2020 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. Introduction to “new” resources

II. Objectives of webinar

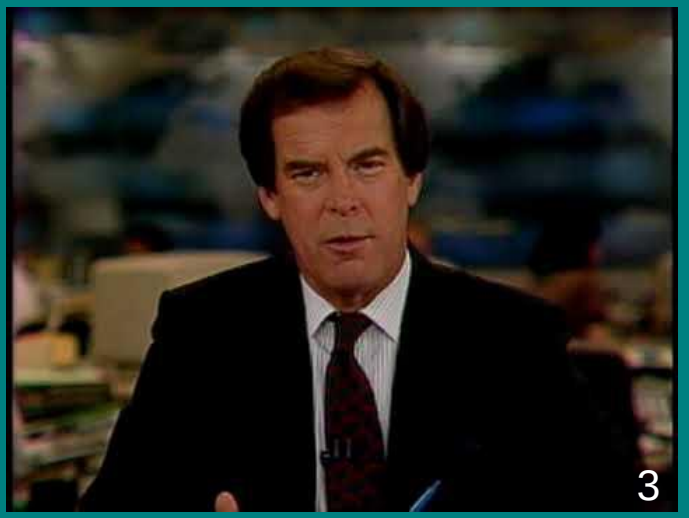
Describe significant changes relevant to pre-existing antimicrobial susceptibility breakpoints…

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 ED30 document.

What's (really exciting and) New?



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MINIREVIEW

Understanding and Addressing CLSI Breakpoint Revisions: a Primer for Clinical Laboratories

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ABSTRACT The Clinical and Laboratory Standards Institute (CLSI) has revised several breakpoints since 2010 for bacteria that grow aerobically. In 2019, these revisions include changes to the ciprofloxacin and levofloxacin breakpoints for the *Enterobacteriaceae* and *Pseudomonas aeruginosa*, daptomycin breakpoints for *Enterococcus* spp., and ceftaroline breakpoints for *Staphylococcus aureus*. Implementation of the revisions is a challenge for all laboratories, as not all systems have FDA clearance for the revised (current) breakpoints, compounded by the need for laboratories to perform validation studies and to make updates to laboratory information system/electronic medical record builds in the setting of limited information technology infrastructure. This minireview describes the breakpoint revisions in the M100 supplement since 2010 and strategies for the laboratory on how to best adopt these in clinical testing.

KEYWORDS CLSI, FDA, antimicrobial susceptibility testing, breakpoints

J. Clin. Microbiol. **57:**e00203-19

WHEN NEEDED (per CLSI M23)?

Recognition of a new resistance mechanism

New PK/PD data indicate existing breakpoint too high/low

Recognition that antimicrobial dosage regimens used in widespread clinical practice differ substantially from dosage regimens used to establish previous breakpoints

Introduction of new formulations of antimicrobial agents, resulting in different PK characteristics

New data emerge to demonstrate the previous breakpoints were not optimal for common uses of antimicrobial agent

J. Clin. Microbiol. **57:**e00203-19

WHEN NEEDED (per CLSI M23) II?

New data demonstrate poor prediction of clinical response using previous breakpoints

Specific public health need not addressed previously

Significant MIC/disk diffusion discordance when testing recent clinical isolates

Changes to CLSI-approved reference methods

Revisions to simplify testing for specific resistance mechanisms

Differences between CLSI and other regulatory organizations

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(MORE) IN SYNCH WITH FDA?

CLSI Revises Breakpoint

CLSI generates a rationale for the revision

CLSI submits the rationale to the federal register

FDA reviews rationale

If acceptable by FDA standards, FDA recognizes CLSI breakpoint on STIC website

cASTs MAN prioritizes breakpoint update with other needs

cASTs MAN redevelops test with revised breakpoint (if needed)

cASTs MAN performs clinical trial to confirm performance (if needed)

cASTs MAN submits for FDA clearance with revised breakpoint

cASTs MAN adds revised breakpoint to software update lists

Revised breakpoint available on cASTs for clinical laboratory

MAYBE

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(MORE) IN SYNCH WITH FDA?

TABLE 4 cASTs with FDA clearance for current CLSI breakpoints[a]

Organism group	Antimicrobial agent	BD phoenix	Beckman coulter MicroScan	bioMérieux Vitek 2	Thermo Fisher Sensititre
Enterobacteriaceae	Cefepime	Y	N	Y	Y
	Cefotaxime	N	Y	Y	Y
	Ceftriaxone	Y	Y	Y	Y
	Ceftazidime	N	N	N	N
	Ertapenem	Y	Y	Y	Y
	Imipenem	Y	Y	Y	Y
	Meropenem	Y	N	N	Y
Enterobacteriaceae (Salmonella)	Ciprofloxacin		*S. typhi*	*S. typhi*; *S. enteritidis*	
Pseudomonas aeruginosa	Imipenem	Y	Y	Y	Y
	Meropenem	Y	Y	N	Y
	Piperacillin-tazobactam	Y	N	N	Y
Acinetobacter spp.	Imipenem	Y	Y	Y	Y

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(LESS) IN SYNCH WITH FDA?

TABLE 5 Agents for which current CLSI breakpoints are not recognized by the FDA[a]

Organism group	Antimicrobial agent
Enterobacteriaceae	Cefazolin
	Ciprofloxacin
	Levofloxacin
Enterobacteriaceae (*Salmonella*)	Levofloxacin
Pseudomonas aeruginosa	Cefepime[b]
	Ceftazidime[b]
	Ciprofloxacin
	Levofloxacin
Acinetobacter spp.	Meropenem
S. aureus	Ceftaroline
Enterococcus spp.	Daptomycin

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PRIORITY 1 (implement now)

Enterobacteriaceae:	carbapenem breakpoints
Enterobacteriaceae:	aztreonam ceftriaxone cefotaxime ceftazidime ceftizoxime cefepime breakpoints
Salmonella spp.:	fluoroquinolone breakpoints
P. aeruginosa *Acinetobacter* spp.:	carbapenem breakpoints
P. aeruginosa:	piperacillin-tazobactam breakpoints

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PRIORITY 2 (institutional need)

Enterobacteriaceae:	cefazolin breakpoints
Enterobacteriaceae:	fluoroquinolone breakpoints
Pseudomonas aeruginosa:	fluoroquinolone breakpoints
Enterococcus spp.:	daptomycin breakpoints

PRIORITY 3 (may need to implement)

Pseudomonas aeruginosa:	colistin breakpoints
Staphylococcus aureus:	ceftaroline breakpoints

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Fluoroquinolone Breakpoints for *Enterobacteriaceae* and *Pseudomonas aeruginosa*

CLSI rationale document MR02
February 2019

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M100, 30th ed.
January 2020
Replaces M100, 29th ed.

Performance Standards for Antimicrobial Susceptibility Testing

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CLSI MR02; 2019

HOW DOES THIS HAPPEN?

- CLSI voluntary consensus process

  Members
  Advisors
  Observers (public)

- Subcommittee on antimicrobial susceptibility testing

  In vitro data
  Pharmacokinetic/pharmacodynamic (PK/PD)
  Clinical studies

- Establish AST methods, breakpoints (M100, M45), quality control ranges

CLSI MR02; 2019

WHY DID THIS ONE HAPPEN??

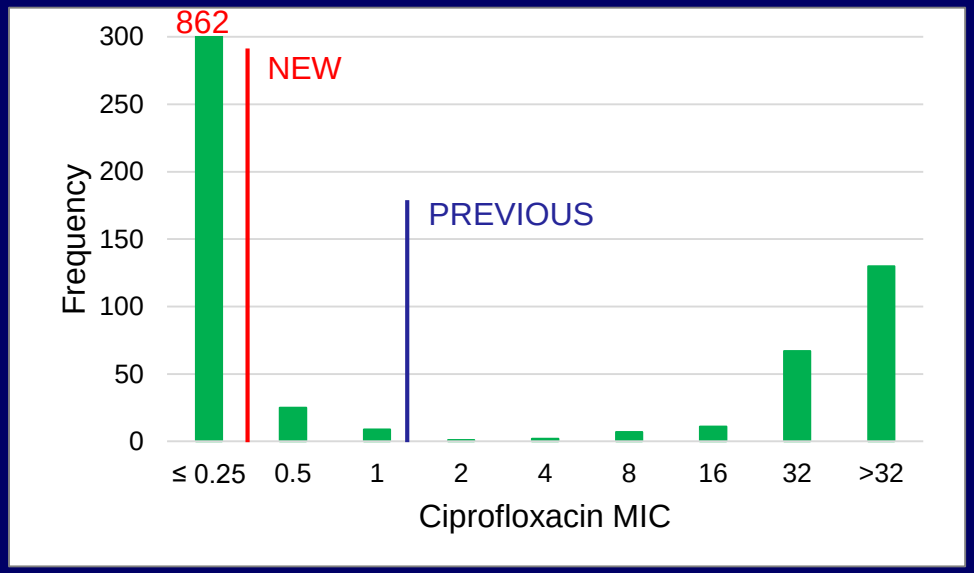
- Susceptibility may decrease over time, resulting in lack of clinical efficacy and/or safety
- Methods for analysis become more refined
- WRT fluoroquinolones, association with debilitating and potentially irreversible adverse reactions (tendinitis, tendon rupture, peripheral neuropathy, CNS effects)

CLSI MR02; 2019

FLASHBACK

Organism	Method	Ciprofloxacin Previous			Ciprofloxacin New		
		S	I	R	S	I	R
Enterobacteriaceae	BMD	≤ 1	2	≥ 4	≤ 0.25	0.5	≥ 1
P. aeruginosa	BMD	≤ 1	2	≥ 4	≤ 0.5	1	≥ 2

Organism	Method	Levofloxacin Previous			Levofloxacin New		
		S	I	R	S	I	R
Enterobacteriaceae	BMD	≤ 2	4	≥ 8	≤ 0.5	1	≥ 2
P. aeruginosa	BMD	≤ 2	4	≥ 8	≤ 1	2	≥ 4



1114 Wisconsin clinical *Escherichia coli* isolates

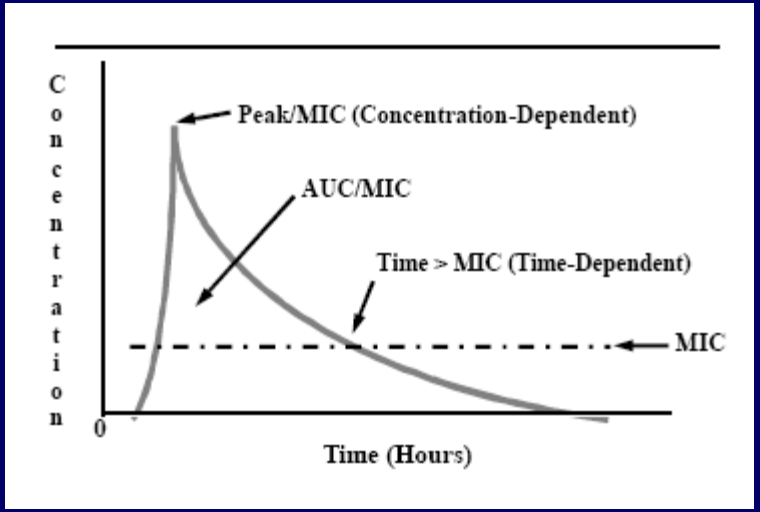
THEORY

- In order for an antimicrobial agent to work:

  Get there
  Get there in enough concentration
  Stay there long enough

- Area Under serum concentration Curve

  Measures how high (concentration) and how long (time) antimicrobial levels remain above target MIC during a dosing interval



METHODS

- AUC:MIC ratios can be calculated (and can vary)

  Fluoroquinolones vs. GP; AUC:MIC ≥ 30
  Fluoroquinolones vs. GNR; AUC:MIC closer to 100

- Two pneumonia studies established clinical (free) AUC:MIC ratio target of 72 for *Enterobacteriaceae*

Table 9. Summary of Nonclinical and Clinical Free-Drug AUC:MIC Ratio Targets for Efficacy[10]
(Reprinted with permission from USCAST, The National Antimicrobial Susceptibility Testing Committee for the United States. Quinolone In Vitro Susceptibility Test Interpretive Criteria Evaluations. Version 1.3, 2018. http://www.uscast.org.)

Organism	Nonclinical Free-Drug AUC:MIC Ratio Targets			Clinical Free-Drug AUC:MIC Ratio Targets
	Net Bacterial Stasis	1-$\log_{10}$ CFU Reduction From Baseline	2-$\log_{10}$ CFU Reduction From Baseline	
Enterobacteriaceae	35.6	67.4	140.0	72.0
P. aeruginosa	34.8	47.3	65.4	72.0

Abbreviations: AUC, area under the curve; CFU, colony-forming unit; MIC, minimal inhibitory concentration.

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Table 10. Percent Probabilities of PK-PD Target Attainment by MIC Based on PK-PD Targets for *Enterobacteriaceae*[10] (Reprinted with permission from USCAST, The National Antimicrobial Susceptibility Testing Committee for the United States. Quinolone In Vitro Susceptibility Test Interpretive Criteria Evaluations. Version 1.3, 2018. http://www.uscast.org.)

Antimicrobial Agent	Route of Administration	Dosing Regimen	Population	MIC, µg/mL	End Points for Nonclinical Free-Drug AUC:MIC Ratio Targets (Magnitude of Target)			Clinical Free-Drug AUC:MIC Ratio Target (72)
					Net Bacterial Stasis (35.6)	1-$\log_{10}$ CFU Reduction From Baseline (67.4)	2-$\log_{10}$ CFU Reduction From Baseline (140)	
Ciprofloxacin	PO	500 mg every 12 hours	Healthy subjects with inflated variance	0.03	100	100	100	100
				0.06	100	100	95.8	100
				0.12	100	96.7	53.6	95.0
				0.25	94.4	53.3	4.16	47.2
				0.5	48.1	5.28	0.02	3.76
				1	3.88	0.04	0	0
				2	0	0	0	0
				4	0	0	0	0
				8	0	0	0	0
Ciprofloxacin	PO	750 mg every 12 hours	Healthy subjects with inflated variance	0.03	100	100	100	100
				0.06	100	100	98.0	100
				0.12	100	98.2	67.3	97.7
				0.25	97.2	67.1	9.08	61.0
				0.5	62.3	10.7	0.20	7.98
				1	8.52	0.30	0	0.140
				2	0.20	0	0	0
				4	0	0	0	0
				8	0	0	0	0
Ciprofloxacin	IV	400 mg every 8 hours	Infected patients	0.03	100	100	100	100
				0.06	100	100	100	100
				0.12	100	100	99.6	100
				0.25	100	99.6	83.9	99.4
				0.5	99.4	86.0	26.6	82.3
				1	82.9	29.8	1.10	24.5
				2	25.5	1.42	0	0.94
				4	0.98	0	0	0
				8	0	0	0	0

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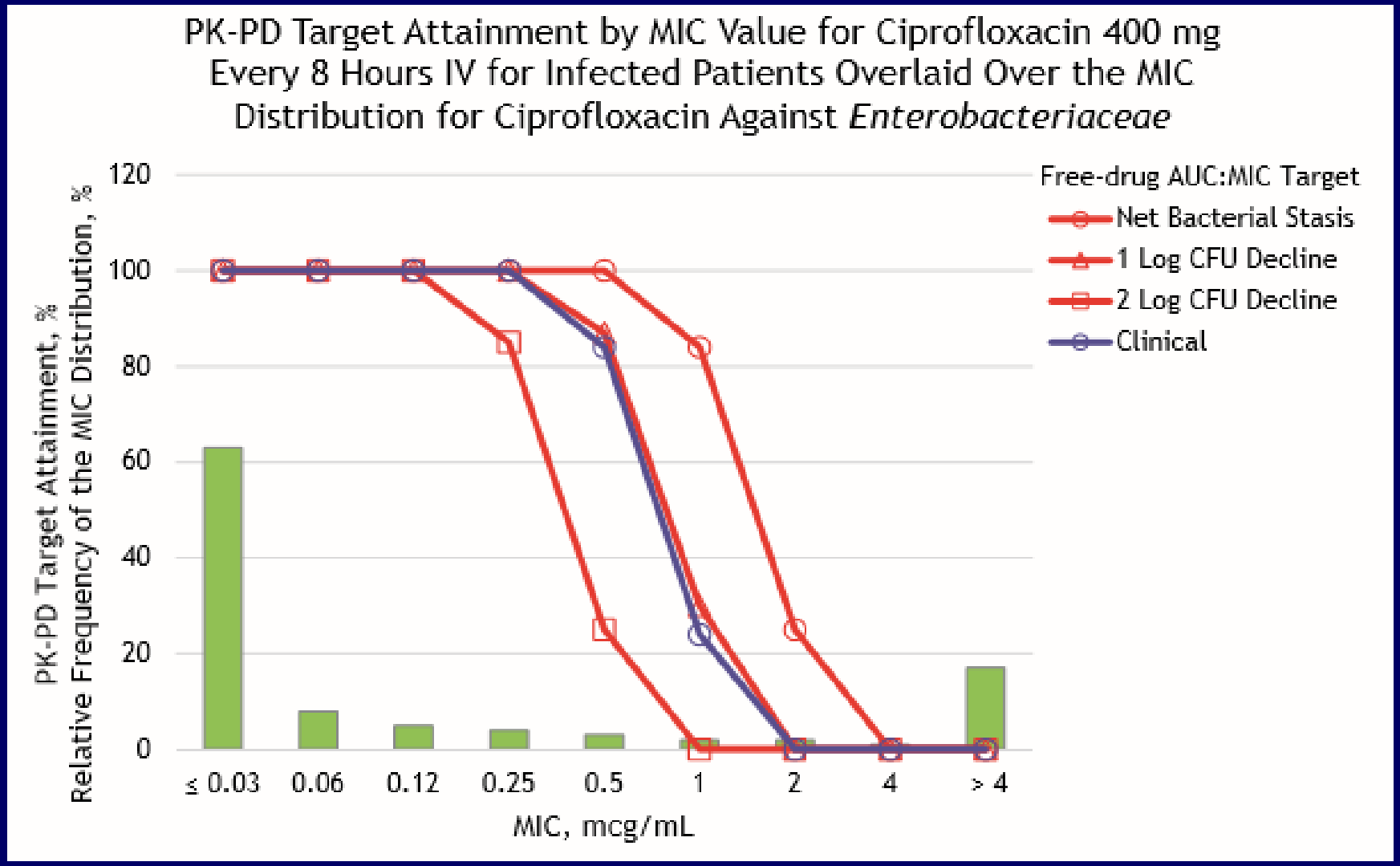
RATIONALE



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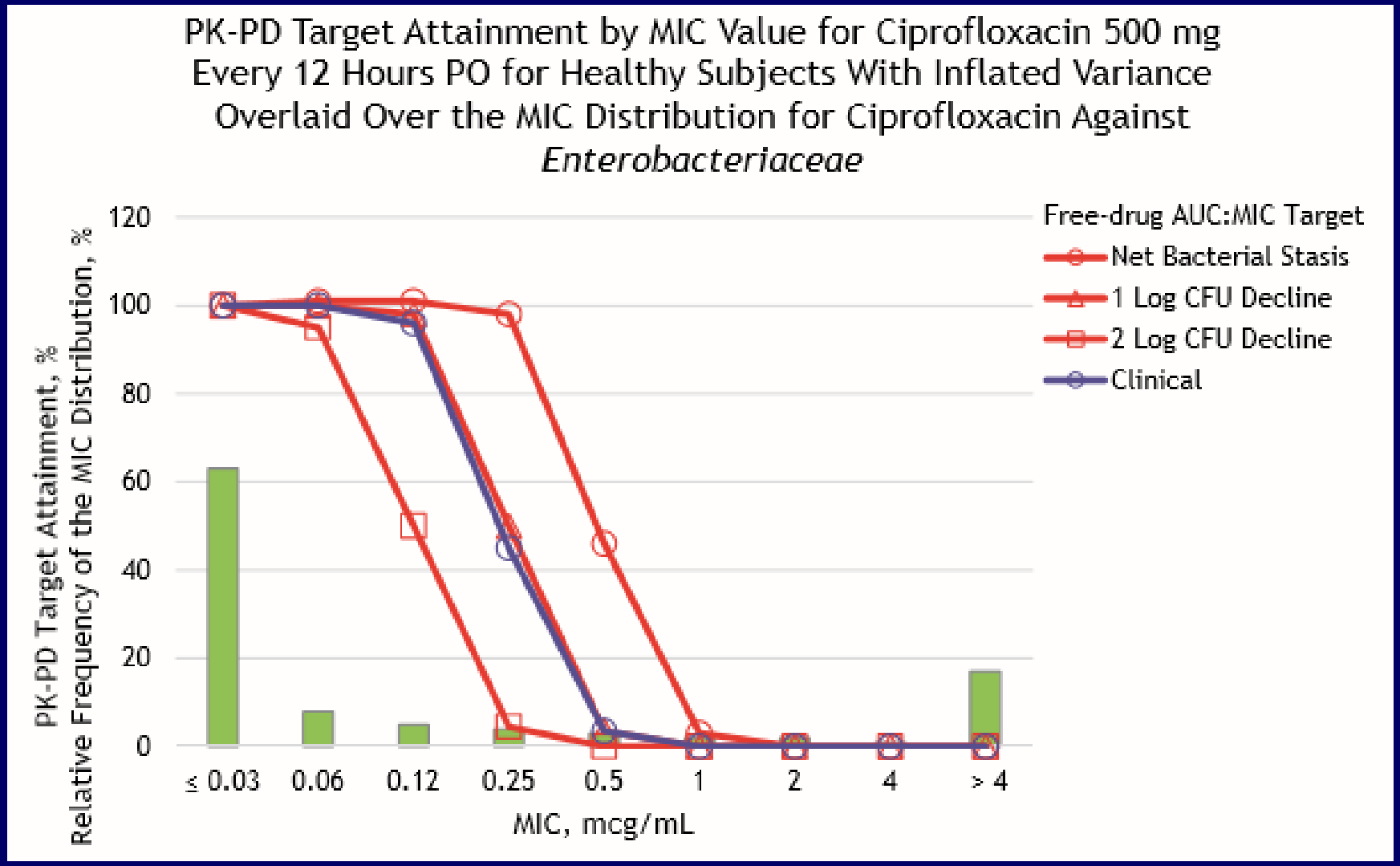
RATIONALE



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Understand AST Breakpoints With Ease

CLSI's annual antimicrobial susceptibility testing (AST) supplement, M100, has been updated and is now available for preorder. This document represents the most current information for drug selection, antimicrobial susceptibility test interpretation, and quality control. The data provided in M100 are relied upon throughout the world by laboratorians, infectious disease practitioners, and AST device manufacturers.

M100 | *Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition*

This document includes updated tables for the Clinical

Support

scroll down a little more…

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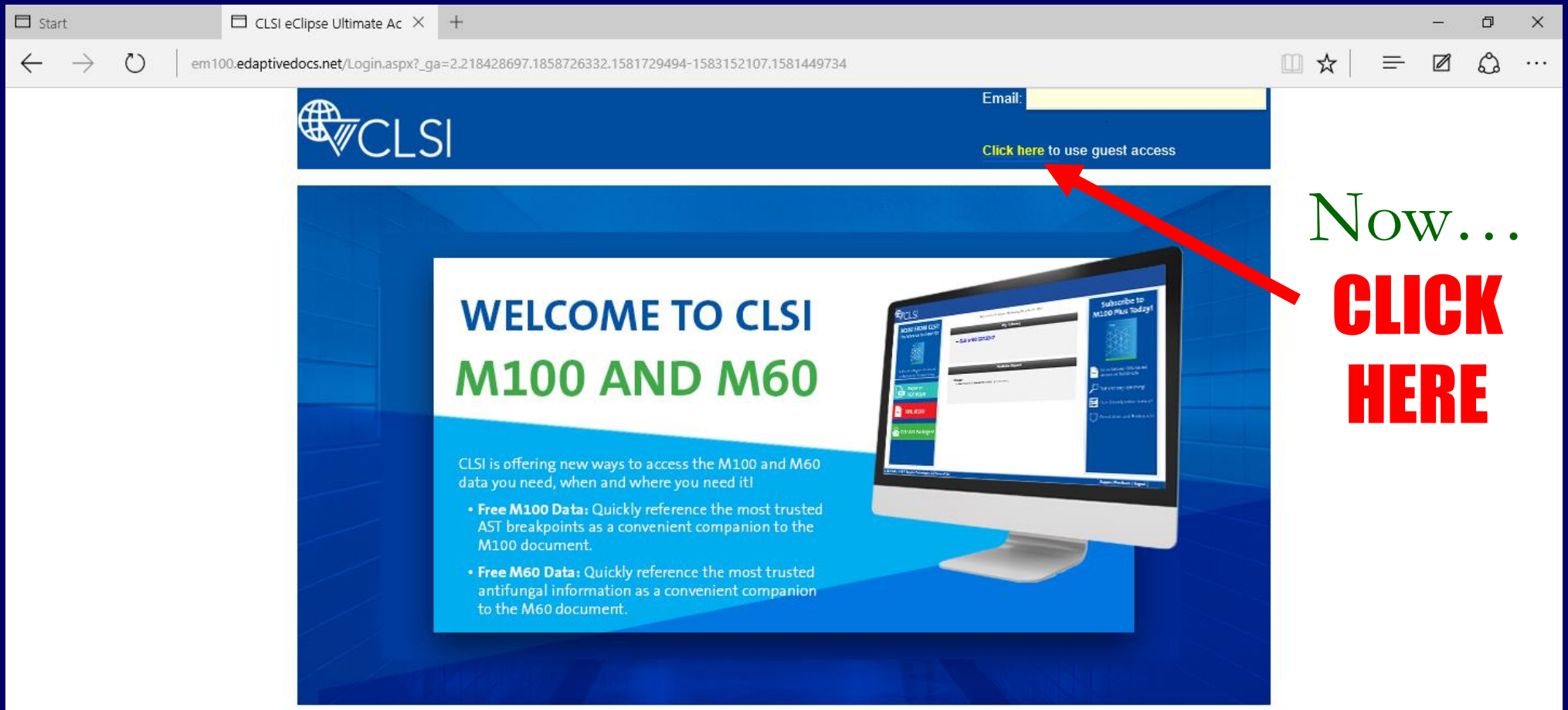
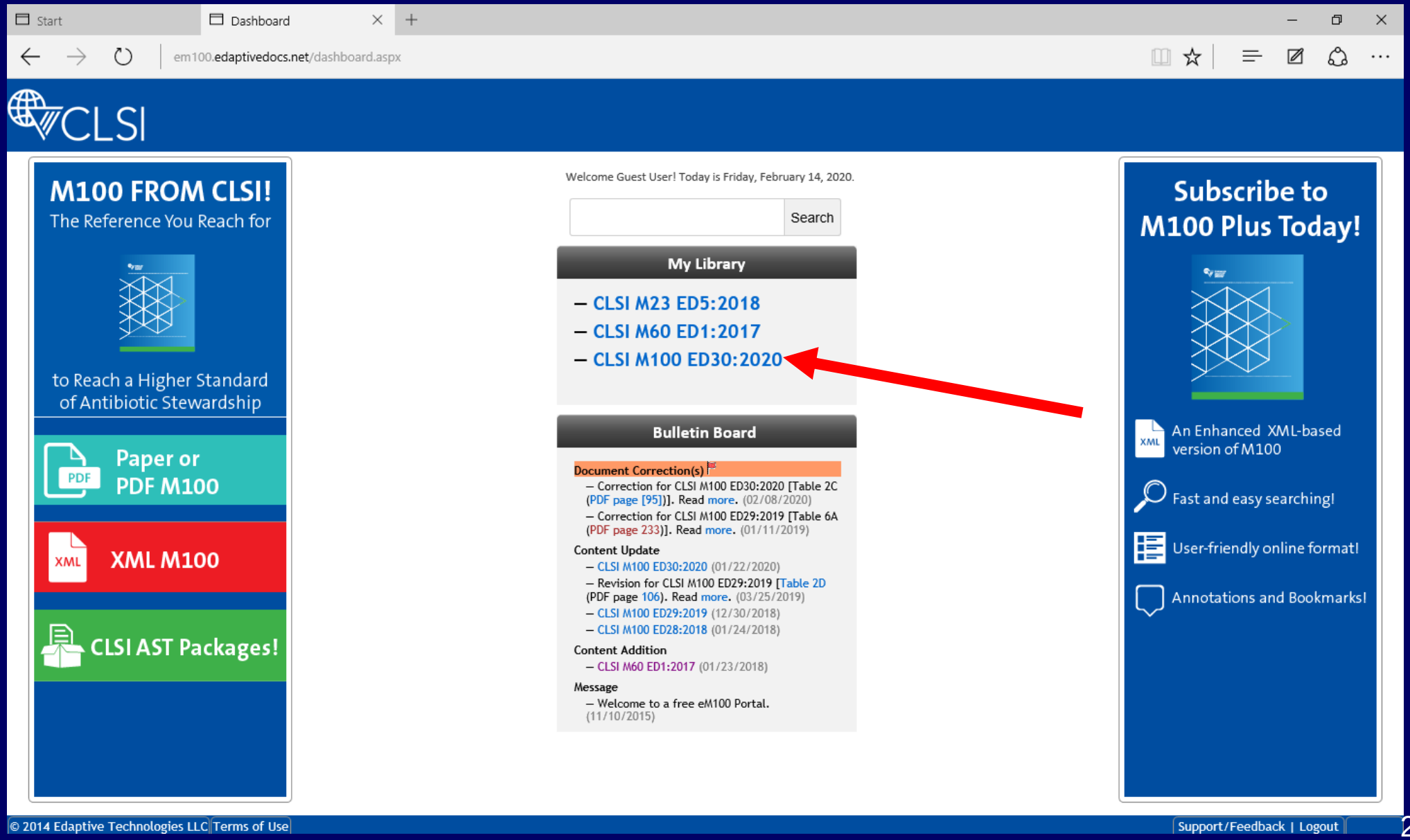


Now…

CLICK HERE

BUT WAIT, THERE'S MORE



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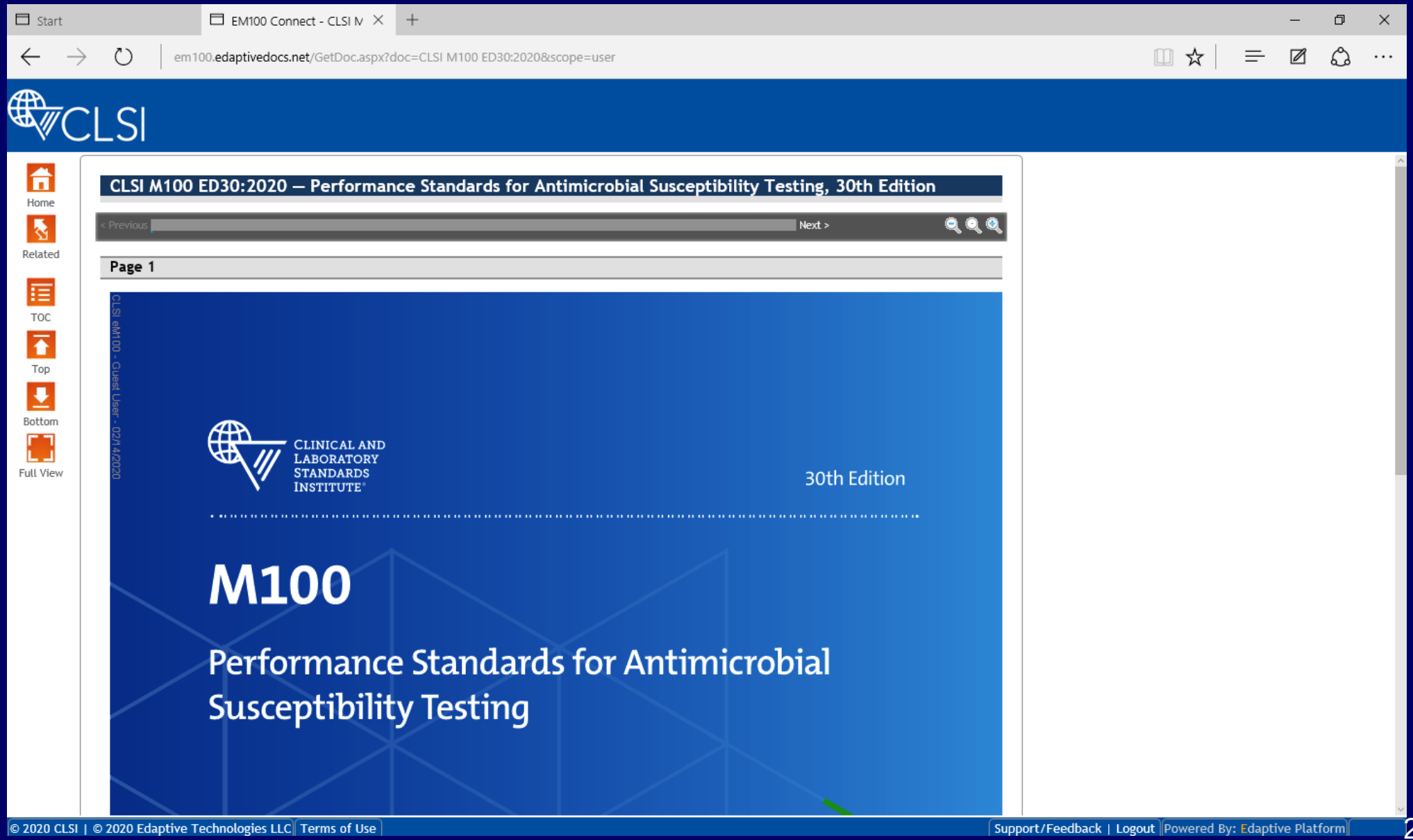
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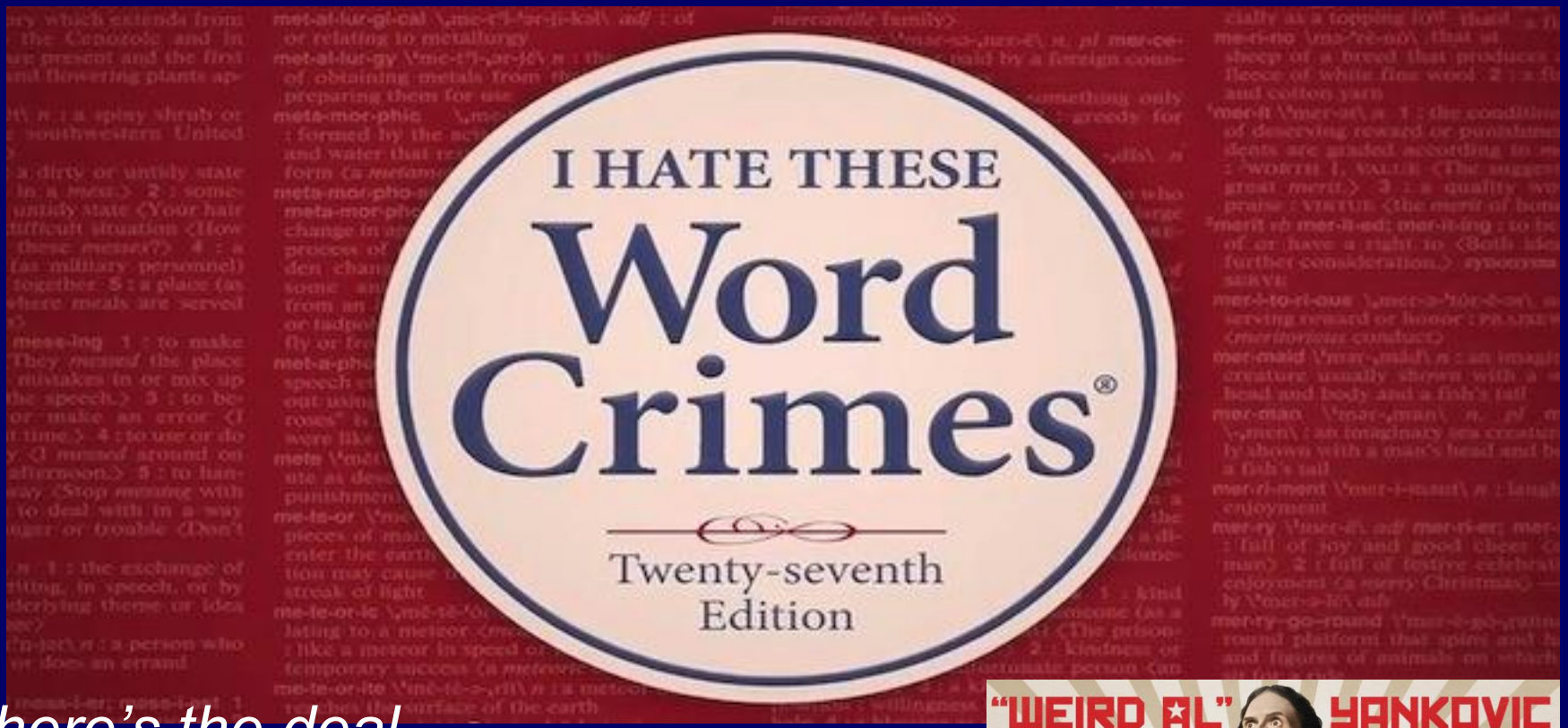

Okay, now here's the deal
I'll try to educate ya
Gonna familiarize
You with the nomenclature
You'll learn the definitions
Of nouns and prepositions
Literacy's your mission

International Journal of Systematic and Evolutionary Microbiology (2016), **66**, 5575–5599 DOI 10.1099/ijsem.0.001485

Genome-based phylogeny and taxonomy of the '*Enterobacteriales*': proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov.

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Enterobacteriaceae

Enterobacterales

Enterobacteriaceae
Erwiniaceae fam. nov.
Pectobacteriaceae fam. nov.
Yersiniaceae fam. nov.**
Hafniaceae fam. nov.**
Morganellaceae fam. nov.**
Budviciaceae fam. nov.

Table 2A. Zone Diameter and MIC Breakpoints for Enterobacterales

Testing Conditions

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

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

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 *Salmonella* enterica ser. Typhi azithromycin disk diffusion testing only; see Table 4A-1)**

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.

OTHER NOMENCLATURE CHANGES

- *Salmonella* Typhi to *Salmonella enterica* ser. Typhi

  Salmonella Paratyphi to
  Salmonella enterica ser. Paratyphi

- Methicillin-resistant to methicillin (oxacillin)-resistant

- Intermediate ranges denoted with ^ in Tables 2 are based on known ability of these agents to concentrate in urine; some can also concentrate in other anatomic sites (epithelial lining); β-lactams, FQ, AG

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NON-*Enterobacterales* (TABLE 2B-5)

Pseudomonas spp., not *Pseudomonas aeruginosa*
Non-fastidious, non-glucose fermentative GNR except:

Acinetobacter spp.
Burkholderia cepacia complex
Stenotrophomonas maltophilia

Aeromonas hydrophila, *Burkholderia pseudomallei*, *Burkholderia mallei*, *Vibrio* spp. (including *V. cholerae*) can be found in CLSI M45

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Instructions for Use of Tables

INSTRUCTIONS FOR USE I

- Susceptible-dose dependent definition modified:

  "…also includes a buffer zone for inherent variability in test methods, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins."

- Intermediate definition modified:

  "…also includes a buffer zone for inherent variability in test methods."

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INSTRUCTIONS FOR USE II

Supplemental Tests (Optional)

Supplemental Test	Organisms	Test Description	Optional for:	Table Location
ESBL	• *E. coli* • *K. pneumoniae* • *Klebsiella oxytoca* • *Proteus mirabilis*	Broth microdilution or disk diffusion clavulanate inhibition test for ESBLs	Isolates demonstrating reduced susceptibility to cephalosporins Results that indicate presence or absence of ESBLs	3A
CarbaNP	• **Enterobacterales** • *P. aeruginosa*	Colorimetric assay for detecting carbapenem hydrolysis	Isolates demonstrating reduced susceptibility to carbapenems Results that indicate presence or absence of certain carbapenemases	3B, 3B-1
mCIM with or without eCIM	• mCIM only: **Enterobacterales** and *P. aeruginosa* • mCIM with eCIM: **Enterobacterales** only	Disk diffusion for detecting carbapenem hydrolysis (inactivation) eCIM add-on enables differentiation of metallo-β-lactamases from serine carbapenemases in **Enterobacterales** isolates that are positive for mCIM	Isolates demonstrating reduced susceptibility to carbapenems Results that indicate presence or absence of certain carbapenemases	3C
Colistin agar test	• **Enterobacterales** • ***P. aeruginosa***	**Modified agar dilution**	**Determining the colistin MIC**	**3D**
Colistin broth disk elution	• **Enterobacterales** • ***P. aeruginosa***	**Tube dilution using colistin disks as antimicrobial agent source**	**Determining the colistin MIC**	**3D**
Oxacillin salt agar	• *S. aureus*	Agar dilution; MHA with 4% NaCl and 6 µg/mL oxacillin	Detecting MRSA; see cefoxitin surrogate agent tests, which are preferred	**3F**

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INSTRUCTIONS FOR USE III

Surrogate Agent Tests

Surrogate Agent	Organisms	Test Description	Results	Table Location
Cefazolin	• *E. coli* • *Klebsiella pneumoniae* • *P. mirabilis*	Broth microdilution or disk diffusion	When used for therapy of uncomplicated UTIs, predicts results for the following oral antimicrobial agents: cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef Cefazolin as a surrogate may overcall resistance to cefdinir, cefpodoxime, and cefuroxime. If cefazolin tests resistant, test these drugs individually if needed for therapy.	1A, 2A
Cefoxitin	• *S. aureus* • *S. lugdunensis* • *S. epidermidis* • Other *Staphylococcus* spp. (excluding *S. pseudintermedius* and *S. schleiferi*)	Broth microdilution: *S. aureus* *S. lugdunensis* Disk diffusion: ***S. aureus*** ***S. lugdunensis*** **Other *Staphylococcus* spp., excluding *S. pseudintermedius* and *S. schleiferi***	Predicts results for *mecA*-mediated **methicillin (oxacillin)** resistance.	1A, 2C, **3F**
Oxacillin	• *S. pneumoniae*	Disk diffusion	Predicts penicillin susceptibility if oxacillin zone is ≥20 mm. If oxacillin zone is ≤19 mm, penicillin MIC must be done.	1B, 2G
Pefloxacin	• *Salmonella* spp.	Disk diffusion	Predicts reduced susceptibility to ciprofloxacin	2A

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Table 1


NEWTON
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TABLE 1A

Table 1A. (Continued)

Group C: Includes alternative or supplemental antimicrobial agents that may require testing in institutions that harbor endemic or epidemic strains resistant to several of the primary drugs, for treatment of patients allergic to primary drugs, for treatment of unusual organisms, or for reporting to infection prevention as an epidemiological aid.			
Enterobacterales	***Pseudomonas aeruginosa***	***Staphylococcus* spp.**	***Enterococcus* spp.[n]**
Aztreonam Ceftazidime		Chloramphenicol[b]	Gentamicin (high-level resistance testing only)
Ceftaroline		Ciprofloxacin or levofloxacin Moxifloxacin	Streptomycin (high-level resistance testing only)
Chloramphenicol[b,d]			Dalbavancin[s,*]
Tetracycline[a]		Gentamicin[m]	Oritavancin[s,*]
		Dalbavancin[l,*]	Telavancin[s,*]
		Oritavancin[l,*]	
		Telavancin[l,*]	
Group U: Includes antimicrobial agents that are used only or primarily for treating UTIs.			
Cefazolin (surrogate test for uncomplicated UTI)[‡]		Nitrofurantoin	Ciprofloxacin Levofloxacin
Fosfomycin[r]		Sulfisoxazole	
Nitrofurantoin		Trimethoprim	Fosfomycin[r]
Sulfisoxazole			Nitrofurantoin
Trimethoprim			Tetracycline[a]
Group A: Includes antimicrobial agents considered appropriate for inclusion in a routine, primary testing panel, as well as for routine reporting of results for the specific organism group.			
***Acinetobacter* spp.**	***Burkholderia cepacia* complex**	***Stenotrophomonas maltophilia***	**Other Non-Enterobacterales[g,*]**
Ampicillin-sulbactam	Levofloxacin[*]	**Levofloxacin**	Ceftazidime
Ceftazidime	Meropenem	**Minocycline**	Gentamicin
Ciprofloxacin Levofloxacin	Trimethoprim-sulfamethoxazole	Trimethoprim-sulfamethoxazole	Tobramycin
Doripenem Imipenem Meropenem			
Gentamicin Tobramycin			

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TABLE 1B

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

Group A: Includes antimicrobial agents considered appropriate for inclusion in a routine, primary testing panel, as well as for routine reporting of results for the specific organism group.				
Haemophilus influenzae*[e] and *Haemophilus parainfluenzae	***Neisseria gonorrhoeae*[j]**	***Streptococcus pneumoniae*[k]**	***Streptococcus* spp. β-Hemolytic Group[q]**	***Streptococcus* spp. Viridans Group[q]**
Ampicillin[e,g]	Azithromycin[*,†]	Erythromycin[a,c]	Clindamycin[c,p]	Ampicillin[n,*] Penicillin[n,*]
	Ceftriaxone[†] Cefixime[†]			
	Ciprofloxacin[†]	Penicillin[l] (oxacillin disk)	Erythromycin[a,c,p]	
	Tetracycline[b,†]	Trimethoprim-sulfamethoxazole	Penicillin[o,†] or ampicillin[o,†]	
Group B: Includes antimicrobial agents that may warrant primary testing but may be reported only selectively, such as when the organism is resistant to agents of the same antimicrobial class, as in Group A.[d]				

p. ***Rx:*** Recommendations for intrapartum prophylaxis for group B streptococci are penicillin or ampicillin. Although cefazolin is recommended for penicillin-allergic women at low risk for anaphylaxis, those at high risk for anaphylaxis may receive clindamycin. Group B streptococci are susceptible to ampicillin, penicillin, and cefazolin, but may be resistant to erythromycin and clindamycin. When group B *Streptococcus* is isolated from a pregnant woman with severe penicillin allergy (high risk for anaphylaxis), erythromycin and clindamycin (including inducible clindamycin resistance [ICR]) should be tested, and only clindamycin should be reported. **Erythromycin, even when tested for determination of ICR, should not be reported.** See Table 3H.

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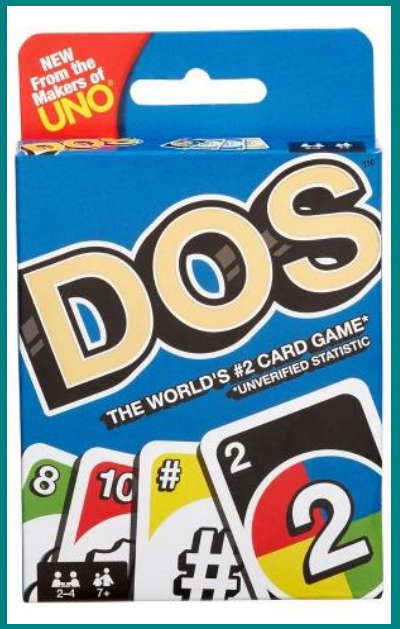
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*UNVERIFIED STATISTIC

Table 2

TABLE 2--CEFIDEROCOL

- Iron-depleted cation-adjusted MH broth for broth microdilution
- Test/report group “Investigational”

Organism	Disk Diffusion (30 μg)			Broth Microdilution		
	S	I	R	S	I	R
Enterobacteriales	**≥ 16**	**12-15 ^**	**≤ 11**	≤ 4	8 ^	≥ 16
P. aeruginosa	**≥ 18**	**13-17 ^**	**≤ 12**	≤ 4	8 ^	≥ 16
Acinetobacter spp.	**≥ 15**	**11-14**	**≤ 10**	≤ 4	8	≥ 16
S. maltophilia	**≥ 17**	**13-16**	**≤ 12**	≤ 4	8	≥ 16

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TABLE 2--POLYMYXINS

- Broth microdilution methods; no disk diffusion
- Test/report group O

Includes antimicrobial agents that have a clinical indication for the organism group, but are generally not candidates for routine testing and reporting in the United States

Organism	Colistin			Polymyxin B		
	S	I	R	S	I	R
Enterobacteriales		≤ 2 ^	≥ 4		≤ 2	≥ 4
P. aeruginosa		≤ 2	≥ 4		≤ 2	≥ 4
Acinetobacter spp.		≤ 2	≥ 4		≤ 2	≥ 4

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TABLE 2--POLYMYXINS

LIPOPEPTIDES

(36) WARNING: Clinical and PK-PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained. Alternative agents are strongly preferred. Colistin and polymyxin B should be used in combination with one or more active antimicrobial agents. Consultation with an infectious diseases specialist is recommended.

(37) Several species are intrinsically resistant to the lipopeptides (colistin and polymyxin B). Refer to Appendix B.

O	Colistin or polymyxin B		– –	– –	– –	– –	– –	– –	≤2^ ≤2	≥4 ≥4	(38) Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see International Consensus Guidelines[8]). (39) Polymyxin B should be given with a loading dose and maximum recommended doses (see International Consensus Guidelines[8]). (40) When colistin or polymyxin B is given systemically, neither is likely to be effective for pneumonia. (41) For colistin, broth microdilution, CBDE, and CAT MIC methods are acceptable. For polymyxin B, broth microdilution is the only approved method. Disk diffusion and gradient diffusion methods should not be performed (see Table 3D).

AMINOGLYCOSIDES

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TABLE 2 (and more)--NORFLOXACIN

- Reinstated norfloxacin disk diffusion and MIC breakpoints for testing and reporting urinary tract isolates
- Test/report group O

Organism	Disk Diffusion (10 μg)			Broth Microdilution		
	S	I	R	S	I	R
Enterobacterales	≥ 17	13-16	≤ 12	≤ 4	8	≥ 16
P. aeruginosa	≥ 17	13-16	≤ 12	≤ 4	8	≥ 16
Non-*Enterobacterales*				≤ 4	8	≥ 16
Staphylococcus spp.	≥ 17	13-16	≤ 12	≤ 4	8	≥ 16
Enterococcus spp.	≥ 17	13-16	≤ 12	≤ 4	8	≥ 16

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TABLE 2C

Organism	Methods for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp.				
	Cefoxitin MIC	Cefoxitin disk diffusion	Oxacillin MIC	Oxacillin disk diffusion	Oxacillin salt agar
S. aureus	Yes **(16–20 h)**	Yes **(16–18 h)**	Yes **(24 h)**	No	Yes **(24 h)**
S. lugdunensis	Yes **(16–20 h)**	Yes **(16–18 h)**	Yes **(24 h)**	No	No
S. epidermidis	No	Yes **(16–18 h)**	Yes **(24 h)**	Yes **(16–18 h)**	No
S. pseudintermedius	No	No	Yes **(24 h)**	Yes **(16–18 h)**	No
S. schleiferi	No	No	Yes **(24 h)**	Yes **(16–18 h)**	No
Other *Staphylococcus* spp. (not listed above)	No	Yes[a] **(24 h)**	Yes[a] **(24 h)**	No	No

Abbreviations: h, hour(s); MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant staphylococci; PBP2a, penicillin-binding protein 2a.

[a] For isolates of "other *Staphylococcus* spp." from serious infections for which the oxacillin MICs are 0.5–2 µg/mL, testing for *mecA* or PBP2a should be considered (see comment [17]). Cefoxitin disk diffusion is not currently recommended.

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TABLE 3F SPOILER ALERT

Table 3F. Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp.

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin			Detecting *mecA*-Mediated Resistance Using Oxacillin			Detecting *mecA*-mediated Resistance Using Oxacillin Salt Agar
Test method	**Disk Diffusion**		**Broth Microdilution**	**Disk Diffusion**	**Broth Microdilution and Agar Dilution**		**Agar Dilution**
Organism group	*S. aureus* and *S. lugdunensis*	Other *Staphylococcus* spp. (excluding *S. pseudintermedius* and *S. schleiferi*)	***S. aureus* and *S. lugdunensis***	***S. epidermidis, S. pseudintermedius,* and *S. schleiferi***	***S. aureus* and *S. lugdunensis***	***Staphylococcus* spp. (excluding *S. aureus* and *S. lugdunensis*)**	*S. aureus*
Medium	MHA		CAMHB	**MHA**	**CAMHB with 2% NaCl (broth microdilution) MHA with 2% NaCl (agar dilution)**		MHA with 4% NaCl
Antimicrobial concentration	30 µg cefoxitin disk		4 µg/mL cefoxitin	**1-µg oxacillin disk**	**2 µg/mL oxacillin**	**0.25 µg/mL oxacillin**	6 µg/mL oxacillin
Inoculum	Standard disk diffusion procedure		Standard broth microdilution Procedure	**Standard disk diffusion procedure**	**Standard broth microdilution procedure or standard agar dilution procedure**		Colony suspension to obtain 0.5 McFarland turbidity Using a 1-µL loop that was dipped in the suspension, spot an area 10–15 mm in diameter. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot a similar area or streak an entire quadrant.
Incubation conditions	33 to 35°C; ambient air[a]		33 to 35°C; ambient air[a]	**33 to 35°C; ambient air[a]**	**33 to 35°C; ambient air[a]**		33 to 35°C; ambient air[a]
Incubation length	16–18 hours	24 hours (may be reported after 18 hours, if resistant)	16–20 hours	**16–18 hours**	**24 hours (may be reported after 18 hours, if resistant)**		24 hours; read with transmitted light
Results	≤ 21 mm = *mecA* positive ≥ 22 mm = *mecA* negative	≤ 24 mm = *mecA* positive ≥ 25 mm = *mecA* negative	≥ 8 µg/mL =*mecA* positive ≤ 4 µg/mL = *mecA* negative	**≤ 17 mm = *mecA* positive ≥ 18 mm = *mecA* negative**	**≥4 µg/mL = *mecA* positive ≤ 2 µg/mL = *mecA* negative**	**≥ 0.5 µg/mL = *mecA* positive ≤ 0.25 µg/mL = *mecA* negative**	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = oxacillin resistant

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TABLE 2C

- Oxacillin MIC breakpoints may overcall resistance

  Some isolates for which oxacillin MIC is 0.5-2.0 μg/mL may be *mecA*-negative

  May test such isolates from serious infections for *mecA* or PBP2a

  Negative results should be reported as oxacillin (methicillin) S

- Erythromycin R / clindamycin I or S

(30) Inducible clindamycin resistance can be detected by disk diffusion using the D-zone test or by broth microdilution (see Table 3G, Subchapter 3.9 in M02,[1] and Subchapter 3.12 in M07[2]).

See comment (26). **2019**

(30) For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR by disk diffusion using the D-zone test or by broth microdilution is required before reporting clindamycin (see Table 3H, Subchapter 3.9 in M02,[1] and Subchapter 3.12 in M07[3]). **2020**

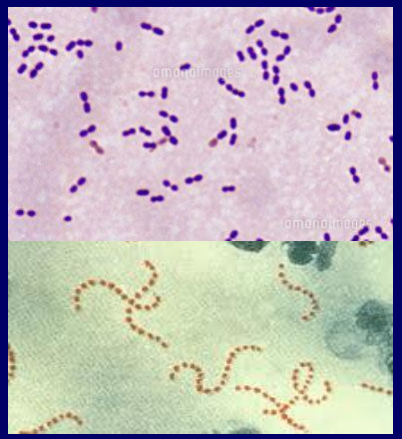

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TABLE 2D

susceptible-dose dependent

LIPOPEPTIDES										
B	Daptomycin *E. faecium* only	–	–	–	–	–	≤ 4	–	≥ 8	(12) Daptomycin should not be reported for isolates from the respiratory tract. (13) The breakpoint for SDD is based on a dosage regimen of 8–12 mg/kg administered every 24 h and is intended for serious infections due to *E. faecium*. Consultation with an infectious diseases specialist is recommended.
B	Daptomycin *Enterococcus* spp. other than *E. faecium*	–	–	–	–	≤2	–	4	≥ 8	(14) The breakpoint for susceptible is based on a dosage regimen of 6 mg/kg administered every 24 h. See comment (12).

originally included in the March 2019 re-released version of M100, 29th ed.

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TABLE 2G

Table 2G
Streptococcus pneumoniae
M02 and M07

Table 2G. Zone Diameter and MIC Breakpoints for *Streptococcus pneumoniae*

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood **or MH-F agar (MHA with 5% defibrinated horse blood and 20 µg/mL NAD)**
Broth dilution: CAMHB with LHB (2.5% to 5% v/v) (see M07[1] for instructions for preparation of LHB)
Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard, prepared using colonies from an overnight (18- to 20-hour) sheep blood agar plate

Incubation: 35°C ± 2°C
Disk diffusion: 5% CO_2; 20–24 hours
Dilution methods: ambient air; 20–24 hours (CO_2 if necessary, for growth with agar dilution)

Routine QC Recommendations (see Tables 4B and 5B for acceptable QC ranges)

S. pneumoniae ATCC®[a] 49619

Disk diffusion: deterioration of oxacillin disk content is best assessed with *S. aureus* ATCC® 25923, with an acceptable range of 18–24 mm on unsupplemented MHA.

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

(5) For disk diffusion, results using MHA with 5% sheep blood and MH-F agar were equivalent when disk contents, testing conditions, and zone diameter breakpoints in Table 2G were used. Disk diffusion QC ranges for *S. pneumoniae* ATCC® 49619 in Table 4B apply to testing using either MHA with 5% sheep blood or MH-F agar.

oral cefuroxime results may be interpreted for isolates other than those from CSF

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Table 3

QC CHANGE FOR ESBL (TABLE 3A)

Table 3A
Tests for ESBLs

Table 3A. (Continued)

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
QC recommendations	When testing antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC®b 700603 is provided as a supplemental QC strain (eg, for training, competence assessment, or test evaluation). Either strain, *K. pneumoniae* ATCC® 700603 or *E. coli* ATCC® 25922, may then be used for routine QC (eg, weekly or daily). *E. coli* ATCC® 25922 (see acceptable QC ranges in Table 4A-1) *K. pneumoniae* ATCC® 700603: Cefpodoxime zone 9–16 mm Ceftazidime zone 10–18 mm Aztreonam zone **10–16** mm Cefotaxime zone 17–25 mm Ceftriaxone zone 16–24 mm	When testing antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC® 700603 is provided as a supplemental QC strain (eg, for training, competence assessment, or test evaluation). Either strain, *K. pneumoniae* ATCC® 700603 or *E. coli* ATCC® 25922, may then be used for routine QC (eg, weekly or daily). *E. coli* ATCC® 25922 = no growth (see acceptable QC ranges listed in Table 5A-1) *K. pneumoniae* ATCC® 700603 = Growth: Cefpodoxime MIC ≥8 µg/mL Ceftazidime MIC ≥2 µg/mL Aztreonam MIC ≥2 µg/mL Cefotaxime MIC ≥2 µg/mL Ceftriaxone MIC ≥2 µg/mL	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be used for routine QC (eg, weekly or daily). **Acceptable QC:** *E. coli* ATCC® 25922: ≤2-mm increase in zone diameter for antimicrobial agent tested in combination with clavulanate vs the zone diameter when tested alone. *K. pneumoniae* ATCC® 700603: ≥5-mm increase in zone diameter of ceftazidime-clavulanate vs ceftazidime alone; ≥3-mm increase in zone diameter of cefotaxime-clavulanate vs cefotaxime alone.	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be tested routinely (eg, weekly or daily). **Acceptable QC:** *E. coli* ATCC® 25922: <3 twofold concentration decrease in MIC for antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone. *K. pneumoniae* ATCC® 700603: ≥3 twofold concentration decrease in MIC for an antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone.

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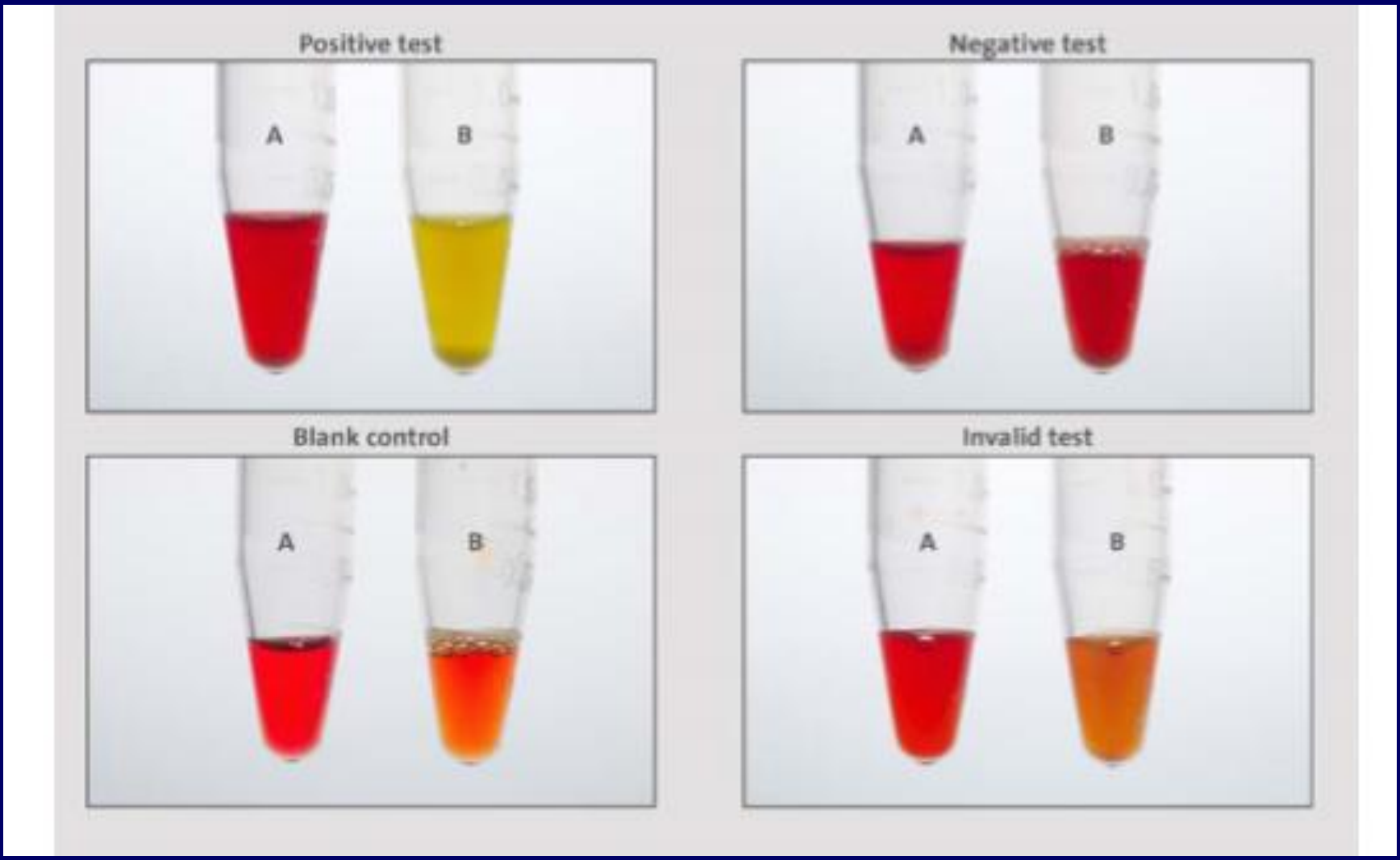
CarbaNP TESTING (TABLE 3B)



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CarbaNP TESTING (TABLE 3B)

- Test recommendations largely derived from testing US isolates of *Enterobacterales* and *P. aeruginosa* and provide >90% sensitivity and >90% specificity for detection of the following carbapenemases:

KPC	NDM	VIM
IMP	SPM	SME

- Ability of this test to detect OXA-48-like producers is poor

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BRAND NEW TABLE (TABLE 3D)

- Colistin testing — broth microdilution
  broth disk elution
  agar dilution

  Polymyxin B testing — broth microdilution

- Colistin and polymyxin B are equivalent agents

  Colistin MIC predict polymyxin MIC (vice versa)
  CLSI has not evaluated polymyxin B methods *per se*
  NO GO for *Acinetobacter* spp.

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BRAND NEW TABLE (TABLE 3D)

Testing multi-drug-resistant isolates for clinical or infection prevention purposes

Parameter	Colistin Broth Disk Elution	Colistin Agar Test
Approved organisms	*Enterobacterales* *Pseudomonas aeruginosa*	*Enterobacterales* *Pseudomonas aeruginosa*
Strengths	No special reagents or media	Test up to 10 at once
Limitations	Hands-on time/cost	Requires special media** (colistin)
Medium	Cation-adjusted MHB (10-mL tubes)	Mueller Hinton Agar** (100 mm)
Antimicrobial	10-μg colistin disks	Special prepared media**
Desired [colistin]	0 μg/mL (growth control), 1 μg/mL, 2 μg/mL, 4 μg/mL	
Inoculum	0.5 McFarland; 50 μL delivery	0.5 McFarland; 1:10 dilution; streak
Incubation	33-35ºC, ambient air; 16-20 hours	

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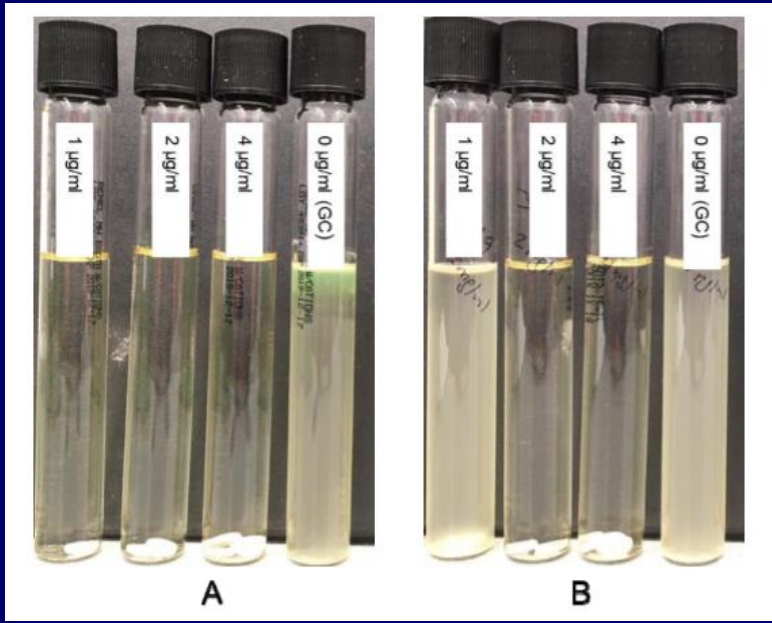
BRAND NEW TABLE (TABLE 3D)

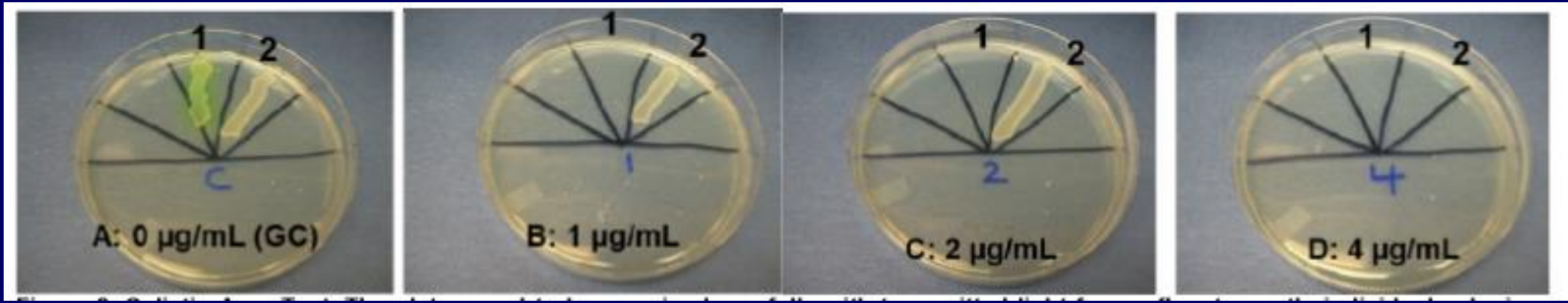


Colistin broth disk elution (CBDE)

QC Recommendations

P. aeruginosa ATCC 27853 (isolate A, 1)
E. coli AR Bank #0349 *mcr-1* (isolate B, 2)

Colistin agar test (CAT)



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INDUCIBLE CLINDAMYCIN (3H)

- All *Staphylococcus* spp.
  Streptococcus pneumoniae
  β-hemolytic *Streptococcus* spp.
- Report as clindamycin resistant

  "This isolate is presumed to be resistant based on detection of ICR, as determined by testing clindamycin in combination with erythromycin."

  ["This group B *Streptococcus* does not demonstrate inducible clindamycin resistance as determined by testing clindamycin in combination with erythromycin."]

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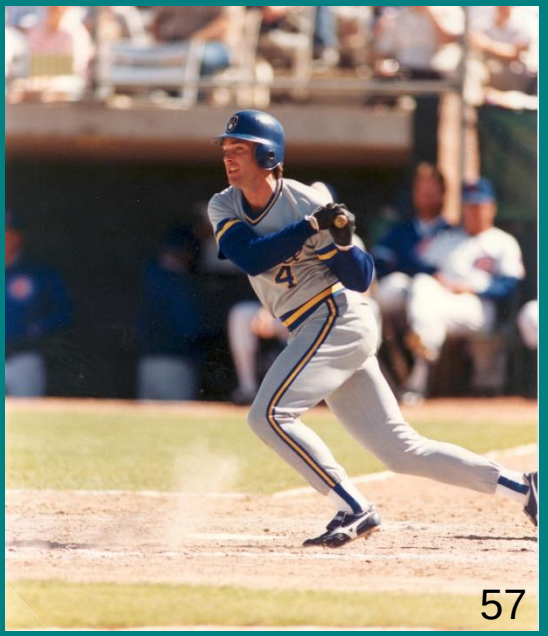
Table 4



DISK DIFFUSION QC RANGES

- Noteworthy additions

  E. faecalis ATCC 29212 for tedizolid
  Norfloxacin for all previous M100, 29th ed. deletions
  Use of MH-F agar for *S. pneumoniae* (only)

- Noteworthy revisions

  E. coli ATCC 25922 for ciprofloxacin (29-38 mm)
  S. pneumoniae ATCC 49619 for tedizolid

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DISK DIFFUSION QC ADDED RANGES

E. coli ATCC 25922	sulopenem cefepime-enmetazobactam cefepime-taniborbactam sulbactam-durlobactam
P. aeruginosa ATCC 27853	cefepime-enmetazobactam cefepime-taniborbactam
K. pneumoniae ATCC 700603	cefepime-enmetazobactam cefepime-taniborbactam
E. coli NCTC 13353	cefepime-enmetazobactam cefepime-taniborbactam
K. pneumoniae ATCC BAA-1705	cefepime-taniborbactam
E. coli ATCC 35218	cefepime cefepime-enmetazobactam cefepime-taniborbactam
A. baumannii NCTC 13304	sulbactam-durlobactam

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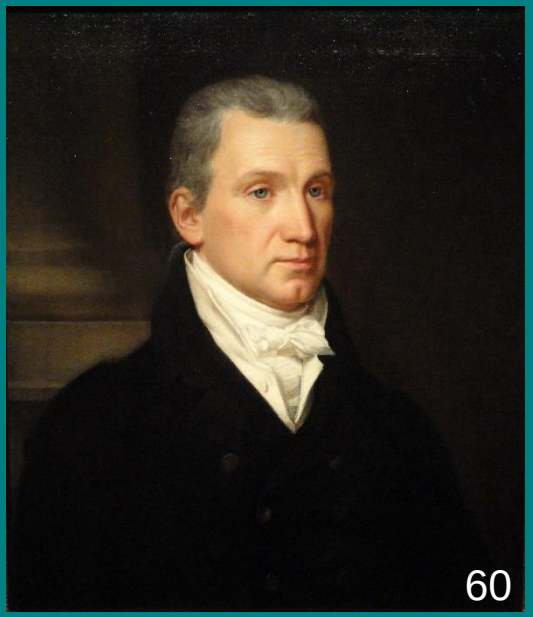
Table 5



MIC QC RANGES

- Noteworthy additions

  Exebacase, ozenoxacin, zoliflodacin for 29213, 29212
  Zoliflodacin for 49226 (agar dilution), 49247, 49619
  Ozenoxacin for 49619
  Norfloxacin for all previous M100, 29th ed. deletions

- Noteworthy revisions

  ATCC BAA-2814 range for imipenem-relebactam
  ATCC 25922 range for eravacycline

- Noteworthy deletion

  ATCC 29212 range for plazomicin

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MIC QC ADDED RANGES

E. coli ATCC 25922	zoliflodacin; sulbactam; durlobactam cefepime-enmetazobactam cefepime-taniborbactam
P. aeruginosa ATCC 27853	cefepime-enmetazobactam cefepime-taniborbactam
E. coli ATCC 35218	cefepime-enmetazobactam cefepime-taniborbactam
K. pneumoniae ATCC 700603	sulbactam cefepime-enmetazobactam cefepime-taniborbactam
E. coli NCTC 13353	cefepime-enmetazobactam cefepime-taniborbactam
K. pneumoniae ATCC BAA-1705	cefepime-taniborbactam
A. baumannii NCTC 13304	sulbactam; durlobactam sulbactam-durlobactam

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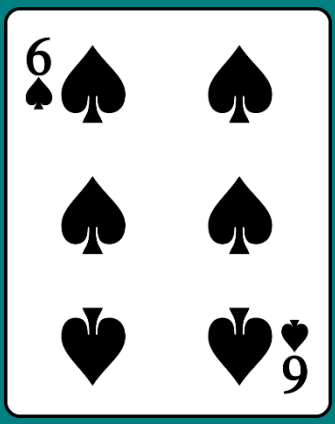
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Tables 6-8


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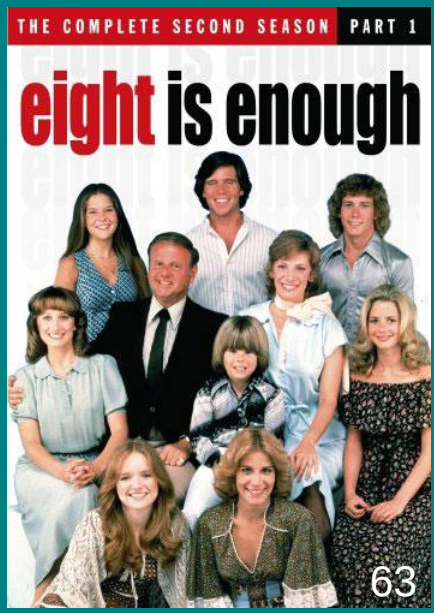
THE COMPLETE SECOND SEASON
PART 1
eight is enough

TABLE 6 PREPARING STOCK SOLNS

- Added solvent and diluent information for:

  Durlobactam
  Enmetazobactam
  Exebacase
  Ozenoxacin
  Taniborbactam
  Zoliflodacin

- Prep instructions for:

  Cefepime-enmetazobactam
  Cefepime-taniborbactam
  Sulbactam-durlobactam

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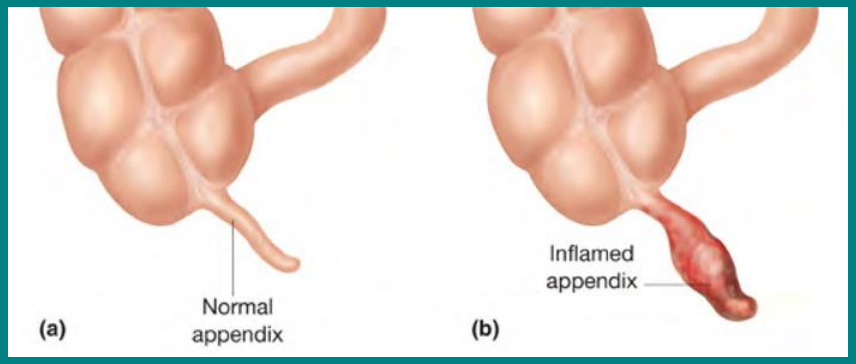

Appendices

NEW APPENDIX I

Appendix I. Cefiderocol Broth Preparation and Reading Broth Microdilution Minimal Inhibitory Concentration End Points

Abbreviations for Appendix I

CAMHB	cation-adjusted Mueller-Hinton broth
ID-CAMHB	iron-depleted cation-adjusted Mueller-Hinton broth
pH	negative logarithm of hydrogen ion concentration

I1. Supplements

I1.1 Calcium and Magnesium Stock Solutions

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

I1.2 Zinc Stock Solution

The steps for preparing zinc stock solution are listed below.

Step	Action	Comment
1	Dissolve 0.29 g $ZnSO_4 \cdot 7H_2O$ in 100 mL deionized water.	This solution contains 10 mg Zn^{++}/mL.
2	Sterilize the solution by membrane filtration.	
3	Store the solution at 15 to 25°C.	

I2. Iron-depleted Cation-adjusted Mueller-Hinton Broth

The steps for preparing iron-depleted cation-adjusted Mueller-Hinton broth (ID-CAMHB) are listed below.[2]

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NEW APPENDIX I

Appendix I. (Continued)

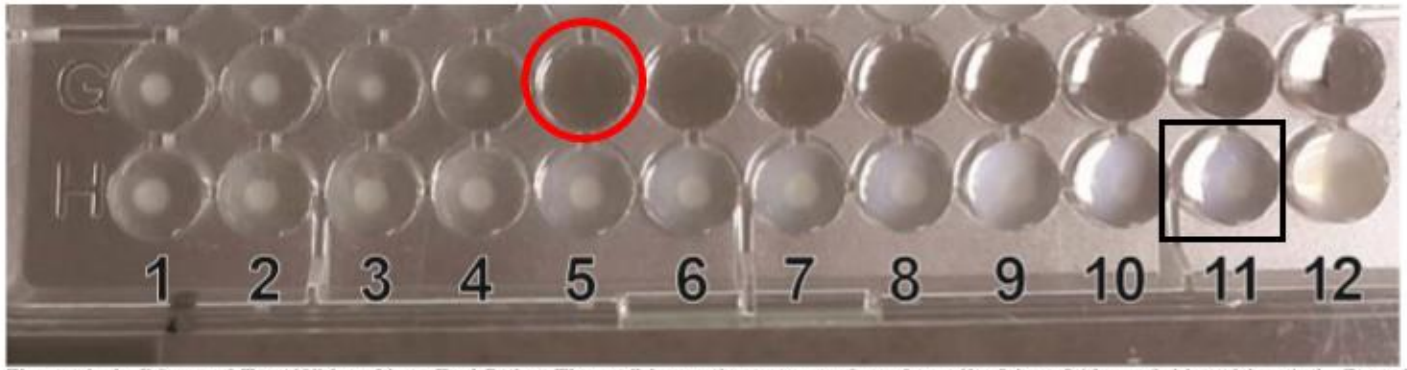

Figure 1. Cefiderocol Test With a Clear End Point. The cefiderocol concentrations in wells G1 to G12 are 0.03 to 64 µg/mL. Row G shows the cefiderocol MIC at 0.5 µg/mL in well G5 (red circle). The growth-control well is H11 (black box). (Courtesy of Meredith M. Hackel, International Health Management Associates. Used with permission.)

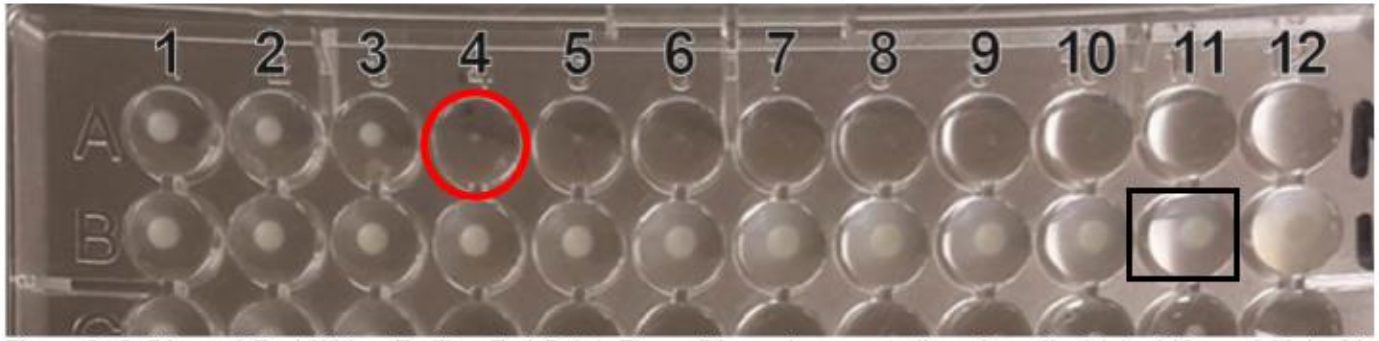

Figure 2. Cefiderocol Test With a Trailing End Point. The cefiderocol concentrations in wells A1 to A12 are 0.03 to 64 µg/mL. Row A shows the cefiderocol MIC at 0.25 µg/mL in well A4 (red circle). The growth control well is B11 (black box). (Courtesy of Meredith M. Hackel, International Health Management Associates. Used with permission.)

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"2020 AST Conference"

Tuesday April 7, 2020

AGENDA

- Keynote address
- Stewardship panel
- Review of automated systems, antibiograms
- Breakout sessions
- Free food


Thank you for your attention.
Have a good 2020.