2022 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 literature reviews relative to major revisions

II. Objectives of webinar

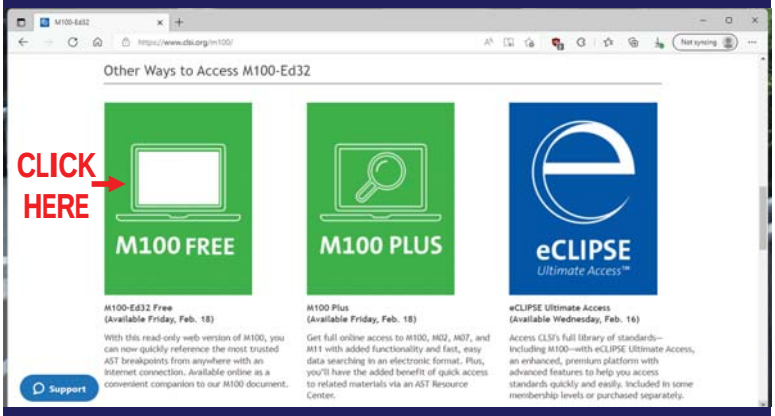
Describe significant changes relevant to pre-existing antimicrobial susceptibility breakpoints…

Describe significant changes relevant to antimicrobial susceptibility testing methodology…

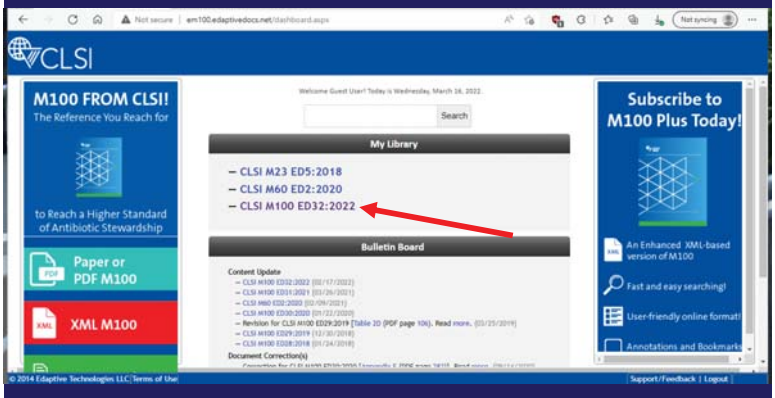
Identify (new) organism/antimicrobial combinations for which susceptibility breakpoints now exist…

as outlined in the CLSI M100-Ed32 document.

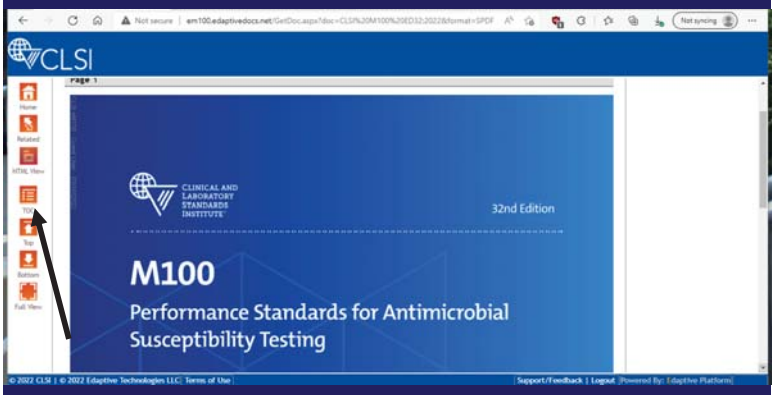
clsi.org/m100, then scroll down a bit…



WOW!!



VOILA!!



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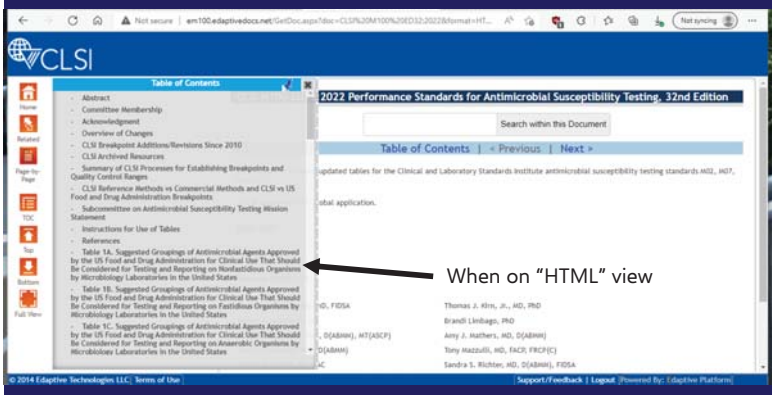
FUTZING WITH THIS…A LITTLE



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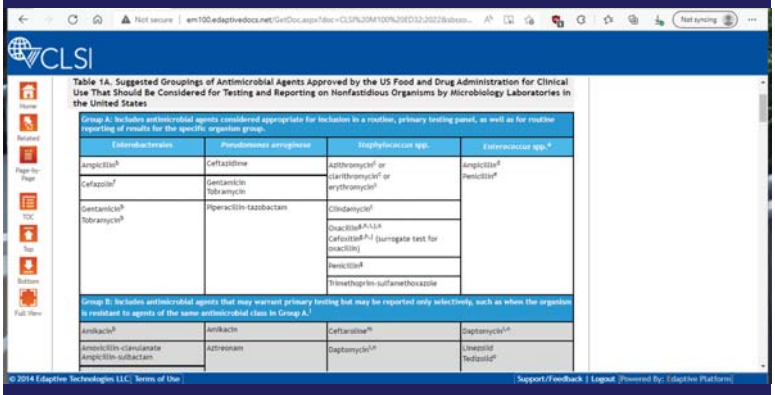
FUTZING WITH THIS…A LITTLE



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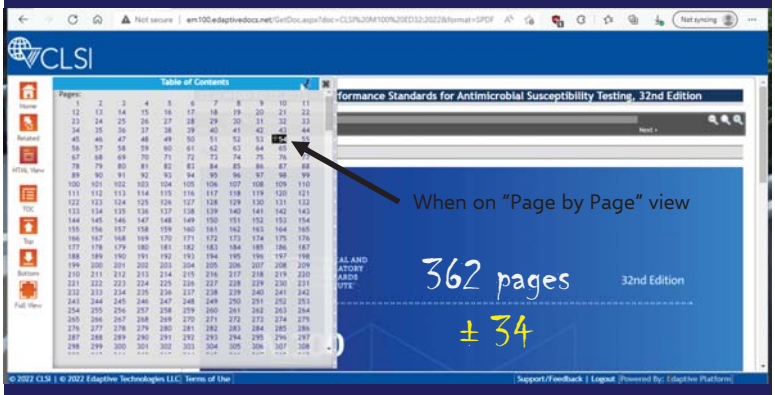
FUTZING WITH THIS…MORE



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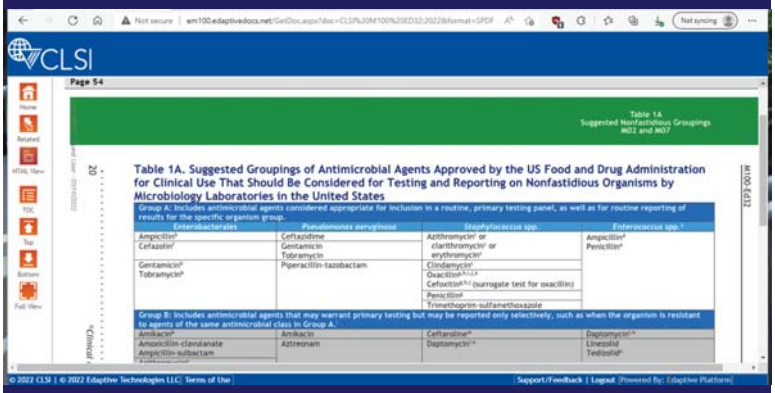
FUTZING WITH THIS…MORE



CLSI

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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

Enterobacterales	*Pseudomonas aeruginosa*	*Staphylococcus* spp.	*Enterococcus* spp.
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.			
Ampicillin[a] Cefazolin[a]	Ceftazidime Gentamicin Tobramycin	Azithromycin[a] or clarithromycin[a] or erythromycin[a]	Ampicillin[a] Penicillin[a]
Gentamicin[b] Tobramycin[b]	Piperacillin-tazobactam	Clindamycin[a] Oxacillin[a,b,c,d] Cefoxitin[a,b,c] (surrogate test for oxacillin) Penicillin[a] Trimethoprim-sulfamethoxazole	
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 in Group A.			
Amikacin[a] Amoxicillin-clavulanate Ampicillin-sulbactam	Amikacin Aztreonam	Ceftaroline[a] Daptomycin[c,e]	Daptomycin[c,e] Linezolid Tedizolid[a]

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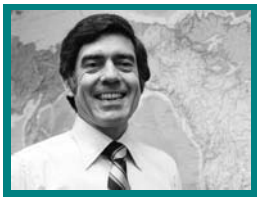

What's (really exciting and) New?

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THE BIG ONE

Table 2A
Enterobacterales
M02 and M07

Table 2A. Enterobacterales (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm S	SDD	I	R	Interpretive Categories and MIC Breakpoints, µg/mL S	SDD	I	R	Comments
B-LACTAM COMBINATION AGENTS (Continued)											
B	Meropenem-vaborbactam	20/10 µg	≥18		15-17[m]	≤14	≤4/8		8/8[m]	≥16/8	(20) Breakpoints are based on a dosage regimen of 4 g every 8 h administered over 3 h.
B	Piperacillin-tazobactam	100/10 µg	**≥25**	**21-24**		**≤20**	**≤8/4**	**16/4**		**≥32/4**	**(21) Breakpoints for susceptible are based on a dosage regimen of 3.375–4.5 g administered every 6 h as a 30-minute infusion. Breakpoints for SDD are based on a dosage regimen of 4.5 g administered every 6 h as a 3-h infusion or 4.5 g administered every 8 h as a 4-h infusion.**				

Method	Piperacillin-tazobactam Previous			Piperacillin-tazobactam New		
	S	I	R	S	SDD	R
BMD	≤ 16	32-64	≥ 128	≤ 8	16	≥ 32
DD	≥ 21	18-20	≤ 17	≥ 25	21-24	≤ 20

CLSI M100-Ed31, 2021; -Ed32, 2022

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Journal of Clinical Microbiology

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

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WHEN NEEDED?

MICROBIOLOGY SAYS:

Significant MIC/disk diffusion discordance when testing recent clinical isolates

Changes to CLSI-approved reference methods

Recognition of a new resistance mechanism

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

WHEN NEEDED?

PHARMACOLOGY SAYS:

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?

THE BOTTOM LINE SAYS:

Specific public health need not addressed previously

Differences between CLSI and other regulatory organizations

New data demonstrate poor prediction of clinical response using previous breakpoints

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

WHY NEEDED?

Recognition of a new resistance mechanism

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

New data demonstrate poor prediction of clinical response using previous breakpoints

CLSI rationale document MR14

Piperacillin-Tazobactam Breakpoints for Enterobacterales

CLSI

CLSI rationale document MR14
February 2022

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REVIEW OF PROCESS

- CLSI voluntary consensus process
  - Members (clinical, industry, government)
  - 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

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THINGS HAPPEN OVER 30 YEARS

- Ureidopenicillin + β-lactamase inhibitor compound
- β-lactamases that are inhibited
  - SHV
  - TEM
  - CTX-M

- β-lactamases less inhibited
  - OXA-1
  - OXA-30

CLSI rationale document MR14

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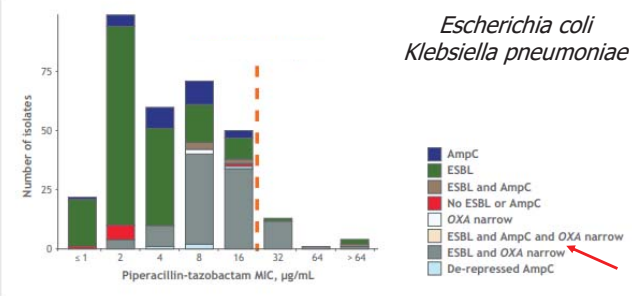

isolates with OXA β-lactamase had higher modal piperacillin-tazobactam MIC than isolates without (8 μg/mL vs. 2 μg/mL; $P < 0.001$)

37% of US isolates with MIC >1 μg/mL to aztreonam, ceftazidime, ceftriaxone harbored OXA β-lactamase

CLSI rationale document MR14

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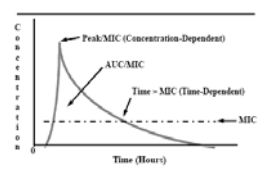
THEORY

- In order for an antimicrobial agent to work:
  - Get there
  - Get there in enough concentration
  - Stay there long enough
- Time > MIC

Once concentration is above MIC, do not observe increased rate of cidal activity with increasing concentrations of (β-lactam) antimicrobial agent



antimicrobe.org

PROBABILITY OF TARGET ATTAINMENT

- Modern methods of PK/PD evaluation determined low PTA for piperacillin-tazobactam when utilizing current CLSI breakpoints (normal renal function)
- No studies revealed high PTA with MIC > 16 μg/mL

Table 5. Summary of Studies Investigating Piperacillin-Tazobactam PK and PD Data

Dosage	Infusion Time	MIC With ≥ 90% PTA[a]
3.375 g every 6 h	30 min	≤ 8 μg/mL[10-12]
4.5 g every 6 h	30 min	≤8 μg/mL[12-15]
3.375 g every 8 h	4 h	≤8 μg/mL[14-17]
4.5 g every 8 h	4 h	≤8 μg/mL[12-15]
4.5 g every 8 h	4 h	≤16 μg/mL[12,14,17,18]
4.5 g every 8 h	3 h	≤16 μg/mL[12,13,18,19]

4.5g q8h dosing used in less than 10% of regimens

CLSI rationale document MR14

CLINICAL TRIAL (Study 1)

- Study of ESBL bacteremia
  - 378 patients
  - *Escherichia coli*, *Klebsiella pneumoniae*
    - S to meropenem and piperacillin-tazobactam
  - 26 hospitals in 9 countries (mostly E. Hemisphere)
- Non-inferiority study
  - Meropenem vs. piperacillin-tazobactam
  - All cause 30-day mortality

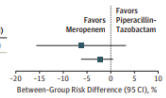
JAMA **320:** 984-994; 2018

CLINICAL TRIAL (Study 1)

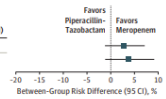
12.3% mortality rate with piperacillin-tazobactam; 3.7% mortality with meropenem

Figure 2. Secondary Outcomes

Measure of Success	Patients Meeting End Point, No./Total No. (%) Piperacillin-Tazobactam	Meropenem	Between-Group Difference (95% CI)
Clinical and microbiological success at day 4[a]	121/177 (68.4)	138/185 (74.6)	-6.2 (-15.5 to 3.1)
Microbiological success at day 4	169/174 (97.1)	184/185 (99.5)	-2.3 (-6.1 to 0.4)



Measure of Failure	Patients Meeting End Point, No./Total No. (%) Piperacillin-Tazobactam	Meropenem	Between-Group Difference (95% CI)
Microbiological relapse	9/187 (4.8)	4/191 (2.1)	2.7 (-1.1 to 7.1)
Secondary infection with multiresistant organism or *Clostridium difficile*	15/187 (8.0)[b]	8/191 (4.2)[c]	3.8 (-1.1 to 9.1)



[a] Clinical and microbiological success defined as survival, negative blood cultures, temperature of 38°C or less, and peripheral white blood cell count of less than or equal to 12 000/μL (to convert to $\times 10^9$/L, multiply by 0.001).

[b] Twelve patients with meropenem- or piperacillin-tazobactam–resistant organism and 3 with *Clostridium difficile* infection.

[c] Six patients with meropenem- or piperacillin-tazobactam–resistant organism and 2 with *Clostridium difficile* infection.

JAMA **320:** 984-994; 2018

CLINICAL TRIAL (Study 2)

Table 1. Logistic Regression Model for Assessment of 30-day Mortality for Patients Treated With Piperacillin/Tazobactam

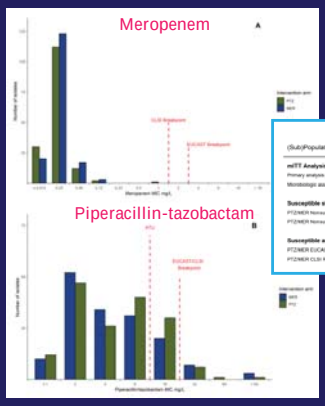
	Bivariate Analysis		Multivariate Analysis	
Variable	OR	*P*	aOR	*P*
Log_2(MIC)	1.2 (0.9–1.6)	.20	…	
MIC > 16 mg/L	10.3 (2.6–41.9)	<.001	14.9 (2.8–87.2)	.002
UTI source	0.4 (0.2–1.1)	.09	0.6 (0.2–1.8)	.3
Charlson comorbidity score	1.6 (1.3–2.0)[a]	<.001	1.7 (1.3–2.2)[a]	<.001

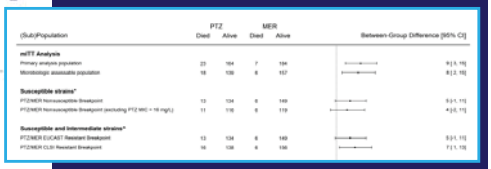
Abbreviations: aOR, adjusted odds ratio; MIC, minimum inhibitory concentration; UTI, urinary tract infection.
[a]Calculated for each numerical increase in Charlson Comorbidity Score.

"There's more to that story…"

Clin. Infect. Dis. **73:** e3842-e3850; 2021

CLINICAL TRIAL (Study 2)



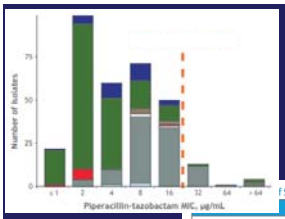


Clin. Infect. Dis. **73:** e3842-e3850; 2021

SUSCEPTIBLE DOSE DEPENDENT

- Intermediate
  - Approach attainable blood and tissue levels but have less clinical response than "susceptible"
  - Also implies clinical efficacy in sites where agents are physiologically concentrated
- Susceptible dose dependent (multiple regimens)
  - Implies that susceptibility of isolate is dependent on dosing regimen
  - Higher dose or more-frequent dosing results in higher drug exposure

CLSI M100; 29th ed.; 2019



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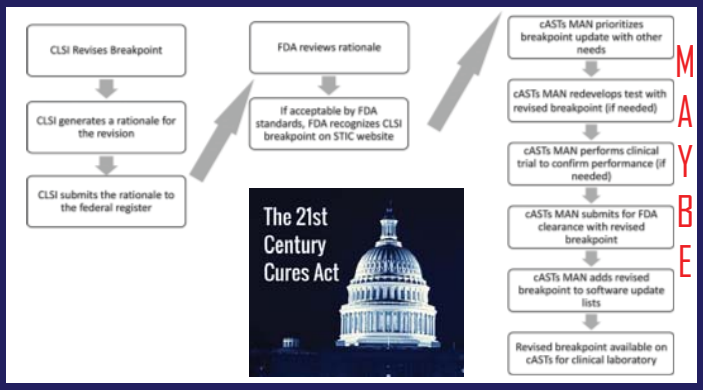
4.5 g every 8 h	4 h	≤8 µg/mL[12-15]
4.5 g every 8 h	4 h	≤16 µg/mL[12,14,17,18]
4.5 g every 8 h	3 h	≤16 µg/mL[12,13,16,19]

≥ 25	21-24		≤ 20	≤ 8/4	16/4 ★	≥ 32/4	(21) Breakpoints for susceptible are based on a dosage regimen of 3.375-4.5 g administered every 6 h as a 30-minute infusion. Breakpoints for SDD are based on a dosage regimen of 4.5 g administered every 6 h as a 3-h infusion or 4.5 g administered every 8 h as a 4-h infusion.

CLSI rationale document MR14; CLSI M100-Ed32

AFTERMATH I



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

THIS CAN GET A BIT HAIRY

Content current as of:
10/14/2021

Susceptibility Test Interpretive Criteria

The table below lists antibacterial drugs and indicates which, if any, susceptibility test interpretive criteria, also known as "breakpoints" (abbreviated as STIC), are recognized or identified by FDA for that drug.

With certain exceptions and additions, identified in the table, FDA recognizes the standard published in:

- Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2021. (CLSI M100)

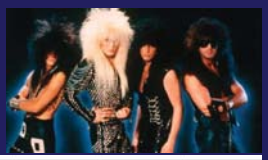
fda.gov/drugs/development-resources

FLUOROQUINOLONES

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

THIS CAN GET A BIT HAIRY



Drug	Route of Administration	STIC for Drug Included in CLSI M100 Standard	Exceptions or Additions to CLSI M100 Standard	Last Updated
Ciprofloxacin	Oral, Injection	Yes	Yes	2/28/20
Levofloxacin	Oral, Injection	Yes	Yes	2/28/20

fda.gov/drugs/development-resources

THIS CAN GET A BIT HAIRY

Piperacillin Tazobactam – Injection products

Recognized Interpretive Criteria

Content current as of: 06/01/2018

	Minimum Inhibitory Concentrations (mcg/mL)			Disk Diffusion (zone diameter in mm)		
Pathogen	S	I	R	S	I	R
Enterobacteriaceae	M100 standard is recognized					

Drug	Route of Administration	STIC for Drug Included in CLSI M100 Standard	Exceptions or Additions to CLSI M100 Standard	Last Updated
Piperacillin and tazobactam	Injection	Yes	Yes	06/26/18

fda.gov/drugs/development-resources

THIS WILL GET A LOT HAIRY

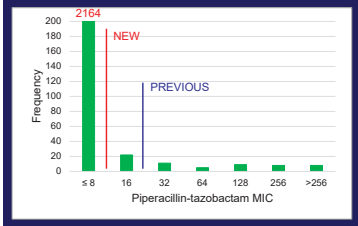
***NEW** 09/22/2021*

MIC.11385 Current Antimicrobial Susceptibility Test Interpretation Breakpoints Phase I

Effective January 1, 2024, the laboratory uses current breakpoints for interpretation of antimicrobial minimum inhibitory concentration (MIC) and disk diffusion test results, and implements new breakpoints within three years of the date of official publication by the FDA or other standards development organization (SDO) used by the laboratory.

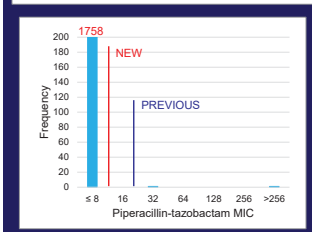
College of American Pathologists Microbiology Checklist

AFTERMATH II



2227 Wisconsin clinical *Escherichia coli* isolates

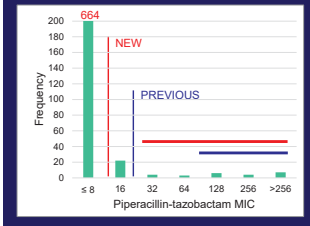
Old:	98.2% S	0.7% I	1.1% R
New:	97.2% S	1.0% SDD	1.8% R



1760 Wisconsin clinical *Proteus mirabilis* isolates

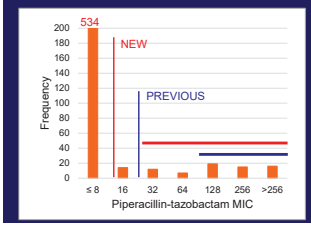
Old:	99.9% S	0.05% I	0.05% R
New:	99.9% S	0.0% SDD	0.1% R

AFTERMATH II



710 Wisconsin clinical *Klebsiella pneumoniae* isolates

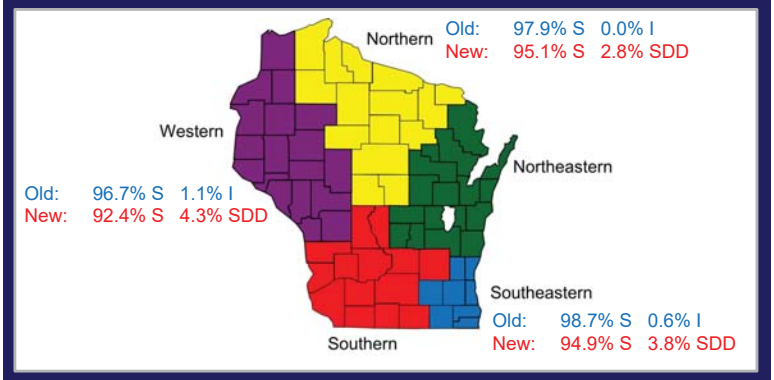
Old:	96.6% S	1.0% I	2.4% R
New:	93.5% S	3.1% SDD	3.4% R



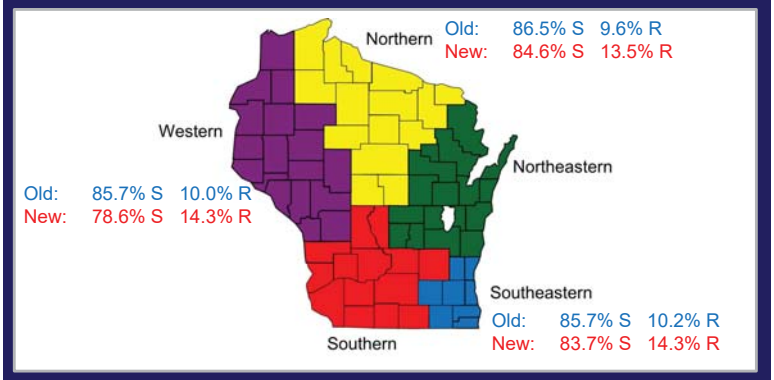
617 Wisconsin clinical *Enterobacter cloacae* isolates

Old:	88.8% S	3.1% I	8.1% R
New:	86.5% S	2.3% SDD	11.2% R

Klebsiella pneumoniae



Enterobacter cloacae



HERE'S ANOTHER BIG ONE

Journal of Clinical Microbiology

BACTERIOLOGY

Direct-from-Blood-Culture Disk Diffusion To Determine Antimicrobial Susceptibility of Gram-Negative Bacteria: Preliminary Report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group

Sukantha Chandrasekaran, April Abbott, Shelley Campeau, Barbara L. Zimmer, Melvin Weinstein, Lauri Thrupp, John Hejna, Lindsey Walker, Tracy Ammann, Thomas Kirn, Robin Patel, Romney M. Humphries

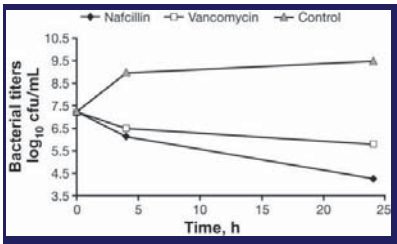
J. Clin. Microbiol. **56:**e01678-17

OUTCOMES

Inappropriate therapy in bacteremia →

Extended duration of hospitalization
Increased patient mortality
Increased cost of treatment

Clin. Infect. Dis. **36:** 1418-1423; 2003
Diagn. Microbiol. Infect. Dis. **52:** 113-122; 2005



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COMMENTARY

Check for updates

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

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THEY'VE BEEN TRYING THIS…I

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1979, p. 347–350
0095-1137/79/03-0347/04$02.00/0
Vol. 9, No. 3

Standardization of Direct Susceptibility Test for Blood Cultures

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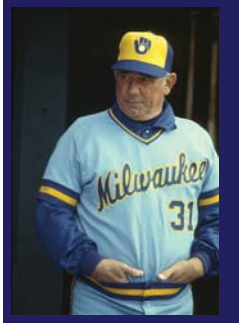
Received for publication 17 December 1978

Insufficient data are available to establish the reliability of direct disk diffusion susceptibility tests performed utilizing positive blood culture broth as inoculum. When *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were used, 0.03 ml of turbid overnight blood culture broth was found to produce zone diameters closely approximating the size of diameters obtained by a standardized method. Results of direct (0.03 ml of inoculum) and standardized susceptibility tests were then compared for 116 positive blood cultures (1,069 individual disk comparisons). There were 1,011 test agreements (94.6%). There were also 48 (4.5%) minor discrepancies (change between sensitive and intermediate or between intermediate and resistant) and 10 (0.9%) major discrepancies (change between sensitive and resistant). The major discrepancies were randomly distributed among several organisms and antibiotics. Discrepancies occurred most frequently in the more clinically acceptable direction; i.e., in 79.3% the direct test indicted greater resistance than the standardized test. These data establish that 0.03 ml of turbid overnight blood culture broth produces results which compare closely to those obtained with standard methods, and in practice yield direct susceptibility results with a clinically acceptable level of reliability.



J. Clin. Microbiol. **9:** 347-350; 1979

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THEY'VE BEEN TRYING THIS…I

Used ATCC *E. coli*, *P. aeruginosa*, *S. aureus* in mock experiments to determine "optimal" inoculum for direct disk

"Prospective" [TSB aerobic (default); TSB anaerobic; Thiol]

n = 116

Two drops (30 μL) directly onto Mueller Hinton (3 planes)

Everything per NCCLS guidelines (18 hours incubation)

J. Clin. Microbiol. **9:** 347-350; 1979

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THEY'VE BEEN TRYING THIS…I

TABLE 2. *Organisms included in the clinical comparison of the direct and standardized susceptibility tests*

Organism	No. of strains tested	Discrepancies		Agreements
		Major	Minor	
E. coli	46	2	22	390
Klebsiella	16	2	12	130
Proteus mirabilis	8	2	3	67
Providencia stuartii	1	0	1	8
Citrobacter diversus	1	0	0	9
Citrobacter freundii	1	0	1	8
Enterobacter aerogenes	3	0	1	26
Enterobacter cloacae	3	2	1	24
Enterobacter agglomerans	1	0	0	9
Serratia marcescens	3	0	0	27
P. aeruginosa	4	0	0	36
Pseudomonas species	2	0	2	16
Bordetella parapertussis	1	0	0	9
Acinetobacter calcoaceticus	1	0	0	9
S. aureus	12	0	0	120
Staphylococcus epidermidis	8	2	4	74
Enterococcus	3	0	1	29
Group D *Streptococcus* (not *Enterococcus*)	1	0	0	10
Viridans *Streptococcus*	1	0	0	10

TABLE 3. *Distribution of discrepancies between direct and standardized susceptibility tests by antibiotic*

Antibiotic	No. of comparisons	Discrepancies		
		Total	Major	Minor
Ampicillin	116	4 (3.8)[a]	1	3
Carbenicillin	91	4 (4.3)	0	4
Cephalothin	116	16 (13.8)	2	14
Chloramphenicol	116	6 (5.2)	3	3
Clindamycin	25	0	0	0
Colistin	91	6 (6.6)	2	4
Erythromycin	25	0	0	0
Gentamicin	116	0	0	0
Kanamycin	116	1 (0.8)	0	1
Methicillin	25	1 (4.0)	0	1
Penicillin	25	3 (12.0)	1	2
Streptomycin	91	9 (9.9)	0	9
Tetracycline	116	8 (6.9)	1	7

Major (0.9%): shift between sensitive and resistant

Minor (4.5%): shift between sensitive and intermediate
shift between intermediate and resistant

J. Clin. Microbiol. **9:** 347-350; 1979

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THEY'VE BEEN TRYING THIS…II

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1981, p. 696–698
0066-4804/81/110696-03$02.00/0 Vol. 20, No. 5

Evaluation of a Direct Blood Culture Disk Diffusion Antimicrobial Susceptibility Test

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Received 10 April 1981/Accepted 6 August 1981

A total of 556 unique blood culture isolates of nonfastidious aerobic and facultatively anaerobic bacteria were examined by direct and standardized disk susceptibility test methods (4,234 antibiotic-organism comparisons). When discrepancies which could be accounted for by the variability inherent in disk diffusion susceptibility tests were excluded, the direct method demonstrated 96.8% overall agreement with the standardized method. A total of 1.6% minor, 1.5% major, and 0.1% very major discrepancies were noted.

Antimicrob. Agents Chemother. **20:** 696-698; 1981

THEY'VE BEEN TRYING THIS…II

"Prospective" (BBL aerobic and anaerobic bottles)

n = 556

Six drops (50 μL) onto Mueller Hinton (GNR; GPC clumps)
Six drops (50 μL) onto Mueller Hinton w/blood (GPC chains)

Everything per NCCLS guidelines (16-18 hours incubation)

Very Major Error: direct susceptible, reference resistant (false-susceptible)
Major Error: direct resistant, reference susceptible (false-resistant)
Minor Error: resistant ↔ intermediate ↔ susceptible

Antimicrob. Agents Chemother. **20:** 696-698; 1981

THEY'VE BEEN TRYING THIS…II

TABLE 1. *Comparison of direct blood culture disk susceptibility test results with standardized disk susceptibility test results*

Antibiotic	No. of discrepancies[a] for: Gram-negative bacilli[b] (n = 171)	Streptococci[c] (n = 166)	Staphylococci and micrococci[d] (n = 219)	Totals
Kanamycin	0, 8, 66 (56.7)	NT	NT	0, 8, 66 (56.7)
Gentamicin	0, 1, 3 (97.7)	NT	NT	0, 1, 3 (97.7)
Tobramycin ←	0, 1, 5 (96.5)	NT	NT	0, 1, 5 (96.5)
Amikacin	0, 4, 17 (87.7)	NT	NT	0, 4, 17 (87.7)
Carbenicillin	0, 6, 14 (88.3)	NT	NT	0, 6, 14 (88.3)
Ampicillin	1, 3, 12 (90.6)	0, 2, 11 (92.2)	NT	1, 5, 23 (91.4)
Cephalothin	0, 4, 50 (68.5)	0, 0, 6 (96.4)	0, 0, 0 (100)	0, 4, 56 (89.2)
Tetracycline	0, 1, 43 (74.3)	1, 5, 15 (87.4)	1, 2, 2 (97.7)	2, 8, 60 (87.4)
Chloramphenicol	0, 1, 13 (91.8)	0, 0, 9 (94.6)	0, 3, 3 (97.2)	0, 4, 25 (94.8)
Erythromycin	NT	1, 1, 9 (93.4)	0, 2, 3 (97.7)	1, 3, 12 (95.8)
Clindamycin	NT	0, 0, 6 (96.4)	0, 2, 0 (99.1)	0, 2, 6 (97.9)
Penicillin	NT	0, 2, 14 (90.4)	0, 5, 25 (96.3)	0, 7, 39 (88.1)
Methicillin	NT	NT	1, 11, 6 (91.8)	1, 11, 6 (91.8)
Totals	1, 29, 223 (83.6)	2, 10, 70 (92.9)	2, 25, 39 (95.7)	5, 64, 332 (90.5)

Very Major Error; Major Error; Minor Error; (percentage concordance)

Antimicrob. Agents Chemother. **20:** 696-698; 1981

THEY'VE BEEN TRYING THIS…III

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1984, p. 473–477
0095-1137/84/090473-05$02.00/0 Vol. 20, No. 3

Rapid Antimicrobial Susceptibility Testing of Isolates from Blood Cultures by Direct Inoculation and Early Reading of Disk Diffusion Tests

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Received 13 January 1984/Accepted 24 May 1984

Disk diffusion tests, inoculated directly from positive blood cultures, were evaluated for accuracy of reading zone diameters after 4- and 6-h and overnight incubation. In comparisons with results from standard disk diffusion tests, the 4-h results were in agreement for 83% of tests with gram-positive organisms and 64% of tests with gram-negative organisms. When minor discrepancies were ignored, the 4-h readings were in agreement for 98% of the tests with gram-positive organisms and 95% of the tests with gram-negative organisms. After 6 h of incubation, 91% of the tests with gram-positive organisms and 86% of the tests with gram-negative organisms agreed with standard results. The agreement was 99% for tests with both gram-positive and gram-negative organisms when minor discrepancies were excluded. Very major discrepancies occurred in two tests (0.1%) with gram-positive organisms and were not observed in tests with gram-negative organisms. The frequencies of major discrepancies were 3.5% after 4 h, 0.6% after 6 h, and 0.7% after overnight incubation. Ampicillin and cephalothin tests with *Escherichia coli* and *Klebsiella* spp. accounted for 81% of the major discrepancies in tests with gram-negative organisms. Oxacillin tests accounted for more than half of the major discrepancies in tests with staphylococci. The results of this study, which did not include the newer antibiotics, indicate that direct susceptibility tests from blood cultures read after 6 h of incubation are more reliable than 4-h results and produce less than 1% major errors in comparisons with standard susceptibility tests.

J. Clin. Microbiol. **20:** 473-477; 1984

THEY'VE BEEN TRYING THIS…III

"Prospective" (aerobic and anaerobic bottles)

n = 403

Swab onto Mueller Hinton (GNR)
Swab onto Mueller Hinton w/blood (GPC)

Everything else per NCCLS guidelines
(except read at 4 hours, 6 hours)

Very Major Error: direct susceptible, reference resistant (false-susceptible)
Major Error: direct resistant, reference susceptible (false-resistant)
Minor Error: resistant ↔ intermediate ↔ susceptible

J. Clin. Microbiol. **20:** 473-477; 1984

THEY'VE BEEN TRYING THIS…III

TABLE 1. Percentage of isolates with direct tests read after 4 or 6 h

Blood culture isolate	No. of isolates	% Read after: 4 h	6 h[a]
Gram positive			
S. aureus	60	22	63
Coagulase-negative staphylococci	87	3	21
Beta-hemolytic streptococci	30	37	87
Enterococci	21	19	52
Pneumococci	21	10	38
Viridans streptococci	14	0	0
Total for gram positive	233	14	44
Gram negative			
E. coli	84	52	85
Klebsiella spp.	38	40	76
Enterobacter spp.	12	42	92
P. aeruginosa	11	0	64
Others[b]	25	36	60
Total for gram negative	170	43	78

TABLE 2. Discrepancies from direct tests compared with standardized tests

Isolate type, time incubated	No. of tests	No. of discrepancies: Very major (%)	Major (%)	Minor (%)	Overall agreement (%)
Gram positive					
4 h	216	1 (0.5)	3 (1.4)	32 (14.8)	83.3
6 h	494	0	3 (0.6)	39 (7.9)	91.4
Overnight	1,307	1 (0.07)	8 (0.6)	65 (5.0)	94.3
Gram negative					
4 h	361	0	17 (4.7)	114 (31.6)	63.7
6 h	438	0	3 (0.7)	59 (13.5)	85.8
Overnight	762	0	6 (0.8)	73 (9.6)	89.6

J. Clin. Microbiol. **20:** 473-477; 1984

IMPETUS

- Resistance in GNR can be multi-factorial; full phenotypic approach may be desirable
- Little standardization; very few laboratories report

J. Clin. Microbiol. **56:**e01678-17

METHODS

- Single site
- Mock inoculation

  0.5 McFarland adjusted
  10^2 CFU inoculum
  BacT/Alert FA Plus
  Bactec Plus aerobic
  VersaTREK Redox 1

TABLE 1 Bacterial isolates used in this study[a]

Isolate no.	Species	Resistance phenotype
15-05-01	*Klebsiella pneumoniae*	CRE (NDM-1)
15-05-02	*K. pneumoniae*	CRE (KPC)
15-05-03	*K. pneumoniae*	ESBL (CTX-M-15)
15-05-04	*Proteus mirabilis*	Wild type
15-05-05	*Enterobacter aerogenes*	Resistant to cephalosporins III, AmpC overexpression
15-05-06	*Enterobacter cloacae*	Wild type
15-05-07	*E. cloacae*	Resistant to cephalosporins III, AmpC overexpression
15-05-08	*Citrobacter freundii*	None
15-05-09	*Escherichia coli*	Plasmid for AmpC CMY-2
15-05-10	*E. coli*	Cefazolin resistant (mechanism not defined)
15-05-11	*E. coli*	Wild type
15-05-12	*E. coli*	ESBL
15-05-14	*Pseudomonas aeruginosa*	Carbapenem resistant
15-05-15	*P. aeruginosa*	Wild type
15-05-16	*P. aeruginosa*	Fluoroquinolone resistant
15-05-17	*Acinetobacter baumannii*	Wild type
15-05-18	*K. pneumoniae*	Wild type
15-05-19	*A. baumannii*	Carbapenem resistant (mechanism not defined)
15-05-20	*P. aeruginosa*	Aminoglycoside resistant
15-05-21	*Serratia marcescens*	SME

- Pulled from instrument within 8 hours of being flagged; tested immediately

J. Clin. Microbiol. **56:**e01678-17

METHODS (CONTINUED)

- Bottles subcultured for reference disk diffusion
- Bottle contents subjected to direct disk diffusion

  4 drops onto Mueller Hinton via venting needle
  Swabbed in three directions
  35°C ambient air; 6 and 18 hours

amikacin	ceftriaxone	minocycline	amoxicillin-clavulate
ampicillin	ciprofloxacin	tigecycline	piperacillin-tazobactam
aztreonam	ertapenem	tobramycin	trimethoprim-sulfa
cefazolin	gentamicin	ceftazidime	cefoxitin
cefepime	imipenem	meropenem	levofloxacin

- Broth microdilution (in-house) final adjudicator

J. Clin. Microbiol. **56:**e01678-17

RESULTS

TABLE 3 Resolved performance of direct-from-blood-culture disk diffusion method at 18 h, by antibiotic

Drug	No. of isolates S	R	% CA	No. (%) of: VME	ME	mE
Amikacin	45	13	96.7	0 (0)	0 (0)	2 (3.3)
Amoxicillin-clavulanate	9	17	88.9	0 (0)	1 (11.1)	2 (7.4)
Ampicillin ←	6	9	93.3	0 (0)	0 (0)	1 (6.7)
Aztreonam ←	21	28	94.3	0 (0)	0 (0)	3 (5.7)
Cefazolin	5	18	73.1	0 (0)	2 (40.0)	5 (19.2)
Cefepime	41	17	91.7	0 (0)	0 (0)	5 (8.3)
Cefoxitin	10	15	85.2	0 (0)	1 (10.0)	3 (11.1)
Ceftazidime ←	25	31	89.8	0 (0)	0 (0)	6 (10.2)
Ceftriaxone ←	16	29	87.5	0 (0)	2 (12.5)	4 (8.3)
Ciprofloxacin ←	26	27	96.6	0 (0)	0 (0)	1 (1.7)
Ertapenem	22	12	83.3	0 (0)	0 (0)	7 (16.7)
Gentamicin	39	18	95.0	0 (0)	1 (2.6)	2 (3.3)
Imipenem	34	21	68.3	0 (0)	3 (8.8)	15 (25.0)
Levofloxacin	33	25	91.7	0 (0)	1 (3.0)	3 (5.0)
Meropenem ←	37	19	84.7	0 (0)	1 (2.7)	8 (13.6)
Minocycline	29	11	80.0	0 (0)	0 (0)	9 (20.0)
Piperacillin-tazobactam	23	30	83.3	0 (0)	0 (0)	10 (16.7)
Tigecycline	35	3	87.2	0 (0)	0 (0)	5 (12.8)
Tobramycin ←	39	17	93.2	0 (0)	0 (0)	4 (6.8)
Trimethoprim-sulfamethoxazole ←	17	30	95.8	0 (0)	0 (0)	2 (4.2)

J. Clin. Microbiol. **56:**e01678-17

RESULTS

TABLE 3 Resolved performanced-culture disk diffusion met 18 h, by antibiotic

Drug	% CA	No. (%) of: VME	ME
Amikacin	96.7	0 (0)	0 (0)
Amoxicillin-clavulanate	88.9	0 (0)	1 (11.1)
Ampicillin	93.3	0 (0)	0 (0)
Aztreonam	94.3	0 (0)	0 (0)
Cefazolin	73.1	0 (0)	2 (40.0)
Cefepime	91.7	0 (0)	0 (0)
Cefoxitin	85.2	0 (0)	1 (10.0)
Ceftazidime	89.8	0 (0)	0 (0)
Ceftriaxone	87.5	0 (0)	2 (12.5)
Ciprofloxacin	96.6	0 (0)	0 (0)
Ertapenem	83.3	0 (0)	0 (0)
Gentamicin	95.0	0 (0)	1 (2.6)
Imipenem	68.3	0 (0)	3 (8.8)
Levofloxacin	91.7	0 (0)	1 (3.0)
Meropenem	84.7	0 (0)	1 (2.7)
Minocycline	80.0	0 (0)	0 (0)
Piperacillin-tazobactam	83.3	0 (0)	0 (0)
Tigecycline	87.2	0 (0)	0 (0)
Tobramycin	93.2	0 (0)	0 (0)
Trimethoprim-sulfamethoxazole	95.8	0 (0)	0 (0)

TABLE 5 Resolved performance of direct-from-blood-culture disk diffusion method at 6 h, by antibiotic

Drug	No. of isolates S	R	% CA	No. (%) of: VME	ME	mE
Amikacin	45	13	62.2	3 (23.1)	2 (4.4)	12 (26.7)
Amoxicillin-clavulanate	9	17	60.0	0 (0)	1 (11.1)	9 (36.0)
Ampicillin ←	6	9	69.2	0 (0)	1 (16.7)	3 (23.1)
Aztreonam ←	21	28	84.2	0 (0)	1 (4.8)	5 (13.2)
Cefazolin	5	18	66.7	1 (5.6)	2 (40.0)	6 (25.0)
Cefepime	41	17	75.6	0 (0)	4 (9.8)	6 (13.3)
Cefoxitin	10	15	68.0	0 (0)	1 (10.0)	7 (28.0)
Ceftazidime ←	25	31	65.9	0 (0)	4 (16.0)	11 (25.0)
Ceftriaxone ←	16	29	77.3	0 (0)	3 (18.8)	7 (15.9)
Ciprofloxacin ←	24	27	57.1	0 (0)	1 (4.2)	16 (39.0)
Ertapenem	22	12	73.7	0 (0)	2 (9.1)	8 (21.1)
Gentamicin	39	18	95.6	0 (0)	0	2 (4.4)
Imipenem	34	21	46.7	0 (0)	6 (17.6)	18 (40.0)
Levofloxacin	33	25	75.6	0 (0)	1 (3.0)	10 (22.2)
Meropenem ←	36	19	52.3	0 (0)	9 (25.0)	11 (25.6)
Minocycline	29	11	65.9	0 (0)	0	12 (29.3)
Piperacillin-tazobactam	22	30	64.4	2 (6.7)	4 (18.2)	11 (25.0)
Tigecycline ←	35	3	45.7	0 (0)	3 (8.6)	16 (45.7)
Tobramycin ←	39	17	95.6	0 (0)	0	2 (4.4)
Trimethoprim-sulfamethoxazole ←	17	30	86.4	1 (3.3)	2 (11.8)	3 (6.8)

J. Clin. Microbiol. **56:**e01678-17

Table 3E-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 **and *Pseudomonas aeruginosa***
Medium	MHA
Antimicrobial concentration	Standard disk contents for the antimicrobials **are detailed in Table 3E-2 (Enterobacterales) and Table 3E-3 (*P. aeruginosa*)**
Inoculum	Positive blood culture broth with gram-negative bacilli, used within 8 hours 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 hours or** 16-18 hours
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 M02.[1] 3. Measure the zone diameters according to routine disk diffusion recommendations in M02.[1] 4. Report results using the interpretive categories and zone diameter breakpoints in **Table 3E-2 or Table 3E-3** if the gram-negative bacillus tested is confirmed to be an Enterobacterales **or *P. aeruginosa*, respectively**. If species is identified as another organism, do not interpret or report results.

Daily or weekly QC; *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853

CLSI M100-Ed32; 2022

TABLE 3E-2

Table 3E-2. Enterobacterales (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Read Times, hours	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm S	SDD	I	R	Comments
PENICILLINS								
A	Ampicillin	10 µg	8-10	-	-	-	-	(3) Results of ampicillin testing can be used to predict results for amoxicillin.
			16-18	≥ 17	-	14-16	≤ 13	(4) Breakpoints are based on an ampicillin dosage regimen of 2 g parenterally administered every 4-6 h or an amoxicillin dosage regimen of 1-2 g parenterally administered every 6 h.
CEPHEMS (PARENTERAL) (including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
B	Ceftriaxone	30 µg	8-10	≥ 23	-	20-22	≤ 19	(5) Breakpoints are based on a dosage regimen of 1 g administered every 24 h.
			16-18	≥ 23	-	20-22	≤ 19	
C	Ceftazidime	30 µg	8-10	≥ 21	-	18-20	≤ 17	(6) Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
			16-18	≥ 21	-	18-20	≤ 17	
MONOBACTAMS								
C	Aztreonam	30 µg	8-10	≥ 21	-	18-20	≤ 17	(7) Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
			16-18	≥ 21	-	18-20	≤ 17	
AMINOGLYCOSIDES								
A	Tobramycin	10 µg	8-10	≥ 15	-	13-14	≤ 12	
			16-18	≥ 15	-	13-14	≤ 12	
FOLATE PATHWAY ANTAGONISTS								
B	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	8-10	-	-	-	-	
			16-18	≤ 16	-	11-15	≤ 10	

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

CLSI M100-Ed32; 2022

Table 3E-3
Zone Diameter Disk Diffusion Breakpoints for *P. aeruginosa* Direct From Blood Culture

Table 3E-3. Zone Diameter Disk Diffusion Breakpoints for *Pseudomonas aeruginosa* Direct From Blood Culture

M100-Ed32

General Comments

(1) The dosage regimens shown in the Comments column below are necessary to achieve plasma drug exposure (in adults with normal renal and hepatic function) on which breakpoints were derived. When new breakpoints are implemented, it is strongly recommended that laboratories share this information with infectious diseases practitioners, pharmacists, pharmacy and therapeutics committees, infection prevention committees, and the antimicrobial stewardship team.

(2) For additional testing and reporting recommendations, refer to Table 2B-1.

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

Test/Report Group	Antimicrobial Agent	Disk Content	Read Times, hours	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm S	SDD	I	R	Comments
CEPHEMS (PARENTERAL) (including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
A	Ceftazidime	30 µg	8-10	-	-	-	-	(3) Breakpoints are based on a dosage regimen of 1 g administered every 6 h or 2 g administered every 8 h.
			16-18	≥ 18	-	15-17	≤ 14	
CARBAPENEMS								
B	Meropenem	10 µg	8-10	-	-	-	-	(4) Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
			16-18	≥ 19	-	16-18	≤ 15	
AMINOGLYCOSIDES								
A	Tobramycin	10 µg	8-10	≥ 15	-	13-14	≤ 12	
			16-18	≥ 15	-	13-14	≤ 12	
FLUOROQUINOLONES								
B	Ciprofloxacin	5 µg	8-10	≥ 23	-	18-22	≤ 17	(5) Breakpoints are based on a dosage regimen of 400 mg administered parenterally every 8 h.
			16-18	≥ 25	-	19-24	≤ 18	

Abbreviations: I, intermediate; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

Feb 2022

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Other General Comments

Appendix E
Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints

Appendix E. Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints

The evolving science of pharmacokinetics-pharmacodynamics has become increasingly important in recent years in determining minimal inhibitory concentration (MIC) breakpoints. Recently approved susceptible or susceptible-dose dependent (SDD) breakpoints for a number of agents have been based on a specific dosage regimen(s); these dosage regimens are listed in the table below. Proper application of the breakpoints necessitates drug exposure at the site of infection that corresponds to or exceeds the expected systemic drug exposure at the dose listed in adult patients with normal renal function. This information should be shared with pharmacists, infectious diseases staff, and others making dosing recommendations for the institution.

Antimicrobial Agent	Breakpoints and Interpretive Categories: Susceptible MIC	Susceptible Dose	SDD MIC	SDD Dose
Table 2A. Enterobacterales				
Ampicillin (used to predict results for amoxicillin)	≤ 8 µg/mL	Breakpoints are based on an ampicillin dosage regimen of 2 g parenterally administered every 4-6 h or an amoxicillin dosage regimen of 1-2 g parenterally administered every 6 h.	N/A	
Ampicillin (used to predict results for amoxicillin; *E. coli, P. mirabilis, Shigella, and Salmonella* for uncomplicated UTIs only)	≤ 8 µg/mL	Breakpoints when oral ampicillin is used for therapy of uncomplicated UTIs due only to *E. coli, P. mirabilis, Shigella, or Salmonella* are based on an ampicillin dosage regimen of 500 mg orally administered every 6 h or amoxicillin dosage regimen of 250 mg orally administered every 8 h or 500 mg every 12 h.	N/A	

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DOSAGE COMMENT ADDITIONS

- *Enterobacterales*

  ampicillin (IV, PO) amoxicillin-clavulanate (IV, PO)
  ampicillin-sulbactam cefazolin (uncomplicated UTI)
  imipenem-relebactam (for *Morganellaceae*)
  piperacillin-tazobactam

- *Pseudomonas aeruginosa*

  ceftolozane-tazobactam

- *Staphylococcus aureus*

  dalbavancin, oritavancin, tedizolid, telavancin

DOSAGE COMMENT ADDITIONS

- *Enterococcus* spp.

  penicillin (IV, PO) ampicillin (IV, PO)
  dalbavancin (VRE), oritavancin, tedizolid, telavancin

- *Haemophilus influenzae*, *H. parainfluenzae*

  ampicillin (IV, PO) ampicillin-sulbactam
  amoxicillin-clavulanate ceftolozane-tazobactam

- *Streptococcus pneumoniae* (non-CSF comments)

  amoxicillin amoxicillin-clavulanate

DOSAGE COMMENT ADDITIONS

- *Streptococcus* spp. β-hemolytic group

  oritavancin, telavancin
  dalbavancin (only A, B, C), tedizolid (only A, B)

- *Streptococcus* spp. viridans group

  oritavancin, telavancin
  dalbavancin, tedizolid only for *S. anginosus* group

- *Neisseria meningitidis*

  ampicillin

Revisions
N. gonorrhoeae/tetracycline
Enterobacterales/ceftolozane-tazo

CEFIDEROCOL

- Group B (primary test, report selectively); former Inv.

  Enterobacterales *Pseudomonas aeruginosa*
  Acinetobacter spp. *Stenotrophomonas maltophilia*

- Breakpoint revisions

  Disk diffusion *Enterobacterales* (only the I and R)
  Disk diffusion *Acinetobacter* spp. (S only)
  Both formats *Stenotrophomonas maltophilia* (S only)

- Dosage commentary

  Acinetobacter spp., *Stenotrophomonas maltophilia*

THE INTERMEDIATE COMMENT

^ agents that have ability to concentrate in urine

(4) An intermediate (I) with a ^ in Tables 2 indicates agents that have the potential to concentrate in the urine. The I^ is for informational use only. The decision to report I^ is best made by each laboratory based on institution-specific guidelines and in consultation with appropriate medical personnel.

Enterobacterales
Pseudomonas aeruginosa
Enterococcus spp.

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β-LACTAM/β-LACTAMASE INHIBITOR

"Organisms that test S to the β-lactam agent alone are also considered S to the β-lactam combination agent. However, organisms that test S to the β-lactam combination agent cannot be assumed to be S to the β-lactam agent alone."

β-lactam agent alone SDD, I, R → may be S to β-lactam combination agent

- Applies to
  - *Enterobacterales*
  - *Pseudomonas aeruginosa*
  - *Acinetobacter* spp.
  - Other Non-*Enterobacterales*
  - *Haemophilus influenzae* and *H. parainfluenzae*
  - Anaerobes
- Replaces imipenem-relebactam comment (in some)

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Table 2

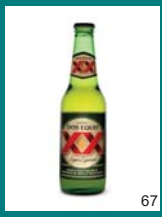


TABLE 2A--*Enterobacterales*

- Ampicillin can predict amoxicillin
- Disk diffusion revision ceftolozane-tazobactam (+1 mm)
- Piperacillin

CLSI M100	Disk Diffusion			Broth Microdilution		
	S	I	R	S	I	R
Old	≥ 21	18-20^	≤ 17	≤ 16	32-64^	≥ 128
New				≤ 8	16	≥ 32

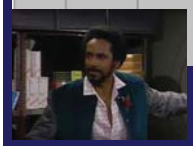
No disk diffusion correlative data for broth microdilution breakpoints

CLSI M100-Ed31, 2021; -Ed32, 2022

TABLE 2C--*Staphylococcus* spp.

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

Test/Report Group	Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm: S	SDD	I	R	Interpretive Categories and MIC Breakpoints, µg/mL: S	SDD	I	R	Comments
GLYCOPEPTIDES												
(21) MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, -intermediate, and -resistant isolates of *Staphylococcus* spp. other than *S. aureus*, all of which give similar size zones of inhibition.												
B	Vancomycin	*S. aureus*, **including MRSA**						≤2		4-8	≥16	(22) For *S. aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy. (23) Send any *S. aureus* for which the vancomycin is ≥ 8 µg/mL to a referral laboratory. See Appendix A. Also refer to Table 3G-1 for *S. aureus*, Subchapter 3.12 in M07,[4] and Subchapter 3.9 in M02.[1]
		Staphylococcus spp. other than *S. aureus*						≤4		8-16	≥32	See comment (20). (24) Send any *Staphylococcus* spp. other than *S. aureus* for which the vancomycin MIC is ≥ 32 µg/mL to a referral laboratory. See Appendix A. See also Subchapter 3.12 in M07[4] and Subchapter 3.9 in M02.[1]



also for lefamulin

CLSI M100-Ed32; 2022

DIESELBE!!!!!!!!!!!!!!!!!!!!!!

Detection of methicillin (oxacillin) resistance in staphylococci is achieved by using specific methods as listed in Table 2C and further described in Tables 3G-1 and 3G-2.

Organism	Phenotypic 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 (24 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
Staphylococcus spp. (not listed above or not identified to the species level)	No	Yes[a] (24 h)	Yes[a] (24 h)	No	No

Staphylococcus aureus complex:
- *S. aureus*
- *S. argenteus*†
- *S. schweitzeri*

† Report as "*S. aureus* complex (*S. argenteus*)"; perform *S. aureus* AST

CLSI M100-Ed32; 2022

TABLE 2G--*H. influenzae*, *parainfluenzae*

- Amoxicillin-clavulanate

CLSI M100	Disk Diffusion			Broth Microdilution		
	S	I	R	S	I	R
Old	≥ 20		≤ 19	≤ 4/2		≥ 8/4
New				≤ 2/1	4/2	≥ 8/4

- Lefamulin

Increased zone size (S) also for *S. pneumoniae*; MIC (S only) stays at ≤ 0.5

CLSI M100	Disk Diffusion			Broth Microdilution		
	S	I	R	S	I	R
Old	≥ 17			≤ 2		
New	≥ 18			≤ 2		

CLSI M100-Ed31, 2021; -Ed32, 2022

LEFAMULIN

TABLE 1 Frequency of occurrence of lefamulin MICs for all pathogens tested

Organism (no. of isolates)	Cumulative % of isolates inhibited at lefamulin MIC (µg/ml) of: ≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
S. pneumoniae (3,923)	0.1	1.8	11.4	55.1	93.7	99.6	99.9	100.0					0.06	0.12
Penicillin nonsusceptible, nonmeningitis (≥4 µg/ml) (189)	0.0	1.1	7.9	64.0	98.4	100.0							0.06	0.12
Ceftriaxone nonsusceptible (≥2 µg/ml) (155)	0.0	0.6	10.3	63.9	99.4	100.0							0.06	0.12
Erythromycin nonsusceptible (≥0.5 µg/ml) (1,348)	0.2	2.2	12.4	52.9	93.1	99.0	99.7	100.0					0.06	0.12
Levofloxacin nonsusceptible (≥4 µg/ml) (47)	0.0	8.5	23.4	68.1	89.4	97.9	97.9	100.0					0.06	0.25
MDR[a] (821)	0.4	2.9	15.8	61.1	96.3	99.8	99.8	100.0					0.06	0.12
XDR[a] (181)	0.0	0.6	7.2	64.6	98.9	100.0							0.06	0.12
S. aureus (2,919)			26.0	88.9	99.2	99.6	99.7	99.8	99.8	99.8	99.8	100.0	0.06	0.12
Methicillin susceptible (1,981)			25.6	95.2	99.7	99.7	99.8	99.9	>99.9	>99.9	>99.9	100.0	0.06	0.06
Methicillin resistant (938)			26.9	75.7	98.2	99.4	99.5	99.6	99.7	99.8	99.8	100.0	0.06	0.12
H. influenzae (1,086)					1.7	20.4	69.4	93.8	99.1	99.9	100.0		0.5	1
β-lactamase negative (835)					1.9	20.0	67.5	93.2	98.9	100.0			0.5	1
β-lactamase positive (251)					1.2	21.9	75.7	96.0	99.6	99.6	100.0		0.5	1
M. catarrhalis (667)	1.0	2.4	11.1	88.3	99.9	100.0							0.06	0.12

"S" only

Antimicrob. Agents Chemother. **63:**e02161-18

Table 3

TABLE 3D AND 3K

- Specialized colistin resistance testing

  E. coli ATCC BAA-3170 formerly known as *E. coli* AR Bank #0349 *mcr-1*

  Adjustments to QC range for this *E. coli* and *P. aeruginosa* ATCC 27853

- High-level aminoglycoside resistance *Enterococcus*

  penicillin, ampicillin MIC ≥ 16 μg/mL are R
  penicillin ≤ 64 μg/mL, ampicillin ≤ 32 μg/mL may be susceptible to synergy with aminoglycosides

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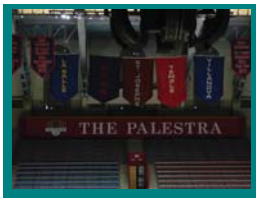

Table 5

SOME MIC QC ADDITIONS/REVISIONS

E. coli ATCC 25922	imipenem imipenem-relebactam meropenem-nacubactam ceftibuten
E. coli NCTC 13353	meropenem ceftibuten
K. pneumoniae ATCC BAA-2814	ceftibuten
K. pneumoniae ATCC BAA-1705	ceftibuten
K. pneumoniae ATCC 700603	imipenem imipenem-relebactam
A. baumannii NCTC 13304	meropenem
E. faecalis ATCC 29212	gepotidacin ozenoxacin
H. influenzae ATCC 49247	grepafloxacin
N. gonorrhoeae ATCC 49226	gentamicin

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MORE MIC QC ADDITIONS/REVISIONS

tebipenem	*S. aureus* ATCC 29213 *Bacteroides fragilis* ATCC 25285 *Bacteroides thetaiotaomicron* ATCC 29741 *Clostridioides difficile* ATCC 700057 *Eggerthella lenta* ATCC 43055
fidaxomicin	*Clostridioides difficile* ATCC 700057

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THE END

- Keynote address
- Stewardship panel
- Review of automated systems, antibiograms
- Surveillance
- CAP and CLSI
- WCLN honoree
- Free food; maybe more