

Test/ Report Group	Body Site	Antimicrobial Agent	Antimicrobial Agent Class or Subclass	Organism	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL				Comments
						S	I	R	S	SDD	I	R	
Horses													
A	Resp, SST	Enrofloxacin	Fluoroquinolones[c]	**Enterobacterales**	–	–	–	–	≤ 0.12	–	0.25	≥ 0.5	

Test/ Report Group	Body Site	Antimicrobial Agent	Antimicrobial Agent Class or Subclass	Organism	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL				Comments
						S	I	R	S	SDD	I	R	
Poultry													
B		Enrofloxacin	Fluoroquinolones[c]	*E. coli*	5 µg	≥ 23	17–22	≤ 16	≤ 0.25	–	0.5–1	≥ 2	**(16)** The US approval for poultry was withdrawn in September 2005.

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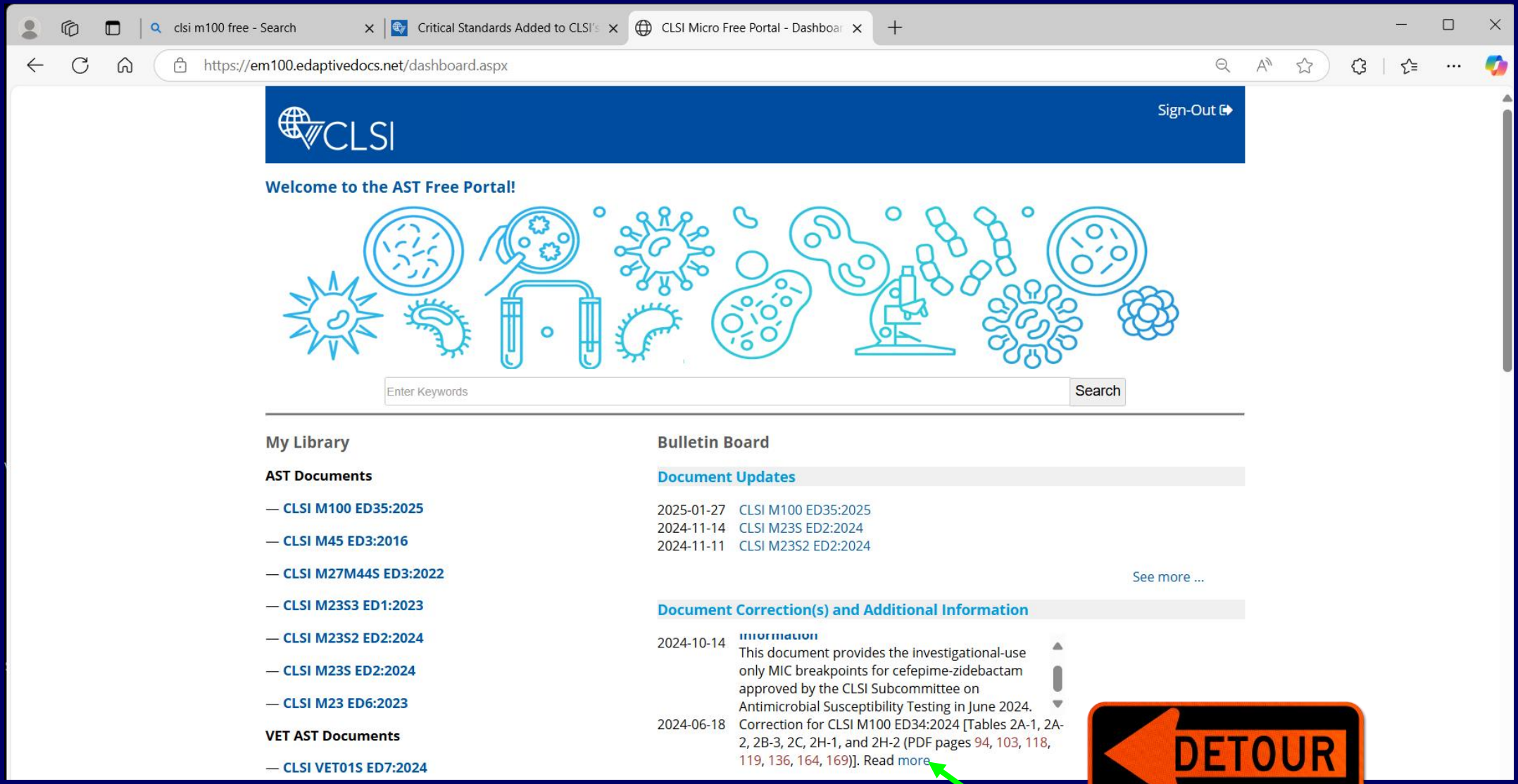
QUICK DETOUR II



WE'LL DO BETTER NEXT TIME

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Setting the standard in laboratory medicine for a healthier world.

6 June 2024

To: Recipients of CLSI M100-Ed34

From: Jennifer K. Adams, MLS(ASCP), MSHA
Vice President, Standards and Quality

Subject: Corrections

This notice is intended to inform users of corrections made to CLSI M100, *Performance Standards for Antimicrobial Susceptibility Testing*, 34th ed. The corrections are described below and shown as highlighted text in the table excerpts.

Table 2A-1. Zone Diameter and MIC Breakpoints for Enterobacterales (excluding *Salmonella/Shigella*) (Correction applies to print and PDF versions of the document):

The breakpoints for ceftriaxone are incorrectly aligned. The word "ceftriaxone" has been moved down one line so that it aligns with the appropriate breakpoints.

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

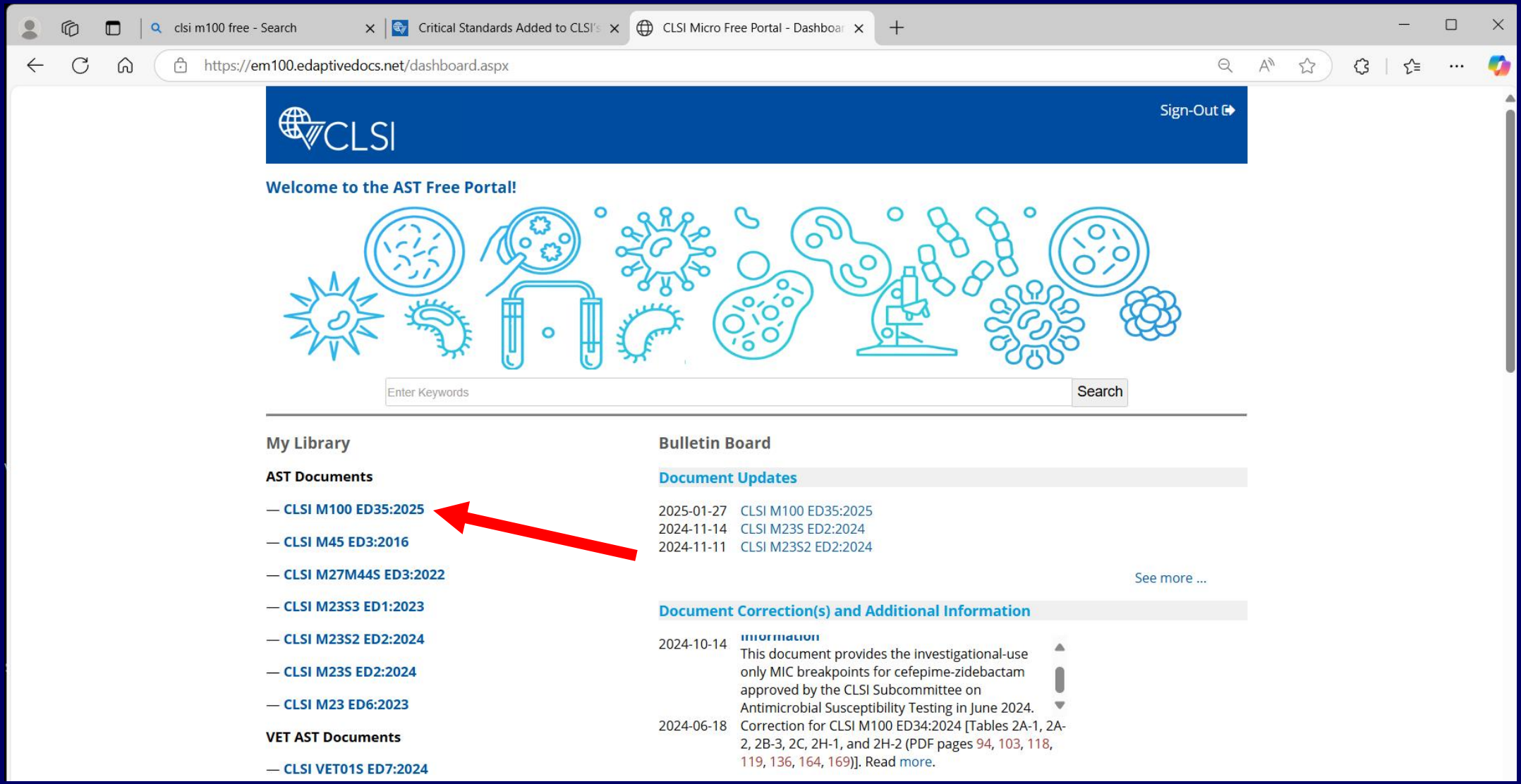
Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)										
Cefotaxime or ceftriaxone	30 µg 30 µg	≥ 26 ≥ 23	-	23-25^ 20-22^	≤ 22 ≤ 19	≤ 1 ≤ 1	-	2^ 2^	≥ 4 ≥ 4	See comment (14).

LARGELY COSMETIC

Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.

Antimicrobial Agent	*Staphylococcus* spp. Indications	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	
OXAZOLIDINONES											
(27) *S. aureus* that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that test resistant to linezolid may be susceptible to tedizolid.											
Linezolid	All staphylococci	30 µg	≥ 26	-	23-25	≤ 22	≤ 4	-	-	≥ 8	
Tedizolid	*S. aureus*, including MRSA	2 µg	≥ 19	-	16-18	≤ 15	≤ 0.5	-	1	≥ 2	

What We're Supposed To Be Doing



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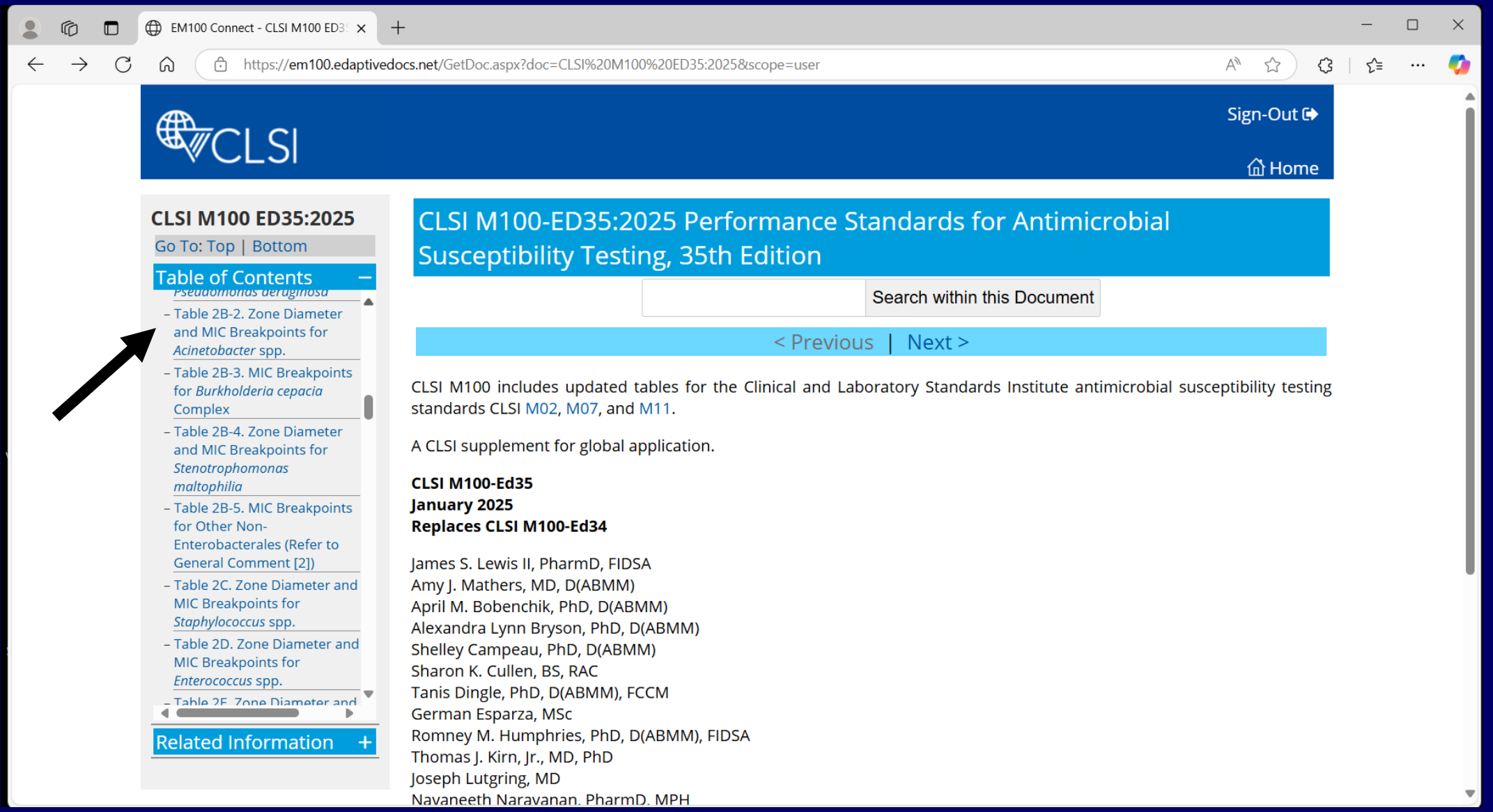
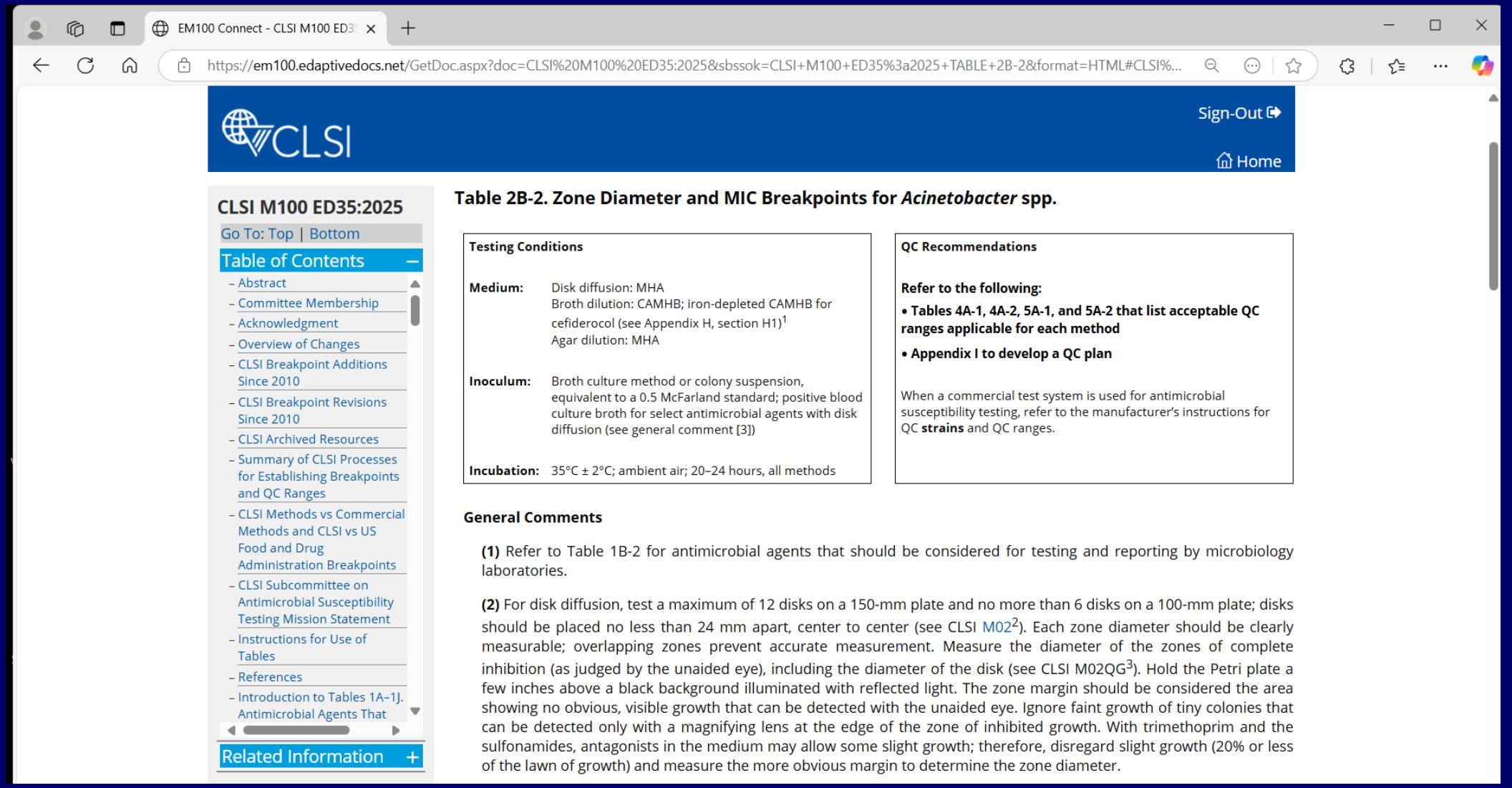


Acinetobacter spp.



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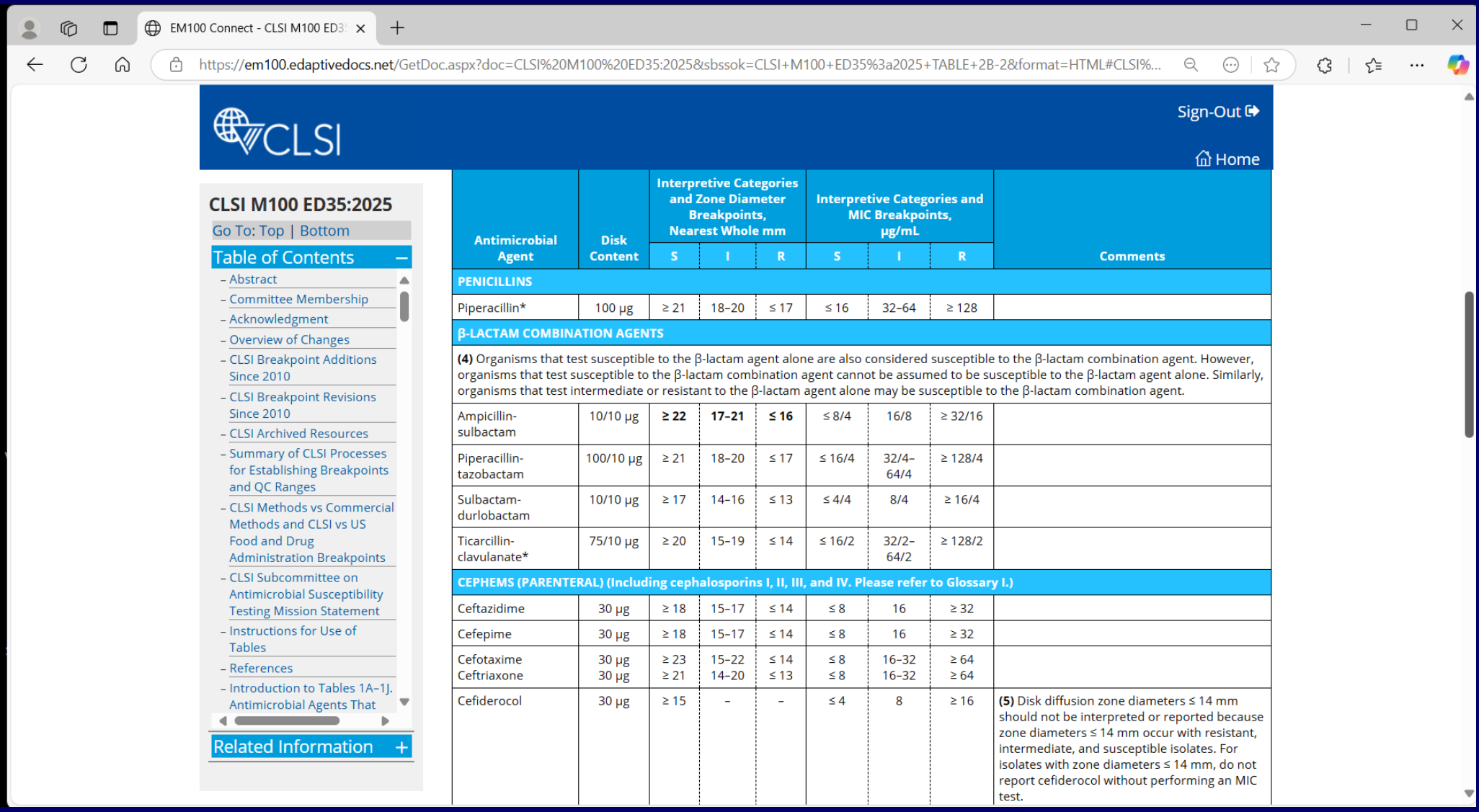
Acinetobacter spp.



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- CLSI Subcommittee on Antimicrobial Susceptibility Testing Mission Statement
- Instructions for Use of Tables
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- Introduction to Tables 1A–1J. Antimicrobial Agents That

Related Information

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Piperacillin*	100 µg	≥ 21	18–20	≤ 17	≤ 16	32–64	≥ 128	
β-LACTAM COMBINATION AGENTS								
(4) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Ampicillin-sulbactam	10/10 µg	**≥ 22**	**17–21**	**≤ 16**	≤ 8/4	16/8	≥ 32/16	
Piperacillin-tazobactam	100/10 µg	≥ 21	18–20	≤ 17	≤ 16/4	32/4–64/4	≥ 128/4	
Sulbactam-durlobactam	10/10 µg	≥ 17	14–16	≤ 13	≤ 4/4	8/4	≥ 16/4	
Ticarcillin-clavulanate*	75/10 µg	≥ 20	15–19	≤ 14	≤ 16/2	32/2–64/2	≥ 128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftazidime	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	
Cefepime	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	
Cefotaxime Ceftriaxone	30 µg 30 µg	≥ 23 ≥ 21	15–22 14–20	≤ 14 ≤ 13	≤ 8 ≤ 8	16–32 16–32	≥ 64 ≥ 64	
Cefiderocol	30 µg	≥ 15	–	–	≤ 4	8	≥ 16	**(5)** Disk diffusion zone diameters ≤ 14 mm should not be interpreted or reported because zone diameters ≤ 14 mm occur with resistant, intermediate, and susceptible isolates. For isolates with zone diameters ≤ 14 mm, do not report cefiderocol without performing an MIC test.

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Related Information −

Keywords:

antimicrobial, susceptibility testing

Document Status:

Active

Versions:

- CLSI M100 ED34:2024

References:

- CLSI M02
- CLSI M02QG
- CLSI M07
- CLSI M11
- CLSI M23
- CLSI M39
- CLSI M45
- CLSI M52
- IDSA 2024
- ISO 20776-1

CLSI M100-ED35:2025 Performance Standards for Antimicrobial Susceptibility Testing, 35th Edition

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CLSI M100 includes updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards CLSI M02, M07, and M11.

A CLSI supplement for global application.

CLSI M100-Ed35
January 2025
Replaces CLSI M100-Ed34

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M02	Disk diffusion
M02QG	Reading guide (DD)
M07	Broth microdilution
M11	Anaerobes
M23	Quality control
M39	Antibiogram
M45	Infrequent bacteria
M52	Commercial verification

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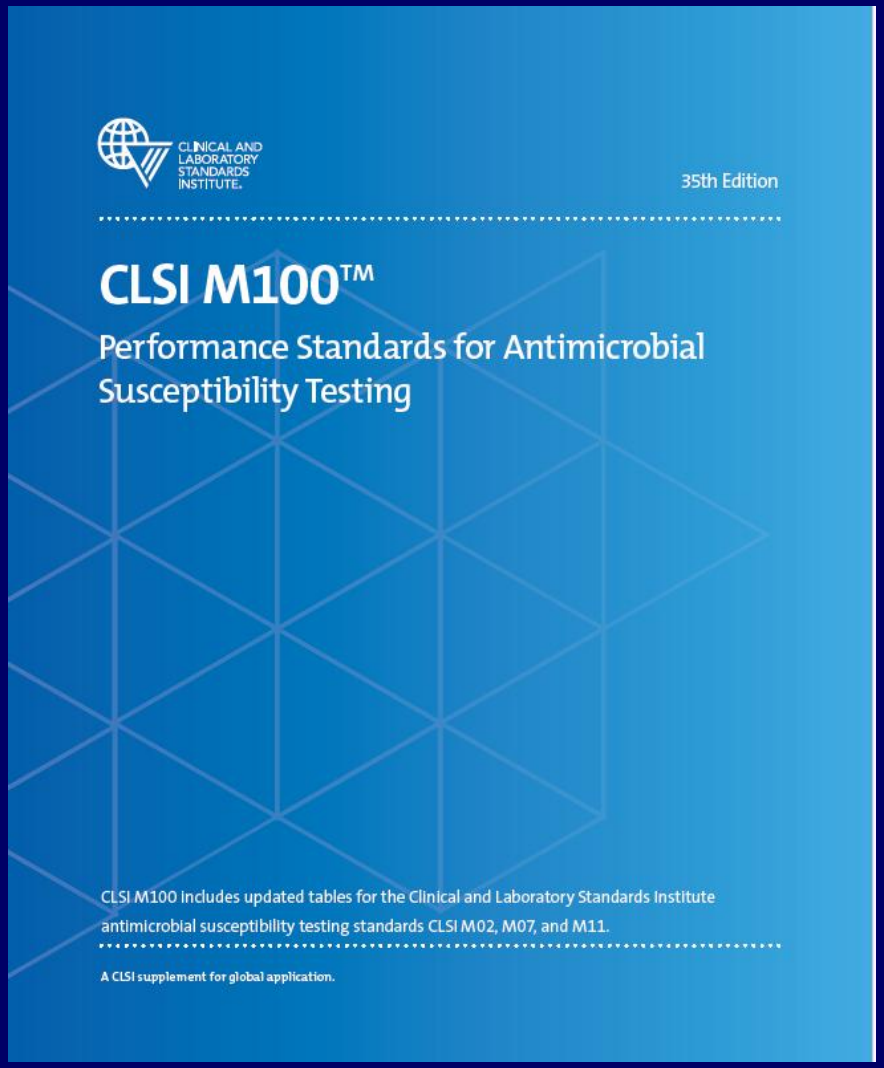

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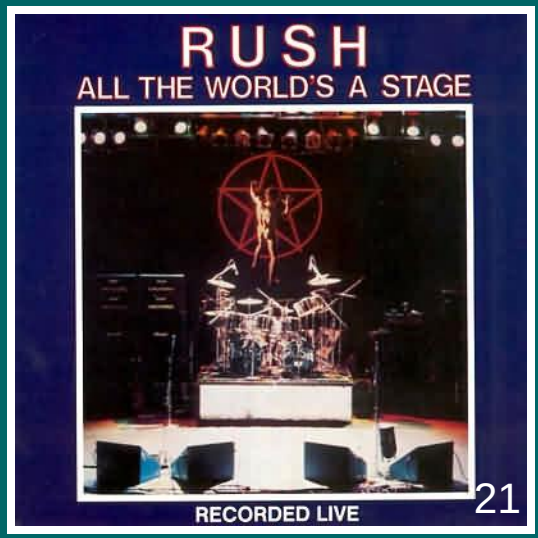

BACK IN THE OLD DAYS…

- Group A Primary test and report
- Group B Optional primary test, report selectively
- Group C Supplemental report selectively
- Group U Supplemental for urine only

CLSI M100 29th ed., 2019

TABLES 1

Table 1A
Suggested Nonfastidious Groupings
M02 and M07

Table 1A. Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Nonfastidious Organisms by Microbiology Laboratories in the United States

	Enterobacteriaceae	*Pseudomonas aeruginosa*	*Staphylococcus* spp.	*Enterococcus* spp.[m]

Table 1B
Suggested Fastidious Groupings
M02 and M07

Table 1B. Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Fastidious Organisms by Microbiology Laboratories in the United States

	Haemophilus influenzae[d] and *Haemophilus parainfluenzae*	*Neisseria gonorrhoeae*[l]	*Streptococcus pneumoniae*[j]	*Streptococcus* spp. β-Hemolytic Group[p]	*Streptococcus* spp. Viridans Group[p]

Table 1C
Suggested Anaerobe Groupings
M11

Table 1C. Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Anaerobic Organisms by Microbiology Laboratories in the United States

	Gram-Negative Anaerobes	Gram-Positive Anaerobes[a]

CLSI M100 29th ed., 2019

TABLE 1 GROUPINGS

- 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

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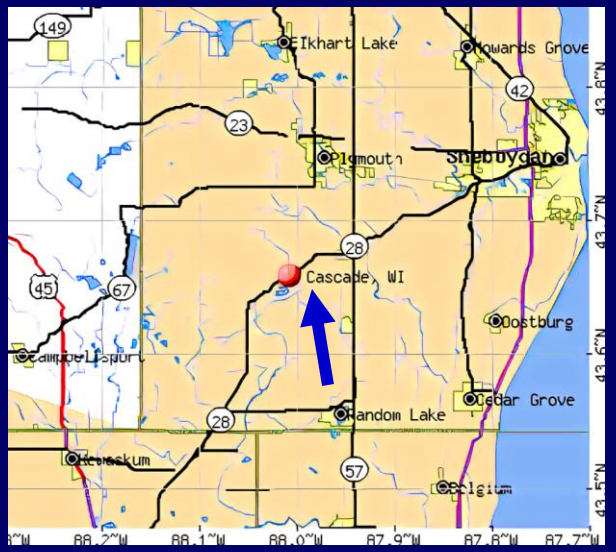
TABLE 1 GROUPINGS

- 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

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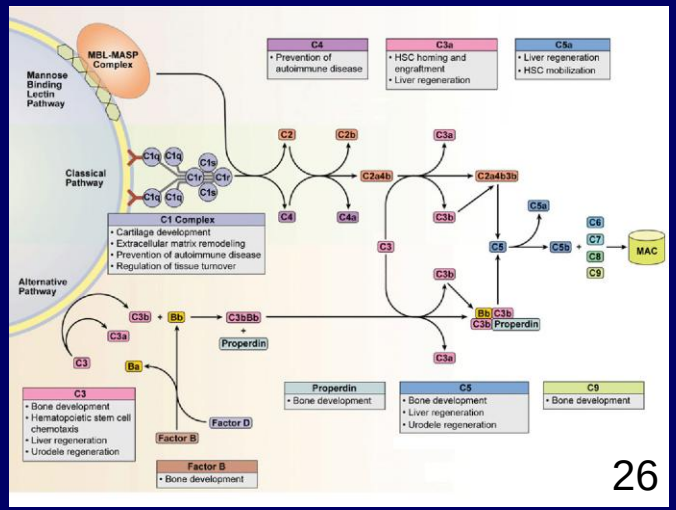
REPORTING

- **Selective** Based on defined criteria unrelated to susceptibility testing data

  Organism ID — Clinical setting
  Site of infection — Patient demographics

- **Cascade** Based on overall antimicrobial susceptibility profile of isolate



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THEY PERFECTLY MATCH UP

Table 1B-2
Acinetobacter spp.
CLSI M02 and CLSI M07

Table 1B-2. *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

Table 2B-2
Acinetobacter spp.
CLSI M02 and CLSI M07

Table 2B-2. Zone Diameter and MIC Breakpoints for *Acinetobacter* spp.

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix H)[1]
Agar dilution: MHA

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard**; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [3])**

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

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

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

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

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

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1,2 A-1	Enterobacterales (excluding *Salmonella*/*Shigella*)
1,2 A-2	*Salmonella* and *Shigella* spp.
1,2 B-1	*Pseudomonas aeruginosa*
1,2 B-2	*Acinetobacter* spp.
1,2 B-3	*Burkholderia cepacia* complex (GUTTED; stay tuned)
1,2 B-4	*Stenotrophomonas maltophilia* (hanging on by a thread)
1,2 B-5	Other Non-Enterobacterales
1,2 C	*Staphylococcus* spp.
1,2 D	*Enterococcus* spp.
1,2 E	*Haemophilus influenzae* and *Haemophilus parainfluenzae*
1,2 F	*Neisseria gonorrhoeae*
1,2 G	*Streptococcus pneumoniae*
1,2 H-1	*Streptococcus* spp. β-Hemolytic Group
1,2 H-2	*Streptococcus* spp. Viridans Group
1,2 I	*Neisseria meningitidis*
1,2 J	Anaerobes (combined Gram-positive and Gram-negative)

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General Comments

THROUGHOUT DOCUMENT

- Disk diffusion no longer categorized as reference method; now a standard method
- QC testing frequency revised from “daily or weekly” to “daily per IQCP” (stay tuned)
- SOSA = staphylococci other than *Staphylococcus aureus*
- Deleted several mentions of sulfisoxazole

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THROUGHOUT DOCUMENT

- Isolates that test susceptible to tetracycline are considered susceptible to doxy/minocycline. Isolates that test intermediate or resistant to tetracycline should be tested against doxycycline or minocycline if those results are needed for Rx.

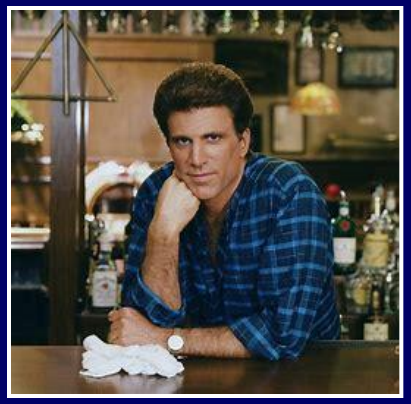
Enterobacterales (including *Salmonella* spp. and *Shigella* spp.)
Haemophilus influenzae and *Haemophilus parainfluenzae*
Streptococcus spp. β-hemolytic group
Streptococcus spp. Viridans group
Other non-*Enterobacterales*
Enterococcus spp.
Staphylococcus spp.
Neisseria gonorrhoeae

Just doxycycline
Streptococcus pneumoniae

CLSI M100-Ed35, 2025

THROUGHOUT DOCUMENT

- Isolates that test susceptible to linezolid are considered susceptible to tedizolid. Isolates that test zzz to linezolid should be tested against tedizolid if that result is needed for therapy.



Resistant
Staphylococcus aureus

Resistant or intermediate
Enterococcus faecalis

Non-susceptible
Streptococcus pyogenes
Streptococcus agalactiae
Streptococcus anginosus group

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Five Big Ones

HISTORIC TABLE

"Warning": The following antimicrobial agents that are included in this document should not be routinely reported for bacteria isolated from CSF. These antimicrobial agents are not the drugs of choice and may not be effective for treating CSF infections caused by these organisms (ie, the bacteria included in Tables 2A through 2J):

- Agents administered by oral route only
- 1st- and 2nd-generation cephalosporins and cephamycins
- Clindamycin
- Macrolides
- Tetracyclines
- Fluoroquinolones

CLSI M100-Ed28, 2018

UPON FURTHER REVIEW

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

https://clsi.org/media/epube0cq/2024_january_ast_meeting_agenda_summary_minutes.pdf

UPON FURTHER REVIEW

Ciprofloxacin	Levofloxacin	Moxifloxacin

Locations	Organisms	Antimicrobial Agents
"Warning": The following antimicrobial agent–organism combinations may appear active *in vitro* but are not effective clinically and must not be reported as susceptible.		
Table 2A-2	*Salmonella* spp., *Shigella* spp.	First- and second-generation cephalosporins, cephamycins, and aminoglycosides
Table 2D	*Enterococcus* spp.	Aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole
"Warning": Do not report the following antimicrobial agents for bacteria isolated from CSF. These are not the drugs of choice and may not be effective for treating CSF infections caused by the bacteria included in Tables 2A–2J:		
Tables 2A–2J	Bacteria isolated from CSF	Agents administered by oral route only, first- and second-generation cephalosporins and cephamycins, doripenem, ertapenem, imipenem, clindamycin, lefamulin, macrolides, and tetracyclines.

Abbreviation: CSF, cerebrospinal fluid.

guidelines	resources	guidelines

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PENEM RESISTANCE

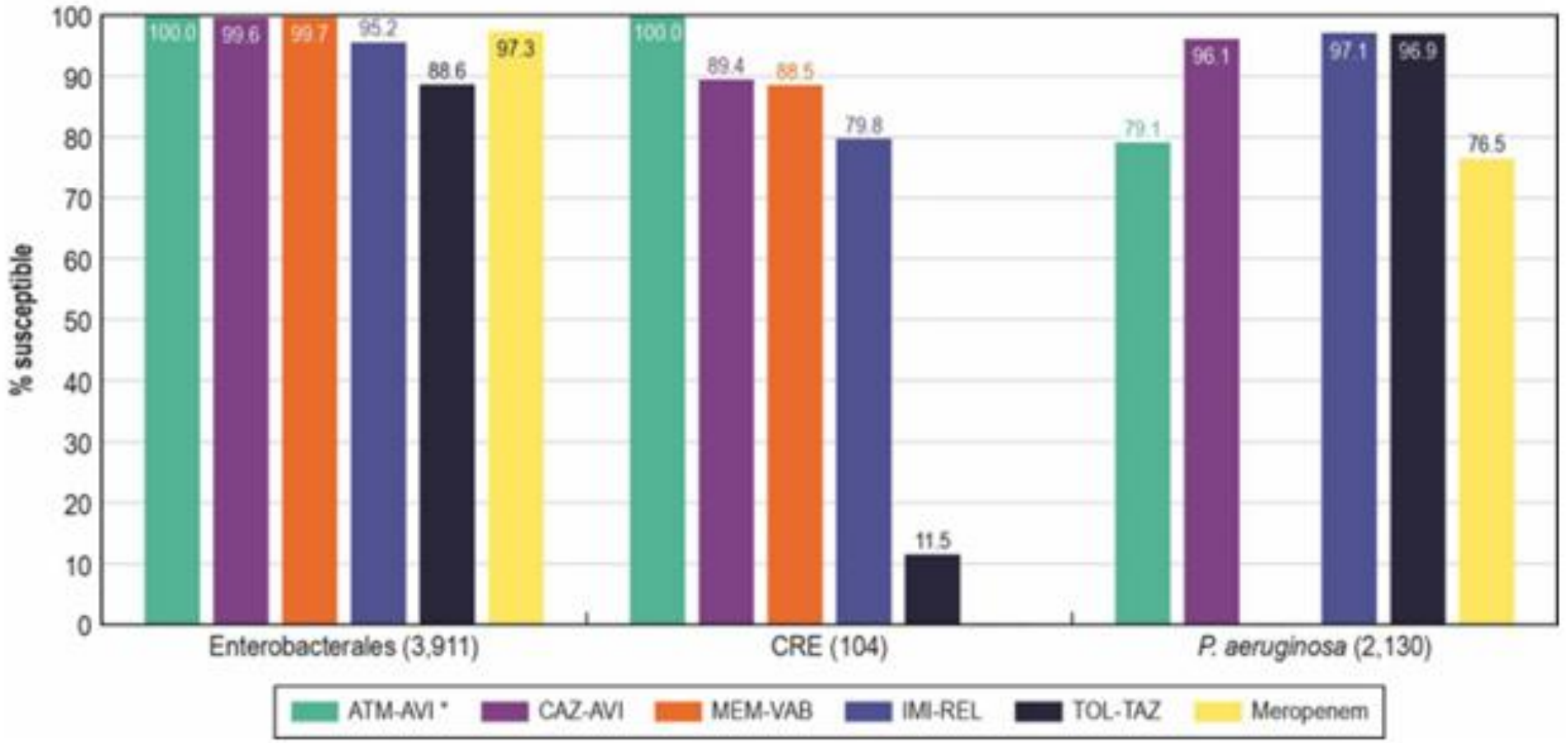
- Antecedent ESBL or ampC + alteration of porin channels in cell wall, reducing permeability (CRE)
- Carbapenemase production (CPE…and CRE)

  Serine carbapenemases (class A β-lactamase)
  Metallo-β-lactamase (class B β-lactamase)
  Oxacillinase (class D β-lactamase)

- CREs and CPEs commonly carry other resistance determinants

Infect Control Hosp Epidemiol. 29:1107-1109; 2008

β-LACTAM/β-LACTAMASE INHIBITOR



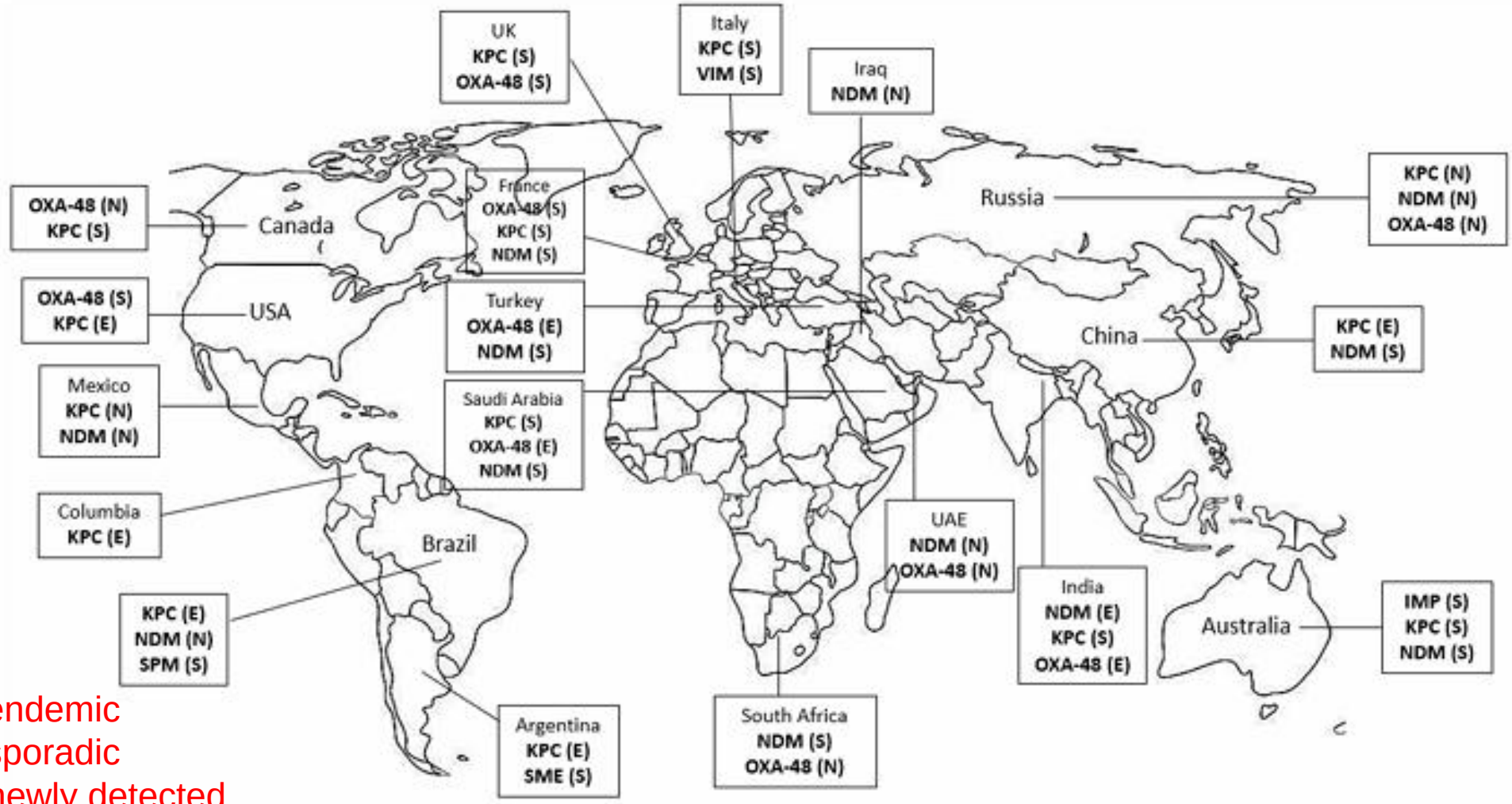
Aztreonam-avibactam
Ceftazidime-avibactam
Meropenem-vaborbactam
Imipenem-relebactam
Ceftolozane-tazobactam

BMC Pulm Med. 25:38; 2025

AMBLER CARBAPENEMASE GROUPS

Group	Examples	Sample targets of hydrolysis	Doesn't touch	Inhibited by
A	KPC IMI SME	penicillins 1°, 2° cephems aztreonam carbapenems	cephamycins	clavulanic acid tazobactam
B	NDM IMP VIM	penicillins 1°, 2° cephems carbapenems	aztreonam	EDTA (chelators)
D	OXA	higher penicillins higher cephems		none of the above

Antibiotics 9:186; 2020



E endemic
S sporadic
N newly detected

Antibiotics 9:186; 2020

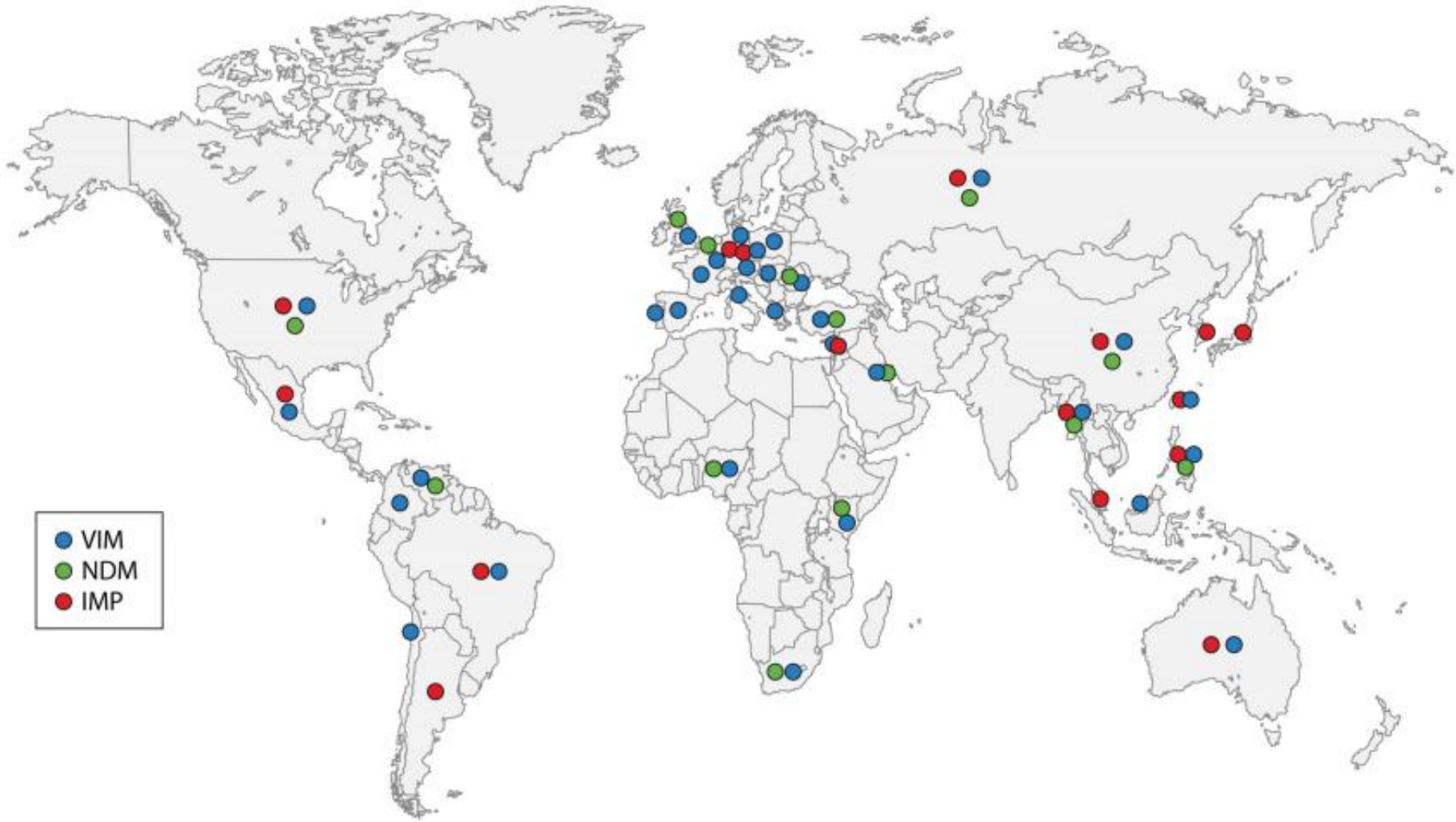
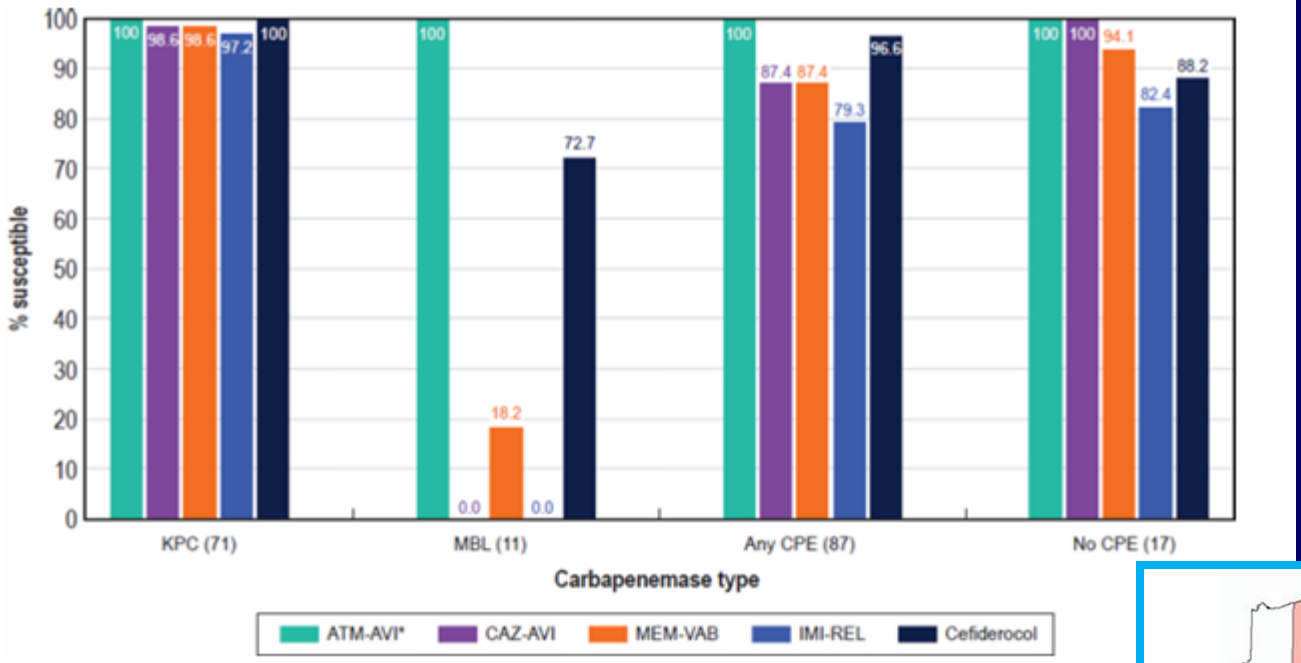
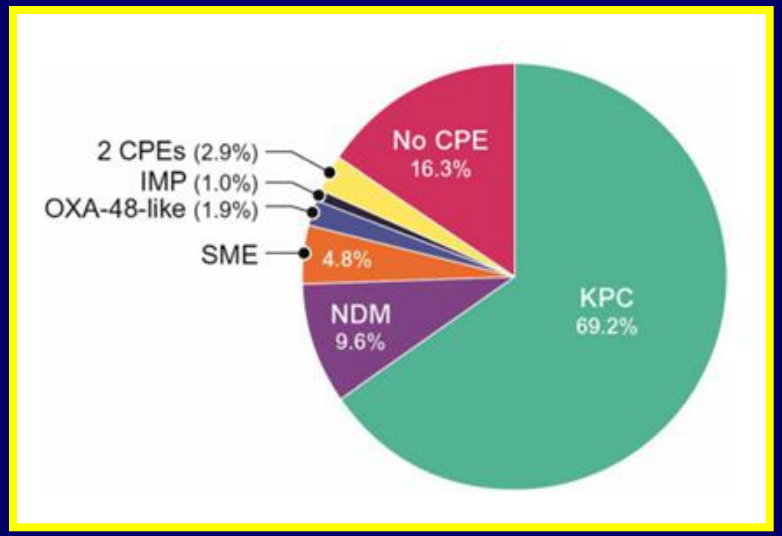


FIG 5 Global distribution of metallo-β-lactamase-positive *Enterobacteriaceae* and *P. aeruginosa*, including NDM-type enzymes collected from 2012 to 2014 from surveillance. (Republished from reference 287).

Clin Microbiol Rev. 33:e00047-19; 2020

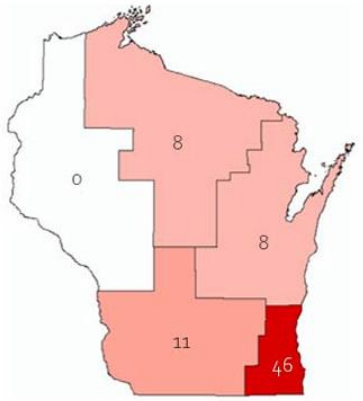
AN OPTION FOR SOME



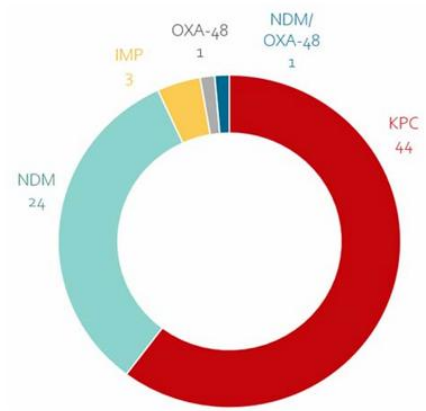


Aztreonam-avibactam
Ceftazidime-avibactam
Meropenem-vaborbactam
Imipenem-relebactam

Laboratory-Based Surveillance Plan 2024-2025



Carbapenamase detections in CRE isolates, by region of the state.



Carbapenamase genes detected in CRE isolates by AR-targeted RT-PCR.

BMC Pulm Med. 25:38; 2025

ENHANCED RECOMMENDATIONS

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										
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.										

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

Institutional treatment guidelines, infection prevention procedures, or epidemiological investigations may necessitate identification of carbapenemase-producing Enterobacterales and *P. aeruginosa*.[1] **Tests that detect the type of carbapenemase are recommended to inform treatment decisions in carbapenem-resistant Enterobacterales isolates.**

Carbapenemase-producing isolates of Enterobacterales usually test intermediate or resistant to one or more carbapenems using the current breakpoints as listed in Table 2A-1 (**NOTE:** Testing not susceptible to ertapenem is often the most sensitive indicator of carbapenemase production. **Depending on local epidemiology and available resources, carbapenemase testing for *Enterobacter cloacae* complex and *Klebsiella aerogenes* isolates that are only resistant to ertapenem might not be necessary. Ertapenem resistance in these species is often due to mechanisms other than carbapenemase production and**

CLSI M100-Ed35, 2025

WHAT CAN BE DONE?

	Tests Used for Carbapenemase Detection			
	Carba NP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Organisms	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales that are positive by mCIM	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by Carba NP or mCIM
Strengths	Rapid	No special reagents or media necessary	No special reagents or media necessary	Determines type of carbapenemase in addition to absence or presence of the enzyme
Limitations	Special reagents are needed, some of which necessitate in-house preparation (and have a short shelf life). Invalid results occur with some isolates. Certain carbapenemase types (eg, OXA-type, chromosomally encoded) are not consistently detected. **Does not determine the type of carbapenemase**	Requires overnight incubation **Does not determine the type of carbapenemase**	Requires overnight incubation **False-negative results are likely to occur for isolates coproducing a serine carbapenemase and a metallo-β-lactamase.** **Does not determine the type of serine carbapenemase or metallo-β-lactamase**	Special reagents and equipment are needed. Specific to targeted genes; false-negative result if specific carbapenemase gene present is not targeted.

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

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INSTANT REPLAY

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
β-LACTAM COMBINATION AGENTS								
Ticarcillin-clavulanate*	–	–	–	–	≤ 16/2	32/2–64/2	≥ 128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftazidime					≤ 8	16	≥ 32	
CARBAPENEMS								
Meropenem					≤ 4	8	≥ 16	
TETRACYCLINES								
Minocycline					≤ 4	8	≥ 16	
FLUOROQUINOLONES								
Levofloxacin	–	–	–	–	≤ 2	4	≥ 8	
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole					≤ 2/38	–	≥ 4/76	
PHENICOLS								
Chloramphenicol*	–	–	–	–	≤ 8	16	≥ 32	**(4)** Not routinely reported on organisms isolated from the urinary tract.

CLSI M100-Ed34, 2024

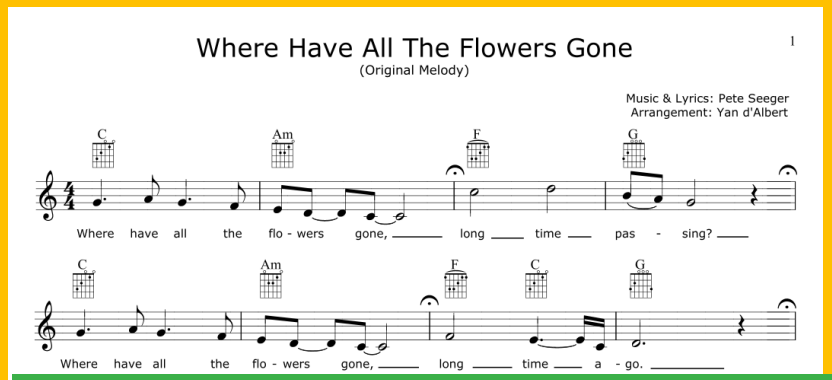


Table 1B-3
Burkholderia cepacia Complex
CLSI M02 and CLSI M07

Table 1B-3. *Burkholderia cepacia* Complex

Refer to Table 2B-3 and Appendix F for information regarding testing of *B. cepacia* complex.

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

CLSI M100-Ed35, 2025

Burkholderia cepacia complex

Table 2B-3
Burkholderia cepacia Complex
CLSI M02 and CLSI M07

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**

NONE

CLSI M100-Ed35, 2025

Burkholderia cepacia complex

(A) Percentage agreement between triplicate testing by BMD_{35}

Antimicrobial agent	% with identical MICs in triplicate testing	% Agreement (with 1 log_2 dilution)	% agreement at 2 log_2 dilutions	% agreement at >2 log_2 dilutions
Minocycline	32.9%	84.5%	12.9%	2.6%
Ciprofloxacin	32.3%	76.8%	16.8%	6.5%
Trimethoprim-sulphamethoxazole[a]	27.1%	72.9%	21.3%	5.8%
Meropenem	31.6%	70.3%	21.9%	7.7%
Ceftazidime	30.3%	73.6%	12.9%	13.5%
Chloramphenicol	25.2%	79.3%	15.5%	5.2%

(C) Percentage essential agreement (EA) between consensus MIC from BMD_{35} and AD_{35}

Minocycline	25.8%	66.5%	21.9%	11.6%
Ciprofloxacin	36.1%	80.7%	12.3%	7.1%
Trimethoprim-sulphamethoxazole[a]	30.9%	80%	14.8%	5.2%
Meropenem	11.6%	32.9%	23.9%	43.2%
Ceftazidime	18.7%	52.3%	18.7%	29%
Chloramphenicol	30.3%	70.3%	20%	9.7%

Gradient diffusion

Antimicrobial agent	CA	mE	ME	VME
Minocycline	79.4%	17.4%	1.3%	1.9%
Trimethoprim-sulphamethoxazole[a]	69.0%	—	0%	30.9%
Meropenem	70.9%	20.0%	6.5%	2.4%
Ceftazidime	81.9%	10.9%	1.9%	5.2%
Chloramphenicol	65.8%	27.7%	4.5%	1.9%

Clin Microbiol Infect. 27:788.e1-788.e4; 2021

EPIDEMIOLOGICAL CUTOFF VALUE

Appendix F
Epidemiological Cutoff Values

Table F1. ECVs for *Burkholderia cepacia* Complex[a]

Antimicrobial Agent	Interpretive Category and MIC, µg/mL		Comment
	WT[b,c]	NWT	
Ceftazidime	≤ 16	≥ 32	
Levofloxacin	≤ 8	≥ 16	
Meropenem	≤ 16	≥ 32	
Minocycline	≤ 8	≥ 16	
Trimethoprim-sulfamethoxazole	≤ 2	≥ 4	

NONE → ECV

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

Table F2. ECVs for Specific Anaerobic Species

Antimicrobial Agent	Interpretive Category and MIC, µg/mL		Comment
	WT[a]	NWT	
Vancomycin	≤ 2	≥ 4	For use with *C.* (formerly *P.*) *acnes*[7-10] and *Clostridioides* (formerly *Clostridium*) *difficile*.[11-13]

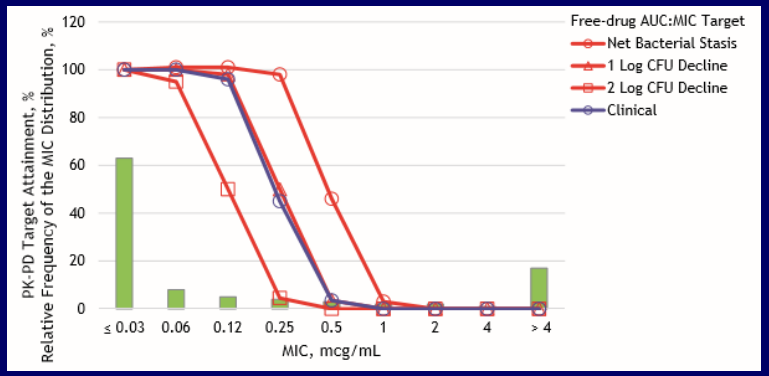
MIC or zone diameter value that separates microbial populations into those with and without phenotypic resistance (NWT or WT, respectively)

CLSI M100-Ed35, 2025

ECV REMINDER

- These are just *in vitro* (using zones and MICs)
- These are not clinical breakpoints

  MIC frequency distributions
  Pharmacokinetic data
  Pharmacodynamic data
  Clinical outcomes



- Don’t report S, I, SDD, or R; instead, NWT or WT

CLSI M100-Ed35, 2025

DISPENSATION

- *B. cepacia* ECV for some agents lie above drug levels in patients achievable by routine dosing
- Forego testing on basis of inaccurate method, lack of attainable levels
- If test (frozen broth microdilution), comment…

"Correlation of MIC values
with clinical outcome is not known."

CLSI M100-Ed35, 2025

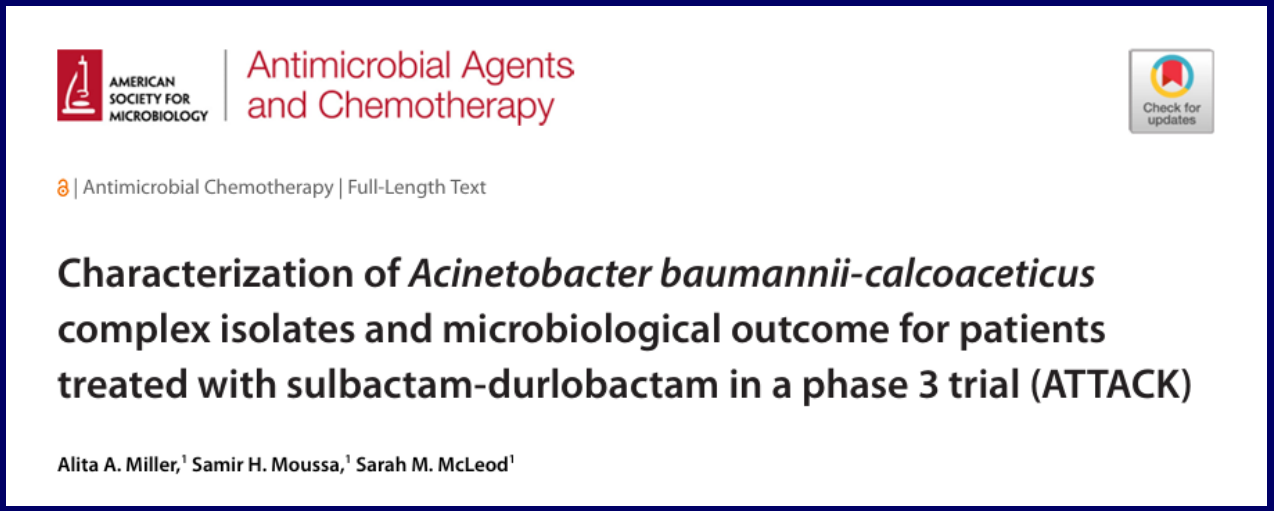
Table 5A-1. (Continued)

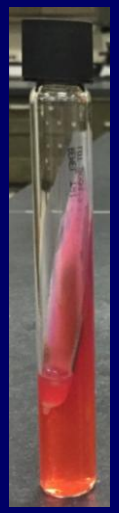
Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212
Telithromycin	–	–	0.06–0.25	0.016–0.12
Tetracycline	0.5–2	8–32	0.12–1	8–32
Ticarcillin	4–16	8–32	2–8	16–64
Tigecycline[t]	0.03–0.25	–	0.03–0.25	0.03–0.12
Tobramycin	0.25–1	0.25–1	0.12–1	8–32
Trimethoprim[v]	0.5–2	> 64	1–4	0.12–0.5
Trimethoprim-sulfamethoxazole[v] (1:19)	≤ 0.5/9.5	8/152–32/608	≤ 0.5/9.5	≤ 0.5/9.5
Trospectomycin	8–32	–	2–16	2–8
Trovafloxacin	0.004–0.016	0.25–2	0.008–0.03	0.06–0.25
Ulifloxacin (prulifloxacin)[y]	0.004–0.016	0.12–0.5	–	–
Upleganan[g,z]	0.06–0.25	0.12–0.5	–	–
Vancomycin[aa]	–	–	0.5–2	1–4
Zidebactam	0.06–0.25	1–8	–	–
Zoliflodacin	1–4	–	0.12–0.5	0.25–2
Zosurabalpin[g,bb,cc]	**–**	**–**	**–**	**–**

QC range for *Acinetobacter baumannii* NCTC 13304 is 0.016-0.12 µg/mL

CLSI M100-Ed35, 2025

CRAB





sulbactam-durlobactam

sulbactam with intrinsic activity vs. *Acinetobacter*
durlobactam active vs. A, C, D serine β-lactamases

CLSI Tier 3; DD and BMD (≤4, 8, ≥16)

Antimicrob Agents Chemother. 68:e0169823; 2024

SULBACTAM-DURLOBACTAM

Antibacterial agent	MIC (µg/mL)			% NS (CLSI)
	Range	MIC_{50}	MIC_{90}	
Amikacin	1 to >64	>64	>64	85
Cefepime	1 to >16	>16	>16	95
Cefoperazone-sulbactam, 2:1	1 to >32	32	>32	NA
Colistin	≤0.25 to >8	0.5	>8	17[b]
Imipenem	0.12 to >8	>8	>8	96
Meropenem	0.06 to >8	>8	>8	96
Levofloxacin	0.06 to >4	>4	>4	96
Minocycline	≤0.12 to >16	4	16	43
Tigecycline	0.06 to >4	1	2	NA
Sulbactam	1 to >64	32	>64	NA
Sulbactam-durlobactam	0.25–16	2	4	4.6

Category	ABC baseline isolates, *N* (%)	SUL-DUR MIC range (µg/mL)	SUL-DUR $MIC_{50/90}$ (µg/mL)
ALL	175 (100)	0.25–16	2/4
CARB-R	168 (96)	0.5–16	2/4
MDR	168 (96)	0.5–16	2/4
XDR	148 (85)	0.5–16	2/4
PDR	26 (15)	1–8	2/4

Antimicrob Agents Chemother. 68:e0169823; 2024

SULBACTAM-DURLOBACTAM

		SUL-DUR MIC of baseline ABC (µg/mL)			
	Total, N (%)	0.5	1	2	4
All evaluable patients who received SUL-DUR[a]					
Number of patients	87	5	28	43	11
(Presumed) Eradication	63 (72%)	3 (60%)	19 (68%)	32 (75%)	9 (82%)
(Presumed) Persistence	18 (21%)	2 (40%)	5 (18%)	10 (23%)	1 (9%)
Indeterminate	6 (7%)	0	4 (14%)	1 (2%)	1 (9%)

Antimicrob Agents Chemother. 68:e0169823; 2024

19% mortality in serious infections (including pneumonia)
32% mortality for colistin in randomized control trial

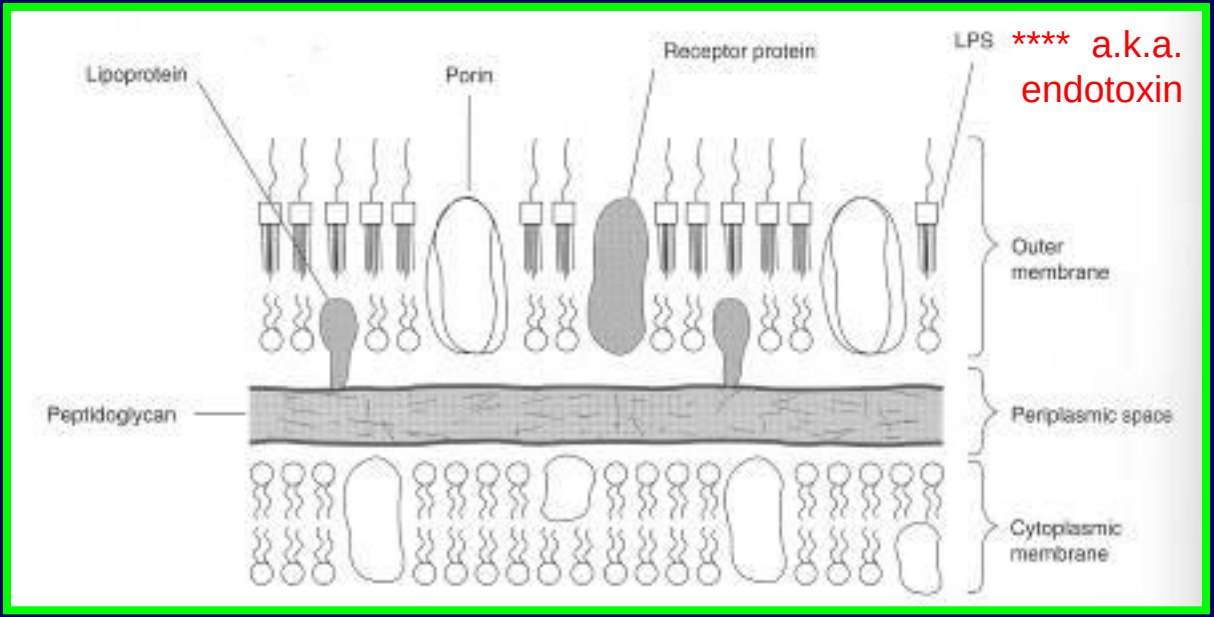
Lancet Infect Dis. 23:1072-1084; 2023

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Acinetobacter baumannii complex	β-Lactam combination agents	Sulbactam-durlobactam – I or R	X		

CLSI M100-Ed35, 2025

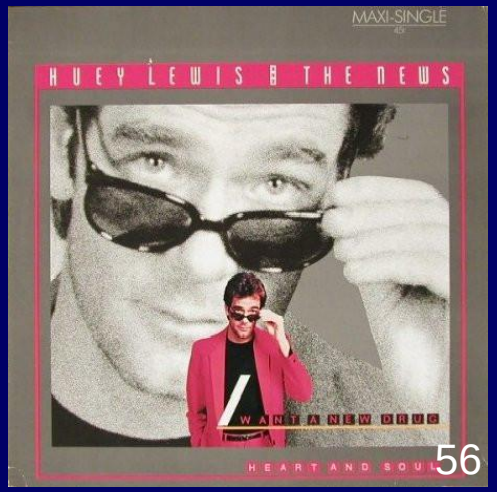
A NEW DRUG (MECHANISM)



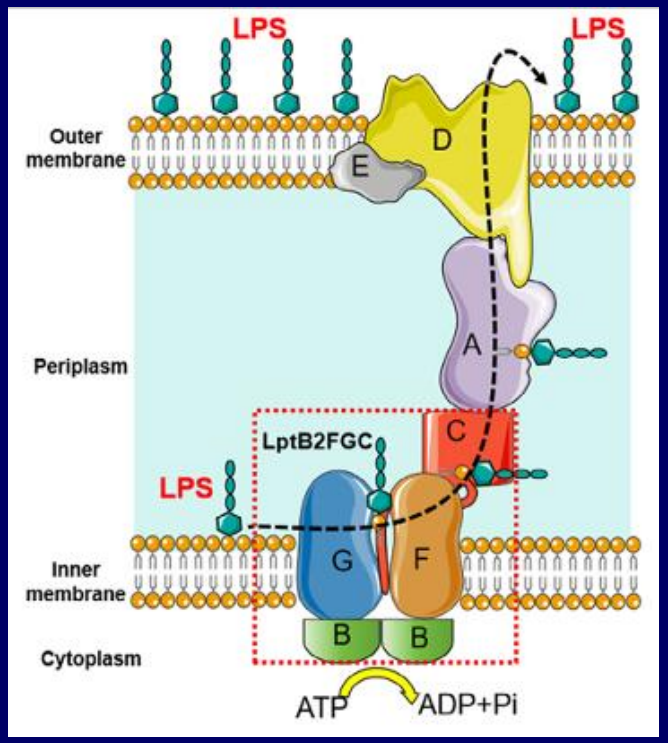
Lpt → lipopolysaccharide transport (3 components)

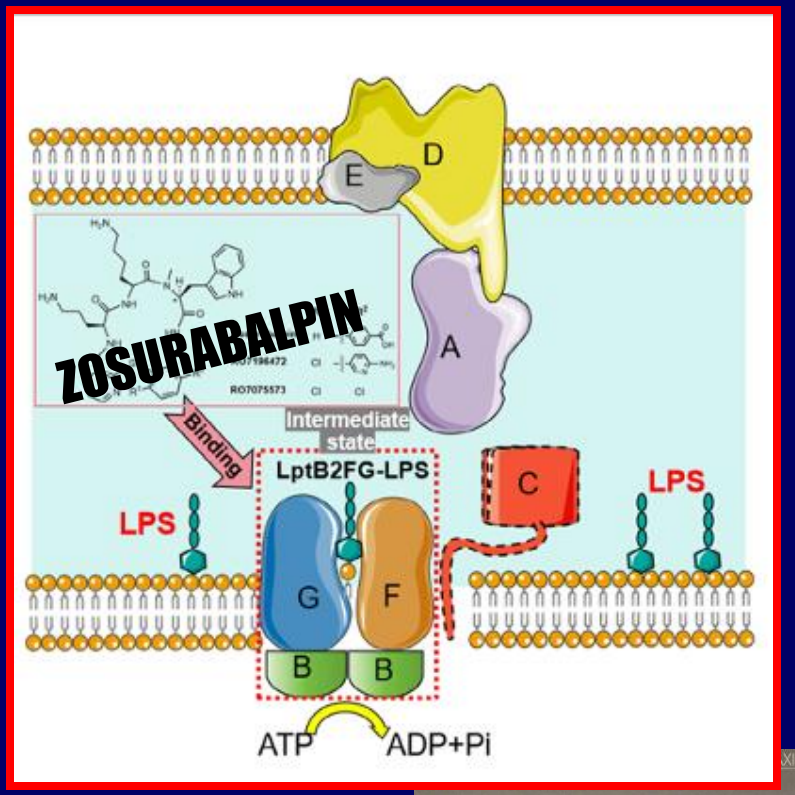
LptB2FGC, inner membrane transport protein complex
LptA, membrane interstitial protein
LptDE, outer membrane protein complex

MedComm. 5:e696; 2024



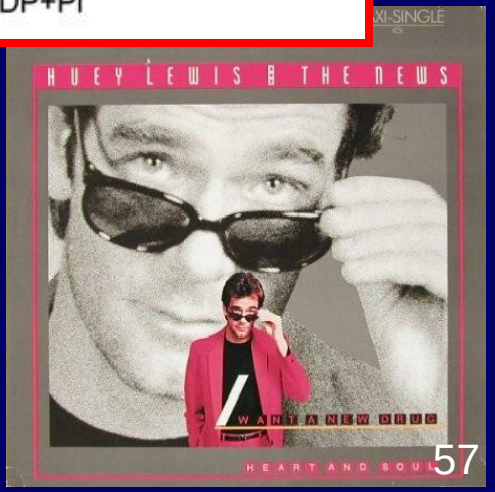
CARPE DIEM



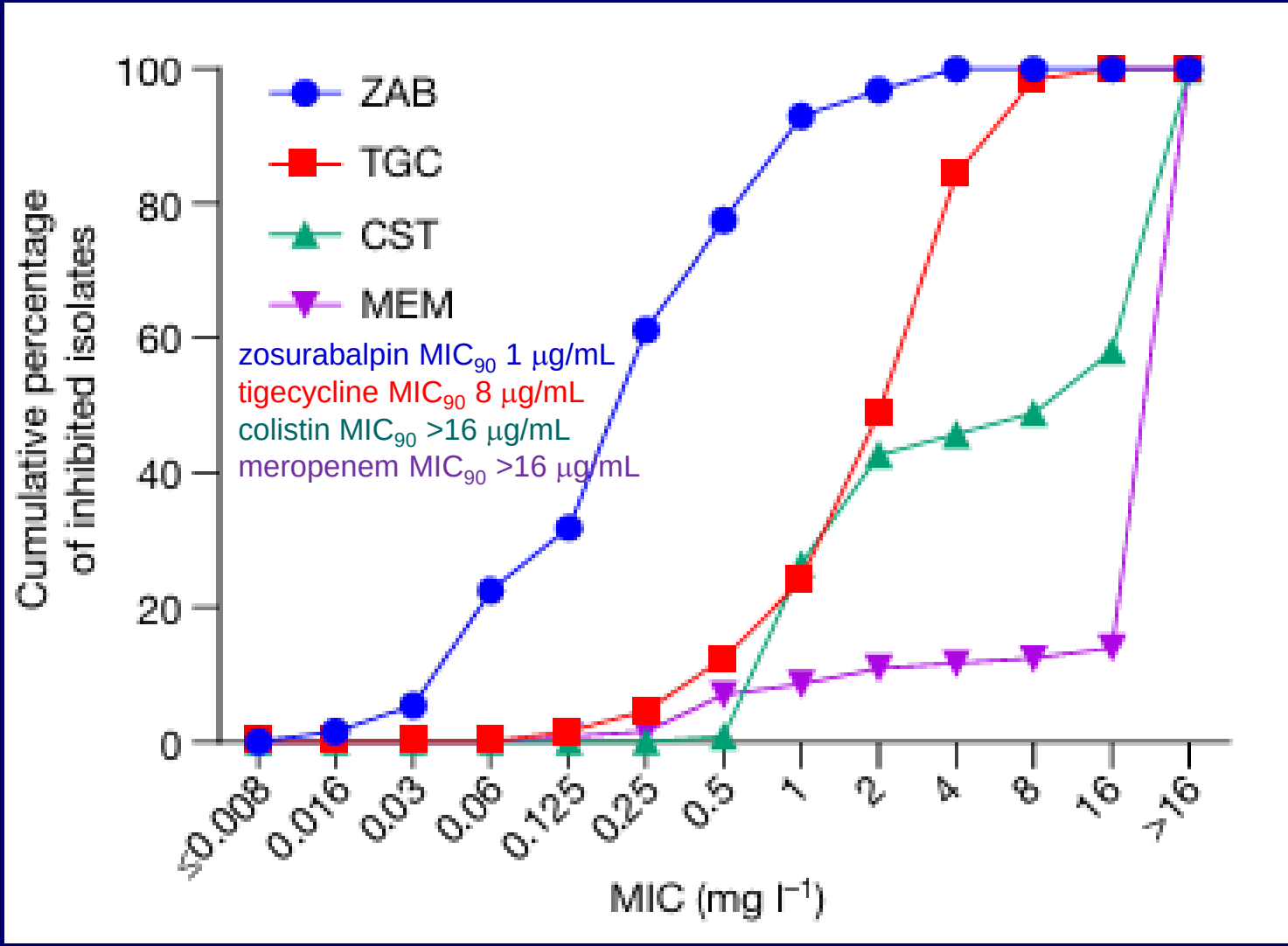


LPS extracted from inner membrane by LptB2FG
Sequentially transported by bridge of LptC, LptA, LptDE
(LptC can dissociate; LptB2FG-LPS is intermediate)

MedComm. 5:e696; 2024

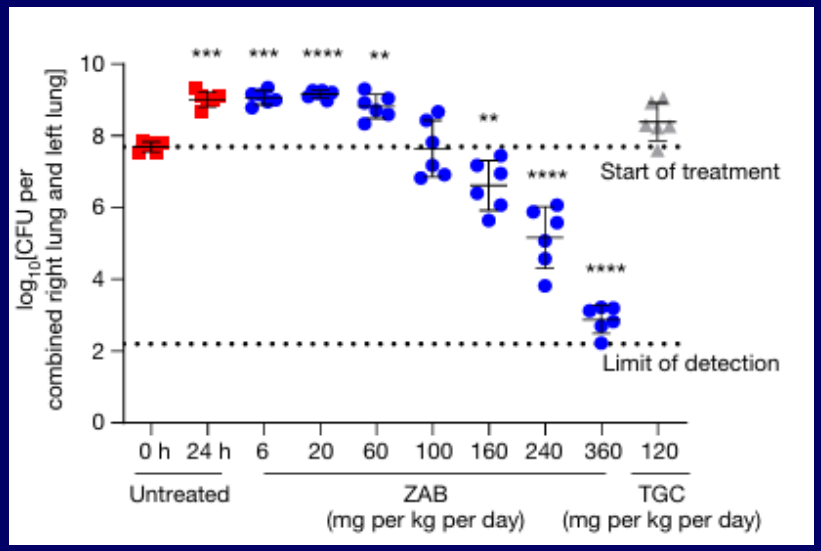


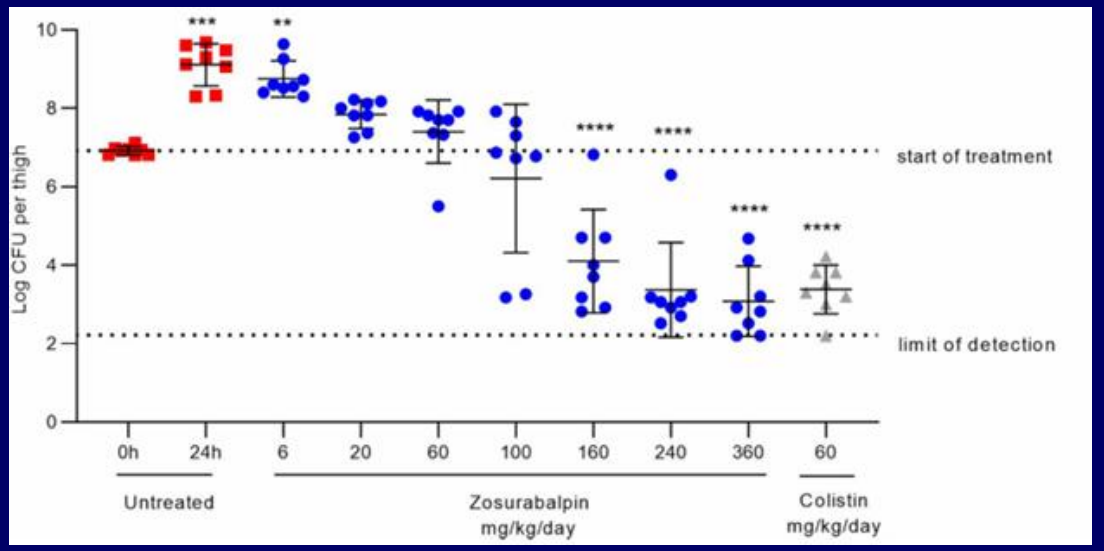
ZOSURABALPIN EARLY DATA I



Nature 625:566-571; 2024

ZOSURABALPIN EARLY DATA II

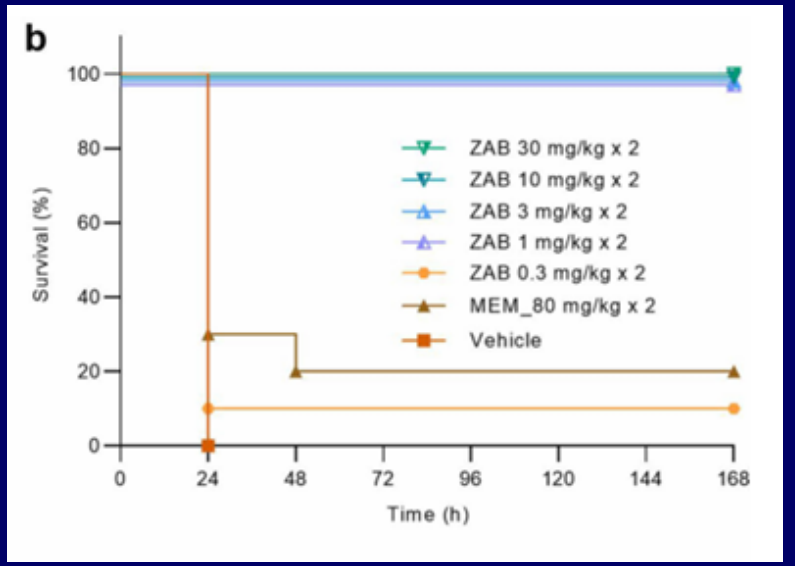




neutropenic mouse lung infection
zosurabalpin MIC 2 μg/mL

neutropenic mouse thigh infection
zosurabalpin MIC 0.5 μg/mL

immunocompetent mouse sepsis
zosurabalpin MIC 0.25 μg/mL



Nature 625:566-571; 2024

ZOSURABALPIN EARLY DATA III

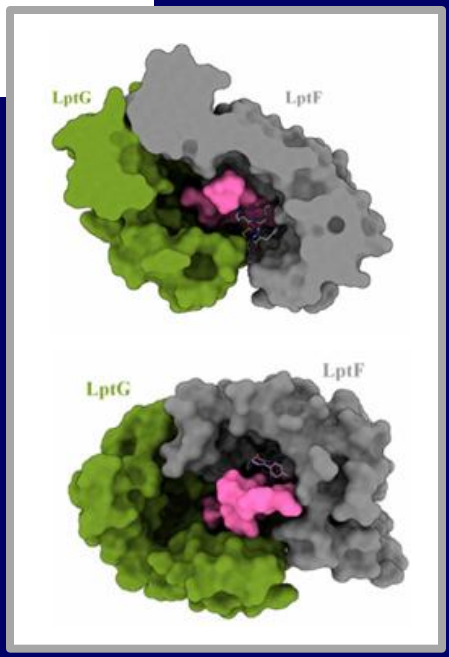
In Silico Pharmacology (2025) 13:62
https://doi.org/10.1007/s40203-025-00343-3

ORIGINAL RESEARCH

In silico analysis of zosurabalpin-LptB2FG binding in *Acinetobacter* spp., *Klebsiella pneumoniae*, and *Shigella flexneri*: mechanisms underlying its differential efficacy

Meryam Magri[1] · Rachid Eljaoudi[1] · Lahcen Belyamani[2,3,4] · Azeddine Ibrahimi[1] · El Mehdi Bouricha[2,3]

	A. Baylyi			
	LptB		LptF	
	Identity	Coverage	Identity	Coverage
K. pneumoniae	66.00%	96.80%	24.00%	98.40%
S. flexneri	64.70%	96.80%	24.50%	98.40%



other zosurabalpin derivatives showed increased binding efficiency for *K. pneumoniae*

In Silico Pharmacol. 13:62; 2025

TABLE 2B-2

- Breakpoint revisions

Organism	Method	Amp-sulbactam Previous			Amp-sulbactam New		
		S	I	R	S	I	R
Acinetobacter	DD	≥ 15	12-14	≤ 11	≥ 22	17-21	≤ 16

Organism	Method	Minocycline Previous			Minocycline New		
		S	I	R	S	I	R
Acinetobacter	BMD	≤ 4	8	≥ 16	≤ 1	2	≥ 4
	DD	≥ 16	13-15	≤ 12	≥ 22	18-21***	≤ 17

- Deleted all doxycycline, tetracycline standards and commentary

CLSI M100-Ed34, 2024; CLSI M100-Ed35, 2025

APPENDIX I

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

Table 2A-2. Zone Diameter and MIC Breakpoints for *Salmonella* and *Shigella* spp.

Testing Conditions

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

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [5])

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours
Dilution methods: 16–20 hours

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

Escherichia coli ATCC®[a] 25922
Pseudomonas aeruginosa ATCC® 27853 (for carbapenems)
Staphylococcus aureus ATCC® 25923 (for disk diffusion) or *S. aureus* ATCC® 29213 (for dilution methods) when testing azithromycin against *Salmonella enterica* ser. Typhi or *Shigella* spp.

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

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

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [5])

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours
Dilution methods: 16–20 hours

and Shigella spp.

QC Recommendations

Refer to the following:

- **Tables 4A-1 and 5A-1 that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

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

CLSI M100-Ed34, 2024; CLSI M100-Ed35, 2025

NON-FASTIDIOUS, DD, Gram-negative

Table I1: Example QC Strain Selection for Disk Diffusion 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	*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]	*E.coli* ATCC® 35218[c]	*E.coli* ATCC® 35218[c]	*E.coli* ATCC® 35218[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; QC, quality control.

CLSI M100-Ed35, 2025

NON-FASTIDIOUS, DD, Gram-positive

Table I2: Example QC Strain Selection for Disk Diffusion Methods When Testing Nonfastidious Gram-Positive Organisms

Antimicrobial Agents	Manufacturer Lot QC[a]	In User's Laboratory		
		New Lot, New Shipment QC	Same Lot, New Shipment QC	Routine QC
Ampicillin Cefoxitin Ciprofloxacin Clindamycin Erythromycin Oxacillin Tetracycline Vancomycin	*Staphylococcus aureus* ATCC®[b] 25923	*S. aureus* ATCC® 25923	*S. aureus* ATCC® 25923	*S. aureus* ATCC® 25923
Trimethoprim-sulfamethoxazole	• *S. aureus* ATCC® 25923 • *E. faecalis* ATCC® 29212	• *S. aureus* ATCC® 25923 • *E. faecalis* ATCC® 29212	*S. aureus* ATCC® 25923	*S. aureus* ATCC® 25923

Abbreviations: ATCC®, American Type Culture Collection; QC, quality control.

CLSI M100-Ed35, 2025

NON-FASTIDIOUS, BMD, Gram-negative

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.

CLSI M100-Ed35, 2025

NON-FASTIDIOUS, BMD, Gram-positive

Table I4: Example QC Strain Selection for MIC Methods when Testing Nonfastidious Gram-Positive Organisms

Antimicrobial Agents	Manufacturer Lot QC[a]	In User's Laboratory		
		New Lot, New Shipment QC	Same Lot, New Shipment QC	Routine QC
Ampicillin Ciprofloxacin Clindamycin Daptomycin Erythromycin Tetracycline Vancomycin	• *S. aureus* ATCC®[b] 29213 • *E. faecalis* ATCC® 29212	• *S. aureus* ATCC® 29213 • *E. faecalis* ATCC® 29212	*S. aureus* ATCC® 29213 or *E. faecalis* ATCC® 29212	*S. aureus* ATCC® 29213 or *E. faecalis* ATCC® 29212
Cefoxitin	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213
Oxacillin	• *S. aureus* ATCC® 29213 • *E. faecalis* ATCC® 29212	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213
Trimethoprim-sulfamethoxazole	• *S. aureus* ATCC® 29213 • *E. faecalis* ATCC® 29212	• *S. aureus* ATCC® 29213 • *E. faecalis* ATCC® 29212	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213

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

CLSI M100-Ed35, 2025

WE KNOW THE DRILL…

- CMS requires laboratories in the United States to perform appropriate QC testing for AST:

  Each lot/batch/shipment of media
  Each lot/batch/shipment of agents
  (before, or concurrent with initial use)

- Also must be performed each day of testing

- IQCP can reduce AST QC workload

CLSI M100-Ed35, 2025

ADDITIONAL APPENDIX I GUIDANCE

- CLSI M23 reference (for IQCP)
- Types of Quality Control errors
- Selection of Quality Control strains
- Quality Control plan (selection of strains, frequency of testing)
- Indicators to detect AST system problems

CLSI M100-Ed35, 2025

BAR TRIVIA

- Imipenem and clavulanate most temperature-labile

  Imipenem *Pseudomonas aeruginosa* ATCC 27853
  Escherichia coli ATCC 35218

  Clavulanate *K. pneumoniae* ATCC 700603

- pH, Ca^{2+}, and Mg^{2+} parameters

  Pseudomonas aeruginosa ATCC 27853

- Thymidine content (trimethoprim-sulfamethoxazole)

  Enterococcus faecalis ATCC 27853

CLSI M100-Ed35, 2025

Old Business

CHANGES (JUST ONE), AGAIN

Table 2C
Staphylococcus spp.
CLSI M02 and CLSI M07

Table 2C. *Staphylococcus* spp. (Continued)

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

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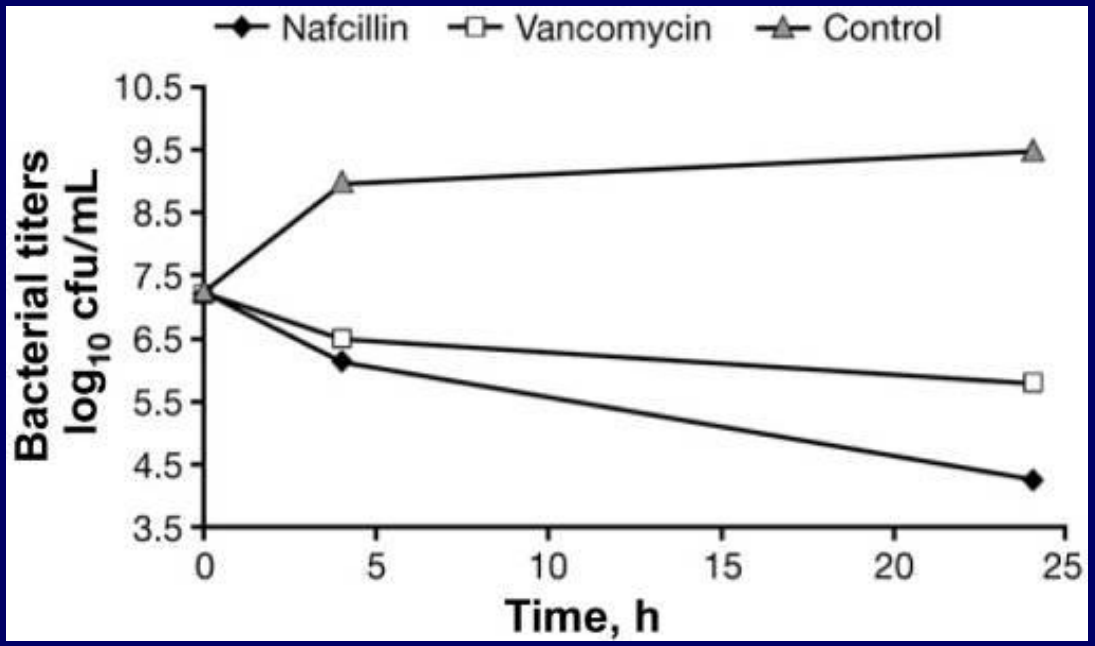

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COMMENTARY

Use of Rapid Diagnostics To Manage Pediatric Bloodstream Infections? You Bet Your ASP!

Mark D. Gonzalez,[a] Melanie L. Yarbrough[b]

[a]Department of Pathology and Laboratory Services, Children's Healthcare of Atlanta, Atlanta, Georgia, USA

[b]Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, Missouri, USA

Table 3F-1. Test for Performing Disk Diffusion Directly From Positive Blood Culture Broth

Test	Direct Disk Diffusion
Test method	Disk diffusion using positive blood culture broth
Organism group	Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter* spp.
Medium	MHA
Antimicrobial concentration	Standard disk contents for the antimicrobial agents are detailed in Table 3F-2 (Enterobacterales), Table 3F-3 (*P. aeruginosa*), and Table 3F-4 (*Acinetobacter* spp.).
Inoculum	Positive blood culture broth with gram-negative bacilli, used within 8 h of flagging positive by the blood culture system
Test procedure	1. Invert blood culture bottle 5–10 times to thoroughly mix. 2. Sterilize the top of the bottle with an alcohol wipe (allow to dry) and insert 20-gauge venting needle into the blood culture bottle. 3. Dispense 4 drops of blood culture broth onto an MHA plate. As a purity check, use an inoculated blood agar plate streaked for isolation. 4. Spread blood culture broth across the entire surface of the MHA plate using a sterile cotton swab. 5. Repeat this procedure by streaking twice more, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. 6. Leave the lid ajar for 3–5 minutes (ideally) but no more than 15 minutes. 7. Dispense antimicrobial disks onto the surface of the inoculated MHA plate. 8. Press each disk down to ensure complete contact with the agar surface. 9. Invert the plate and place in the incubator within 15 minutes of disks being applied.
Incubation conditions	35°C ± 2°C; ambient air
Incubation length	8–10 h or 16–18 h (refer to Tables 3F-2, 3F-3, and 3F-4 for antimicrobial agent–specific incubation lengths)
Results	1. Examine the blood agar purity plate to ensure pure growth. 2. Examine the test plate to ensure confluent lawn of growth appropriate to read disk zone tests per CLSI M02.[1] 3. Measure the zone diameters according to routine disk diffusion recommendations in CLSI M02.[1] 4. Interpret results using the zone diameter breakpoints in Tables 3F-2, 3F-3, and 3F-4 if the gram-negative bacillus tested is confirmed to be an Enterobacterales, *P. aeruginosa*, or *Acinetobacter* spp., respectively. If species is identified as another organism, do not interpret or report results. 5. Report only the interpretive category and not the measured zone size.

4

35 ± 2

8-10 or 16-18

Daily or IQCP; *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 35218

CLSI M100-Ed35, 2025

EARLY-READ QUALITY CONTROL

Supplemental early reading – optional

- Ranges have been established for early reading (8–10 h) of select QC strain/antimicrobial agent combinations as shown below. This testing is performed using a 0.5 McFarland standardized inoculum (standard disk diffusion QC procedures per CLSI M02[1]).
- Early reading of QC strains can be used to train staff or assess competency but is not necessary for routine QC.

Antimicrobial Agent	Disk Content	Optional Early Read (8–10 h) Ranges, mm		
		Escherichia coli ATCC® 25922	*P. aeruginosa* ATCC® 27853	*E. coli* ATCC® 35218
Ampicillin	10 µg	15–22	–	–
Ampicillin-sulbactam	10/10 µg	–	–	13–19
Ciprofloxacin	5 µg	29–38	–	–
Ertapenem	10 µg	–	13–21	–
Tobramycin	10 µg	18–26	–	–
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	23–29	–	–

CLSI M100-Ed35, 2025

TABLE 3F-2 NEWBIES

Agent	Concentration	Incubation time (hours)	Zone Diameters (mm)		
			S	I	R
cefepime	30 µg	8-10	≥ 23	19-22	≤ 18
		16-18	≥ 23	19-22	≤ 18

* fluoroquinolone breakpoints do not apply to *Salmonella* spp.
* aztreonam, ceftazidime, tobramycin do not apply to *Salmonella* spp., *Shigella* spp.

Glucose fermenters
Reduce nitrates to nitrites
Non-spore-forming GNR
Grows on routine media
Facultative
Oxidase-negative (except *Plesiomonas*)

9 agents
17 breakpoints

CLSI M100-Ed35, 2025

TABLE 3F-3 NEWBIES

Agent	Concentration	Incubation time (hours)	Zone Diameters (mm)		
			S	I	R
ceftazidime	30 μg	8-10	≥ 18		≤ 14

* confirmatory cefepime MIC testing for zone diameters 15-17 mm
* if ceftazidime zone is 15-17 mm at 8-10 h, reincubate and read at 16-18 h

5 agents
9 breakpoints

CLSI M100-Ed35, 2025

Acinetobacter spp. NEWBIES

Agent	Concentration	Incubation time (hours)	Zone Diameters (mm)		
			S	I	R
ceftazidime	30 μg	8-10	≥ 17	15-16	≤ 14
ampicillin-sulbactam	10/10 μg	8-10	≥ 22	17-21	≤ 16
piperacillin-tazobactam	100/10 μg	8-10	≥ 19	17-18	≤ 16
		16-18	≥ 19	17-18	≤ 16

9 agents
18 breakpoints

CLSI M100-Ed35, 2025

TABLE 3F-4 REVISION

Agent	Concentration	Incubation time (hours)	Zone Diameters (mm)		
			S	I	R
ampicillin-sulbactam	10/10 μg	16-18	≥ 22	17-21	≤ 16

Everything
Increased
(matches novel 8-10 h breakpoints)

CLSI M100-Ed35, 2025


one
BEE GEES

Table 1


GET WILD UNO
UNO
NOW WITH CUSTOMIZABLE WILD CARDS!
2
3
4
WILD
2-10
7+

TABLE 1 IMPORTANT Δs

- Aztreonam (Table 1A)

  Tier 4 agent
  Can be cascaded as Tier 3 agent at institutions with
  ↑ risk of metallo-β-lactamase *Enterobacterales*

- Anaerobes (Table 1J)

  Penicillin listed as Tier 4 agent vs. Gram-negatives
  Penicillin with good activity vs. *Fusobacterium* spp.
  (therefore, consider for primary testing, reporting)
  β-lactamases have been reported in *Fusobacterium*

CLSI M100-Ed35, 2025

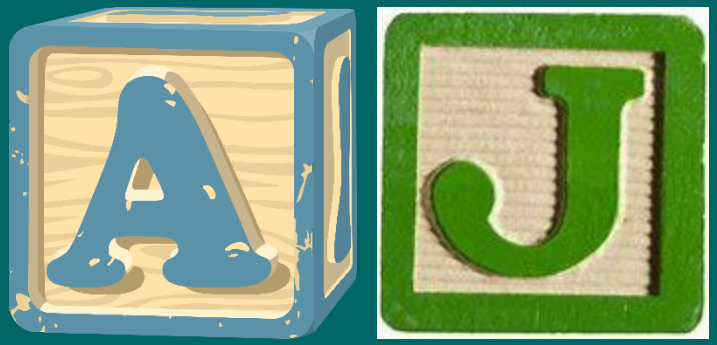

A
J

Table 2

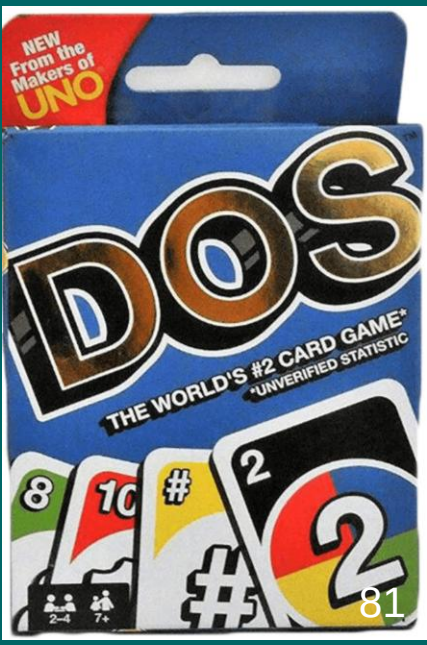

NEW From the Makers of UNO
DOS
THE WORLD'S #2 CARD GAME*
*UNVERIFIED STATISTIC
8
10
#
2
2
#

TABLE 2 IMPORTANT Δs

- *Staphylococcus aureus* complex (Table 2C)

  S. aureus, *S. argenteus*, *S. schweitzeri*
  CLSI method evaluation applicable only to *S. aureus*
  Report as "*S. aureus* complex (*S. argenteus*)" w/ *S. aureus* AST

- Anaerobes (Table 2J)

  Agar dilution (MIC) has been mainstay (42-48 hrs)

  Added broth microdilution method (46-48 hrs)
  Bacteroides fragilis, *Bacteroides thetaiotaomicron*

- Two "dosing table" additions (*Ac*); one revision (*Pa*)

CLSI M100-Ed35, 2025

Table 3

AS A REMINDER…

Table 3A
Tests for ESBLs

Table 3B
CarbaNP Test for Suspected Carbapenemase Production

Table 3C
Modified Carbapenem Inactivation Methods

Table 3D
Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution Method

Table 3E
Tests for Colistin Resistance for
Enterobacterales and *Pseudomonas aeruginosa*

Table 3F-1
Test for Performing Disk Diffusion Directly From Positive Blood Culture Broth

Table 3G
Tests for β-Lactamase Production in *Staphylococcus* spp.

Table 3H
Oxacillin Salt Agar Test for Methicillin (Oxacillin) Resistance in *Staphylococcus aureus*

Table 3I
Vancomycin Agar Screen for *Staphylococcus aureus* and *Enterococcus* spp.

Table 3J
Tests for Inducible Clindamycin Resistance in *Staphylococcus* spp.,
Streptococcus pneumoniae, and *Streptococcus* spp. β-Hemolytic Group

Table 3K
Test for High-Level Mupirocin Resistance in *Staphylococcus aureus*

Table 3L
Test for High-Level Aminoglycoside Resistance in *Enterococcus* spp.

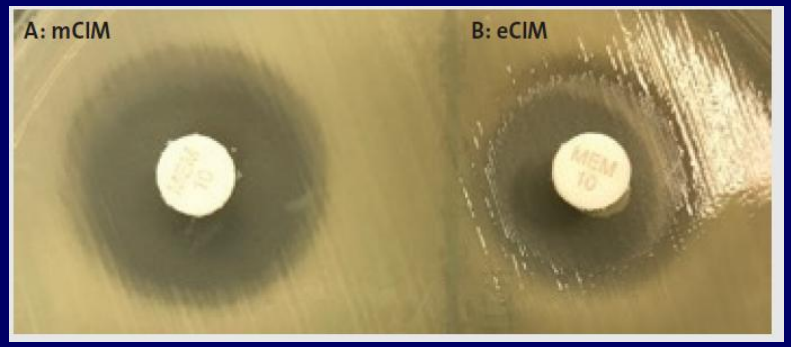
CLSI M100-Ed35, 2025

TABLE 3C

mCIM and eCIM Combination Test		
mCIM Result	**eCIM Result**	**Report**
Negative	Do not interpret	Carbapenemase not detected
Positive	Negative	Serine carbapenemase detected; **metallo-β-lactamase inconclusive.** **Call laboratory to discuss.**[b]
Positive	Positive	Metallo-β-lactamase detected
Inconclusive	Do not interpret	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.[a]

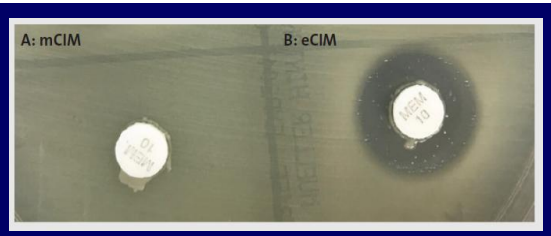
SERINE CARBEPENEMASE

METALLO-β-LACTAMASE (EDTA)



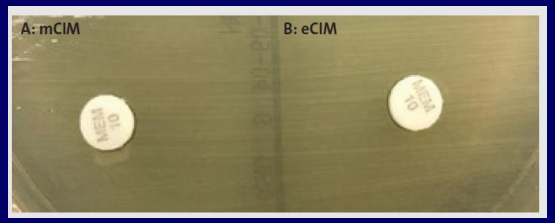
neg non-valid

carbapenemase not detected



pos pos

metallo-β-lactamase detected



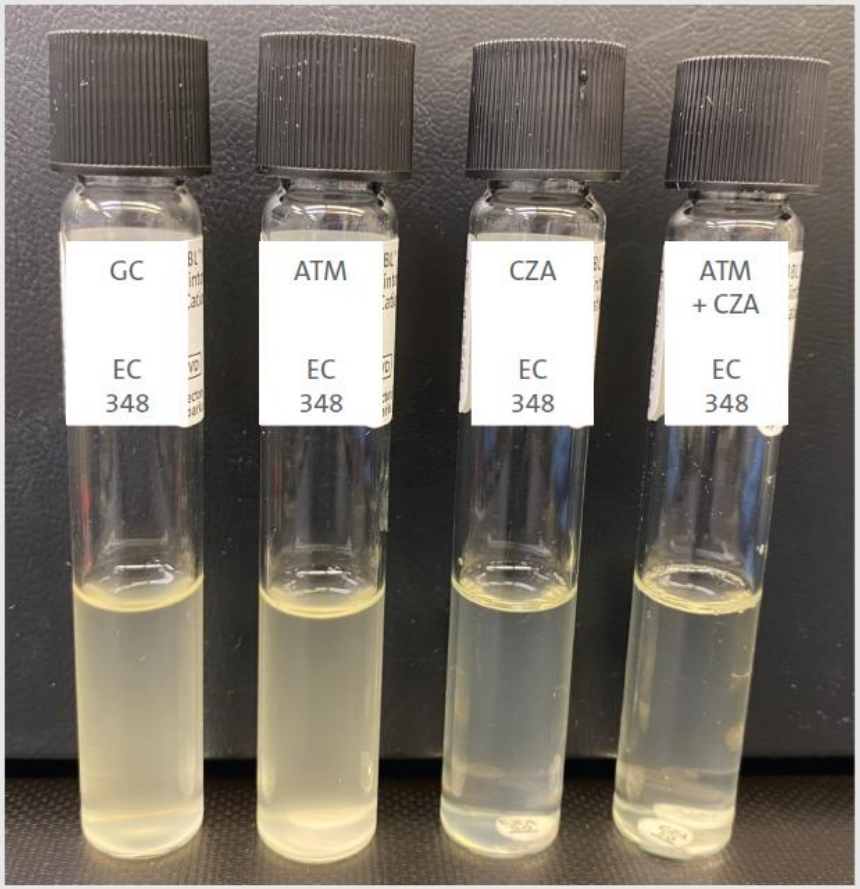
pos neg

serine carbapenemase detected

metallo-β-lactamase inconclusive

CLSI M100-Ed35, 2025

TABLE 3D



Escherichia coli AR Bank #0348

aztreonam plus ceftazidime-avibactam
broth disk elution method

additional QC strains
Escherichia coli AR Bank #0434
Escherichia coli AR Bank #0450

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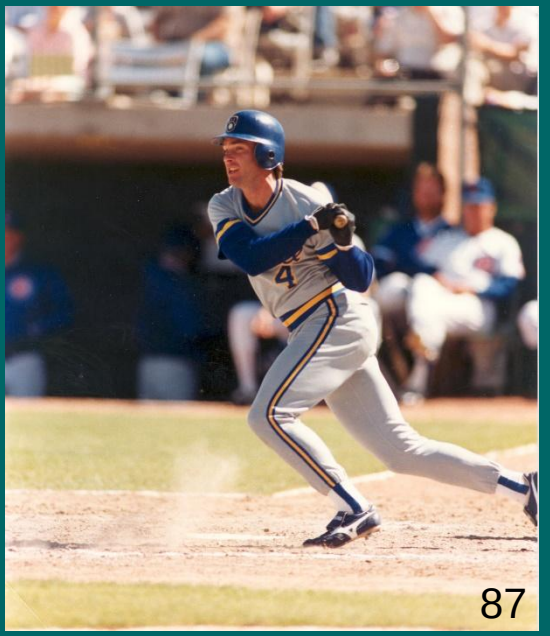
Table 4



SOME DD QC ADDITIONS/REVISIONS

Klebsiella pneumoniae ATCC 700603	ceftibuten-avibactam
Klebsiella pneumoniae ATCC BAA-1705	ceftibuten-avibactam
Klebsiella pneumoniae ATCC BAA-2814	ceftibuten-avibactam
Escherichia coli NCTC 13353	ceftibuten-avibactam
Escherichia coli ATCC 25922	minocycline ceftibuten-avibactam

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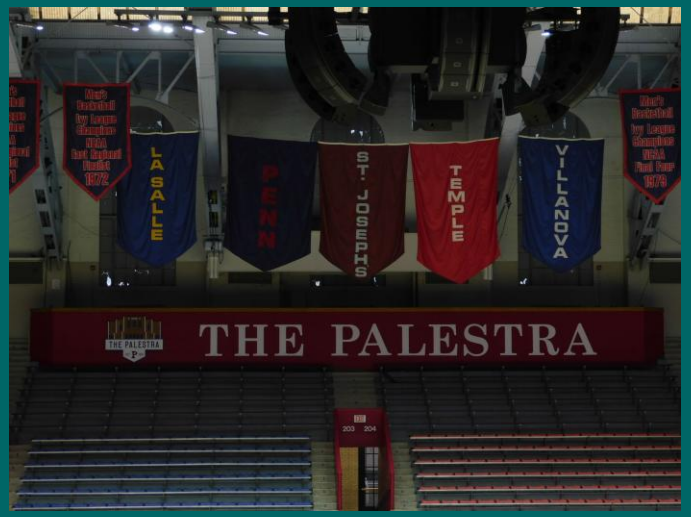
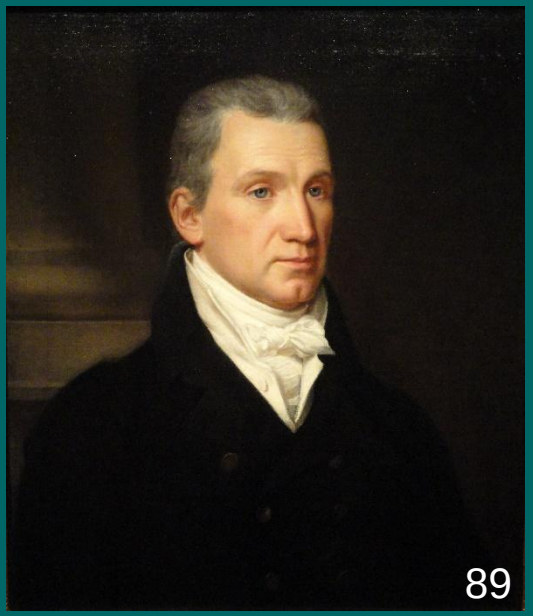


Table 5



SOME MIC QC ADDITIONS/REVISIONS

Klebsiella pneumoniae ATCC BAA-1705	ceftibuten-xeruborbactam
Klebsiella pneumoniae ATCC BAA-2814	ceftibuten-xeruborbactam
Klebsiella pneumoniae ATCC 700603	ceftibuten-xeruborbactam
Acinetobacter baumannii NCTC 13304	zosurabalpin

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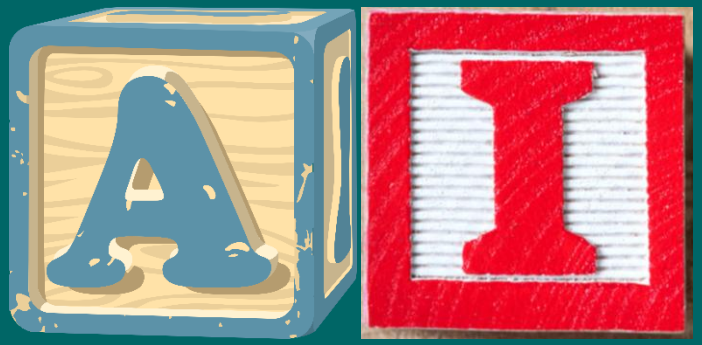
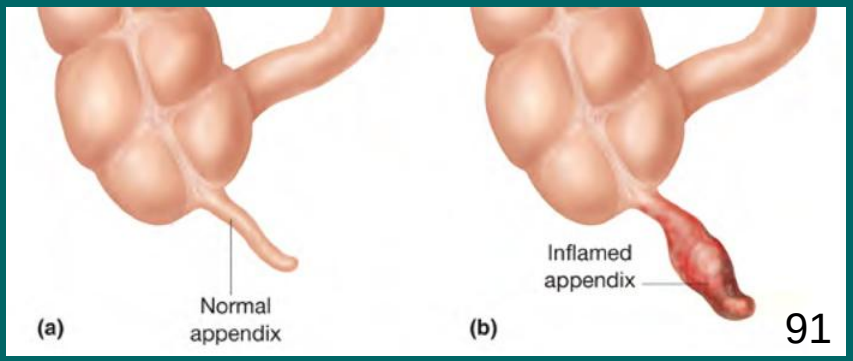


Appendices



REVISED APPENDIX H

- Modifications of the CLSI M07 broth microdilution method may be required (cefiderocol)

- Exebacase (*S. aureus*, SOSA, β-hemolytic *Strep*.)

  CAMHB w/ 25% horse serum, 0.5 mM DL-dithiothreitol
  S. aureus 16-20 hours ambient;
  SOSA 20-24 hours, 5% CO_2
  β-*Strep*. 20-24 hours, ambient
  Read MIC at complete inhibition

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Thank you for your attention.
Have a better 2025.