Updates on Carbapenem Resistant Organisms a Clinical Lab's Perspective

Kevin Alby, PhD, D(ABMM)
Associate Professor, Pathology and Laboratory Medicine
UNC School of Medicine
Associate Director, Clinical Microbiology Laboratory, McLendon Clinical Labs

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Disclosures

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- No relevant disclosures

Outline

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- Overview of antimicrobial resistance (AMR)
- New agents to treat carbapenem resistant organisms (CROs)
- Ways to combat AMR/Detection of AMR
- Future steps

Learning Objectives

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- Identify the methods used by the laboratory to determine carbapenem resistance
- List the antibiotics that are commonly tested for carbapenem resistant organisms
- Discuss the limitations of the methods used to test for antimicrobial resistance

Antimicrobial Resistance is not a new concept

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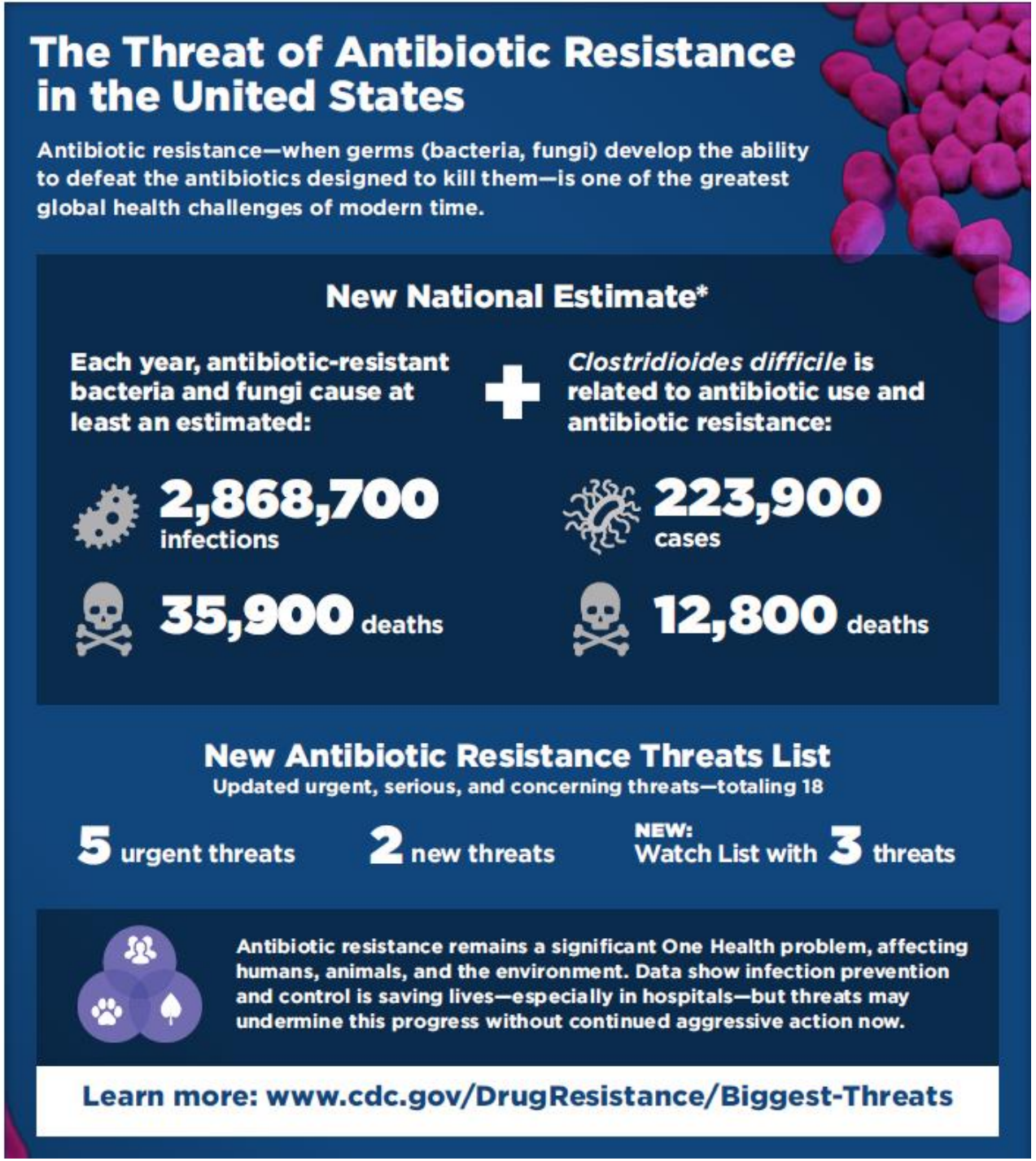
"The greatest possibility of evil in self-medication is the use of too-small doses, so that, instead of clearing up the infection, the microbes are educated to resist penicillin and a host of penicillin-fast organisms is bred out which can be passed on to other individuals and perhaps from there to others until they reach someone who gets a septicemia or a pneumonia which penicillin cannot save.

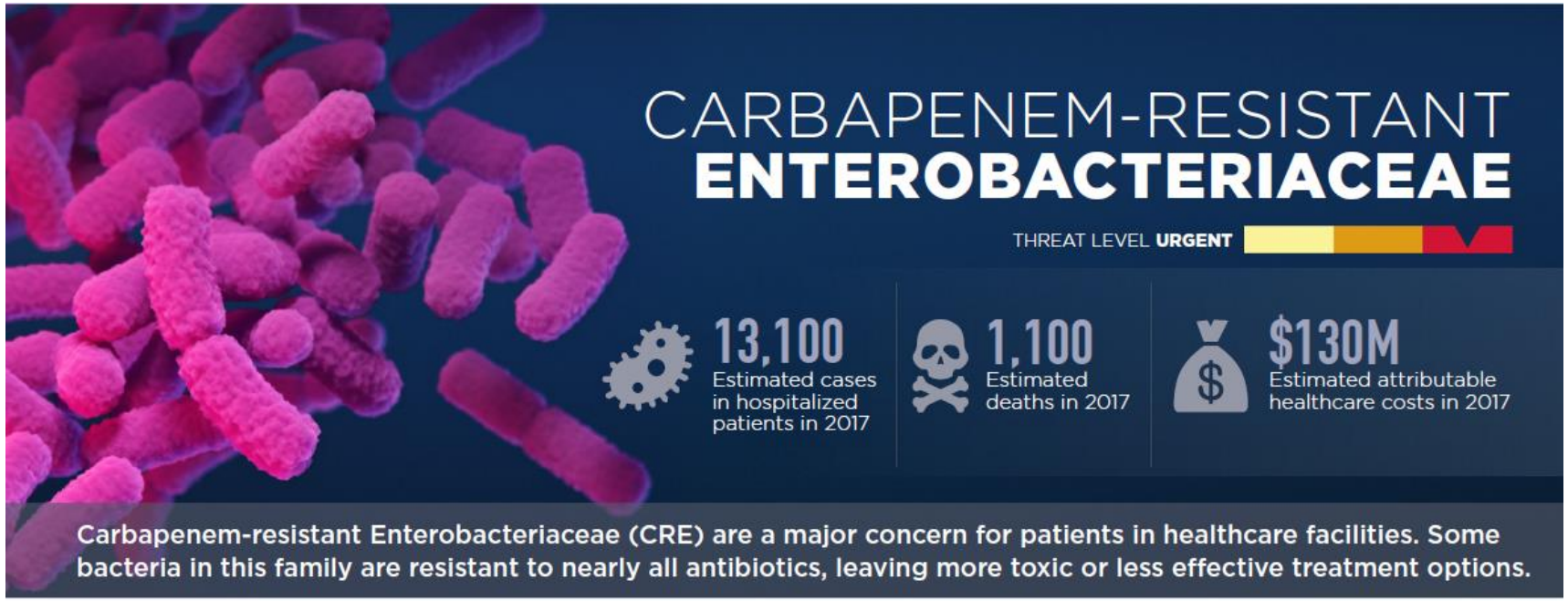
"In such a case the thoughtless person playing with penicillin treatment is morally responsible for the death of the man who finally succumbs to infection with the penicillin-resistant organism. I hope this evil can be averted."

Alexander Fleming, NY Times, June 26th, 1945

Antimicrobial resistance is a big deal



CDC Threats Report 2019



CARBAPENEM-RESISTANT ENTEROBACTERIACEAE

THREAT LEVEL **URGENT**

13,100 Estimated cases in hospitalized patients in 2017

1,100 Estimated deaths in 2017

$130M Estimated attributable healthcare costs in 2017

Carbapenem-resistant Enterobacteriaceae (CRE) are a major concern for patients in healthcare facilities. Some bacteria in this family are resistant to nearly all antibiotics, leaving more toxic or less effective treatment options.

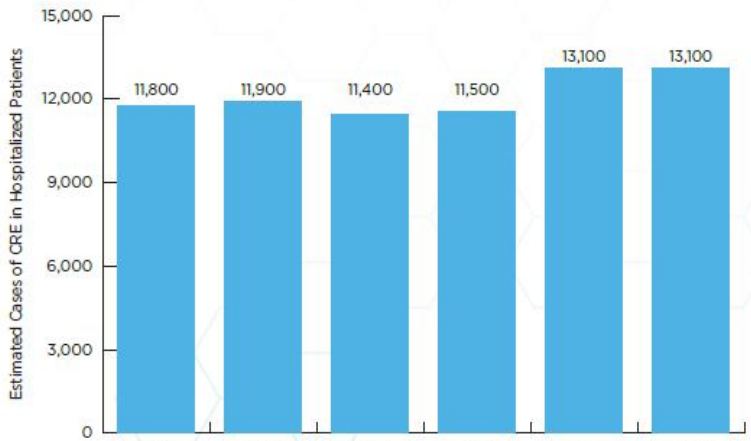
WHAT YOU NEED TO KNOW

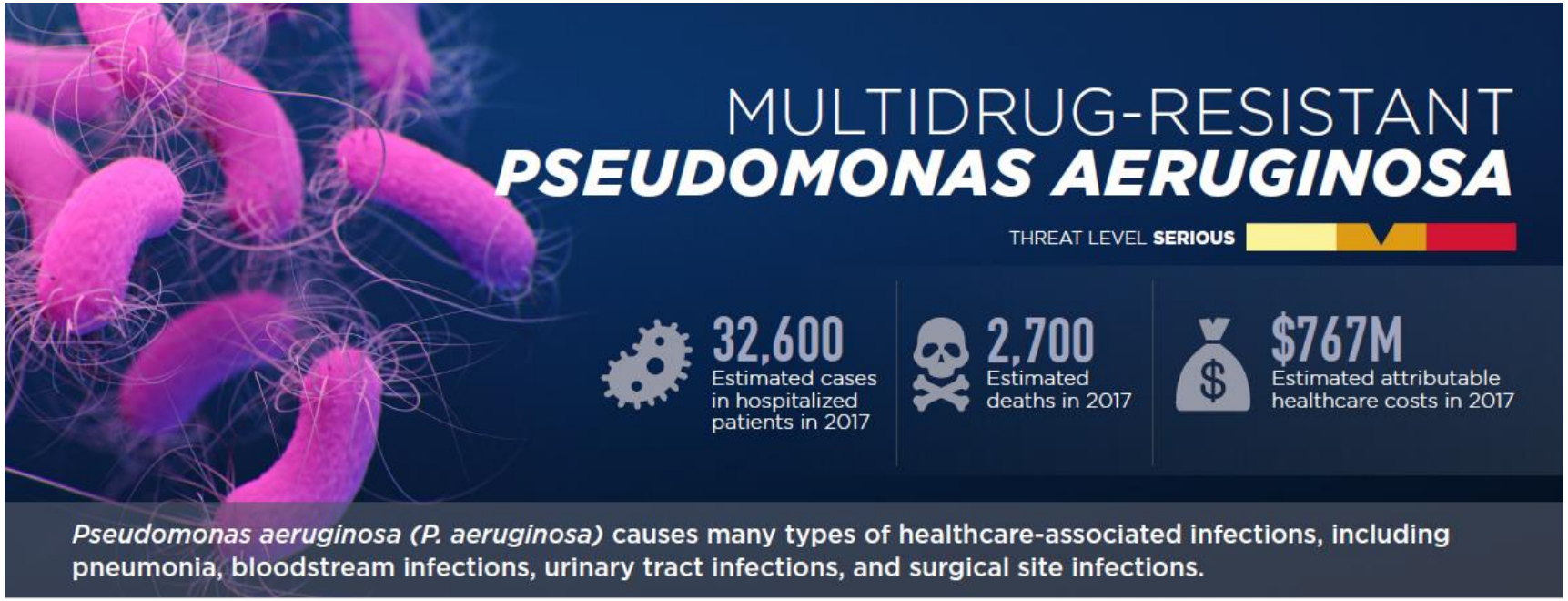
- Patients who require devices (e.g., catheters) and patients taking long courses of some antibiotics are most at risk for CRE infections.
- CRE can carry mobile genetic elements that are easily shared between bacteria. Approximately 30% of CRE carry a mobile genetic element that can make an enzyme, which makes carbapenem antibiotics ineffective and rapidly spreads resistance that destroys these important drugs.
- Preventing CRE infections and containing the spread of carbapenem resistance is important to protect people.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

CASES OVER TIME

Containment strategies have prevented further spread of some types of CRE in the United States, but continued action is needed.





MULTIDRUG-RESISTANT *PSEUDOMONAS AERUGINOSA*

THREAT LEVEL **SERIOUS**

32,600 Estimated cases in hospitalized patients in 2017

2,700 Estimated deaths in 2017

$767M Estimated attributable healthcare costs in 2017

Pseudomonas aeruginosa (P. aeruginosa) **causes many types of healthcare-associated infections, including pneumonia, bloodstream infections, urinary tract infections, and surgical site infections.**

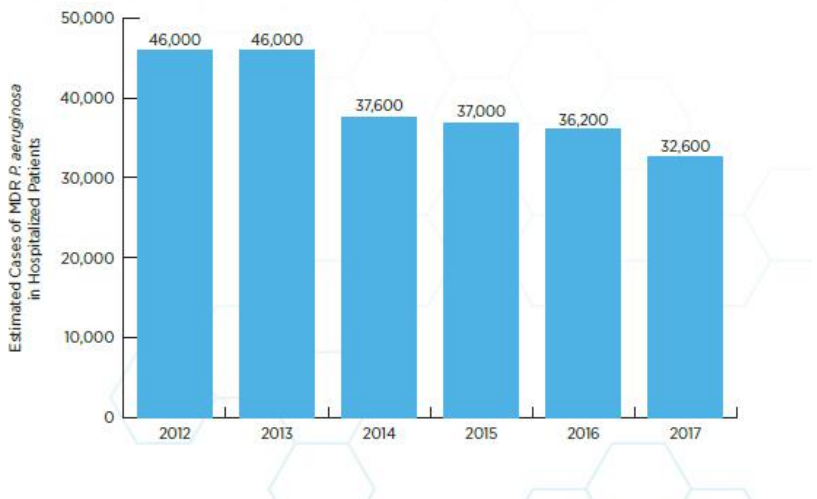
WHAT YOU NEED TO KNOW

- *P. aeruginosa* infections usually occur in people in the hospital or with weakened immune systems. It is particularly dangerous for patients with chronic lung diseases.
- Some types of multidrug-resistant (MDR) *P. aeruginosa* are resistant to nearly all antibiotics, including carbapenems.
- Two to 3% of carbapenem-resistant *P. aeruginosa* carry a mobile genetic element that makes a carbapenemase enzyme. This enzyme makes carbapenem antibiotics ineffective. Mobile genetic elements are easily shared between bacteria, rapidly spreading resistance that destroys these important drugs.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

CASES OVER TIME

Continued infection control and appropriate antibiotic use are important to maintain decreases in MDR *P. aeruginosa* infections.



A primer on beta-lactam antibiotics

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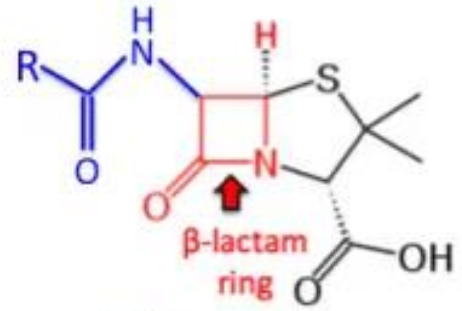
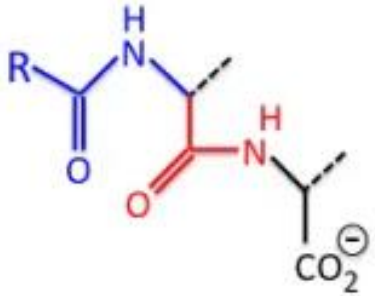
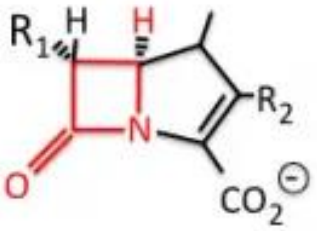


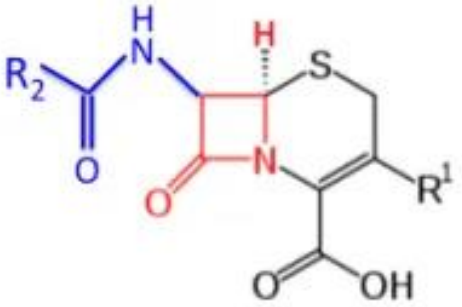
penicillins



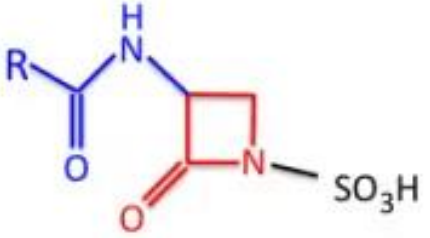
acyl-D-Ala-D-Ala (cell wall precursor)



carbapenems



cephalosporins



monobactams

Microbeonline.com

Introduction to beta-lactamase enzymes

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TABLE 1 Table of Firsts: the dates, organisms, and locations of the first of a series of β-lactamase-producing isolates with long-term clinical significance

Original β-lactamase name (currently recognized name)	Yr of first verified isolation	Organism	Location	First description in literature	Reference(s)
Penicillinase (chromosomal AmpC)	1940	*Bacillus coli* (*Escherichia coli*)	England	1940	1
Penicillinase	1942	*Staphylococcus aureus*	England	1942	65
OXA	1962	*Salmonella enterica* serovar Typhimurium, *Escherichia coli*[a]	England	1965 1967	87, 215
TEM-1	1963	*Escherichia coli*	Greece	1965	85
SHV-1	1972	*Klebsiella pneumoniae*	Unknown	1972	216
Transferable ESBL (SHV-2)	Pre-1983	*K. pneumoniae*	Germany	1983	217
Serine (class A, group 2f) carbapenemase (SME-1)	1982 1985	*Serratia marcescens*	England (London) USA (Minnesota)	1990 1986	148, 150
Plasmid-encoded AmpC (MIR-1)	1988	*K. pneumoniae*	USA (Massachusetts)	1990	141
Plasmid-encoded MBL (IMP-1)	1988	*Pseudomonas aeruginosa*	Japan	1991	151
Inhibitor-resistant TEM (TEM-30)	1991	*E. coli*	France (Paris)	1994	118
KPC-type (KPC-2)	1996	*K. pneumoniae*	USA (North Carolina)	2000	158
NDM-1	2006	*K. pneumoniae*	India (New Delhi)	2009	175, 176

Bush, *Antimicrob Agents Chemother*, 2018

A dissertation on beta-lactamase enzymes

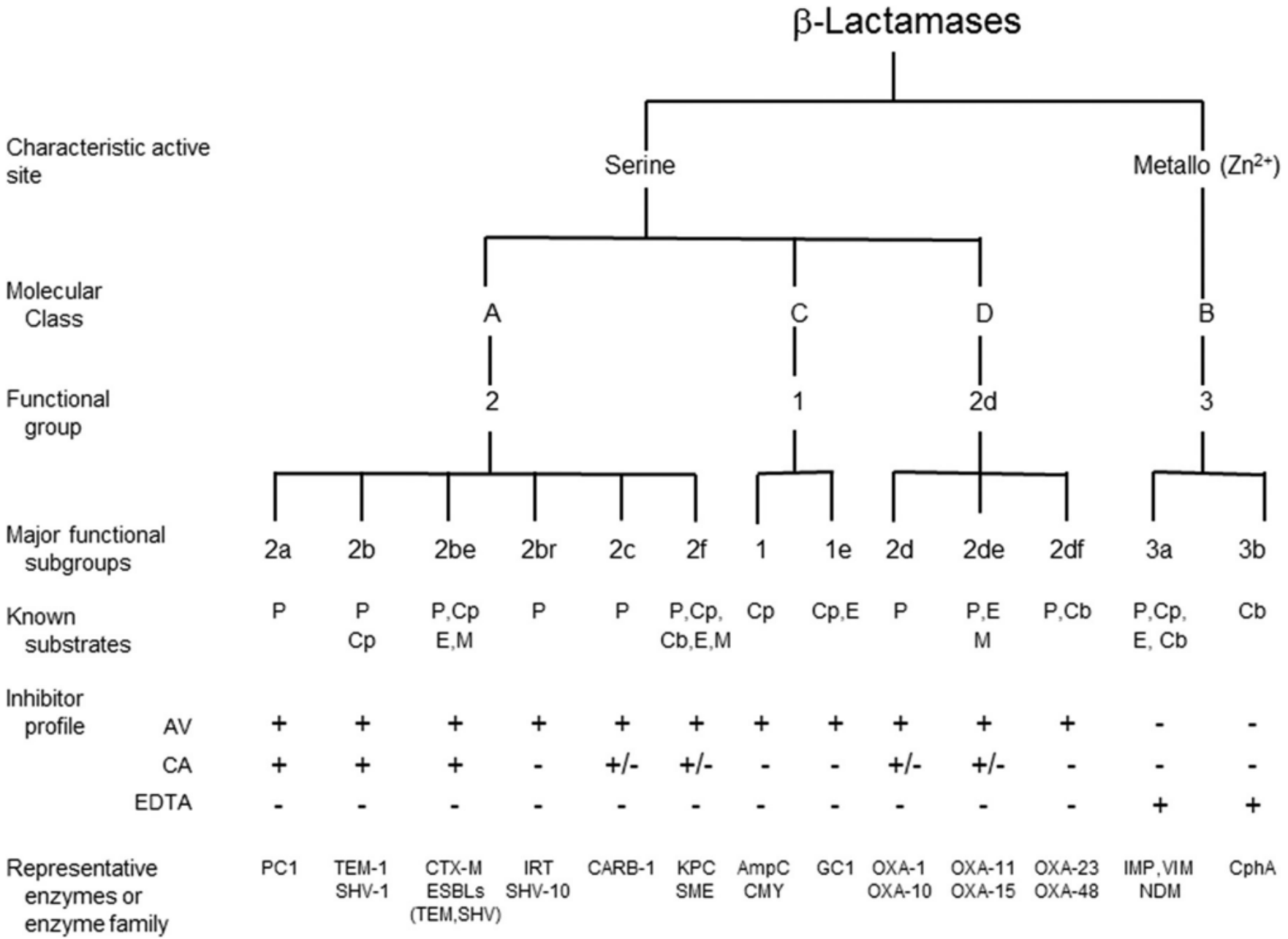

FIG 1 Molecular and functional relationships among β-lactamases (adapted from references 20 and 201 with permission). AV, avibactam; CA, clavulanic acid; Cb, carbapenem; Cp, cephalosporin; E, expanded-spectrum cephalosporin; M, monobactam; P, penicillin.

Bush, *Antimicrob Agents Chemother*, 2018

Beta-lactamase inhibitor activity

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Mechanism	Beta-lactamase inhibitor			
	Tazobactam	Avibactam	Vaborbactam	Relebactam
Class A ESBLs (SHV, TEM, CTX)	+	+	+	+
Class C ESBLs (AmpC, CMY, PDC)	+/-	+	+/-	+
Class A CP (KPC)	-	+	+	+
Class B CP (NDM, VIM, IMP)	-	-	-	-
Class D CP (OXA)	-	+	-	+/-

Ceftolozane-tazobactam

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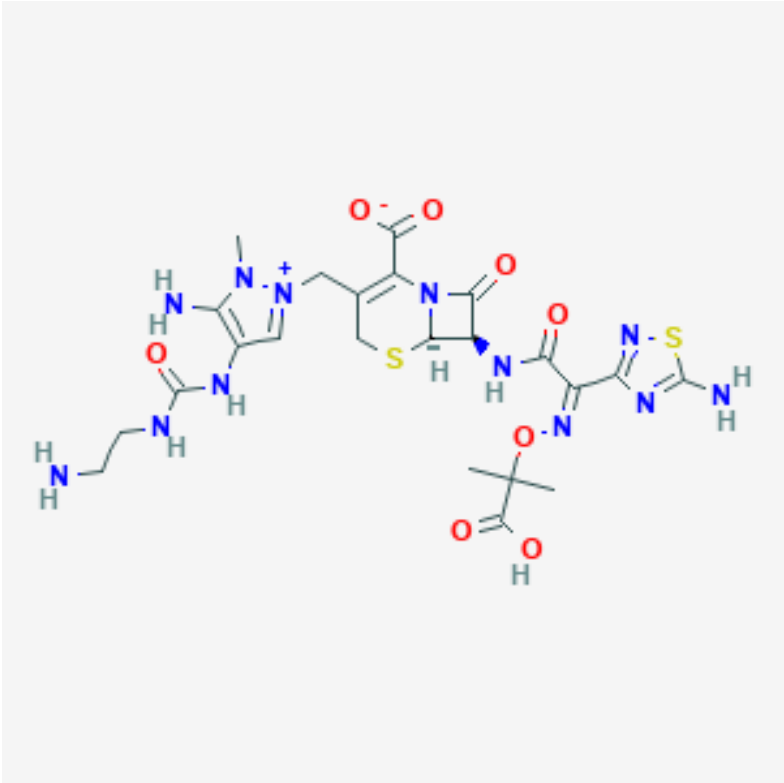

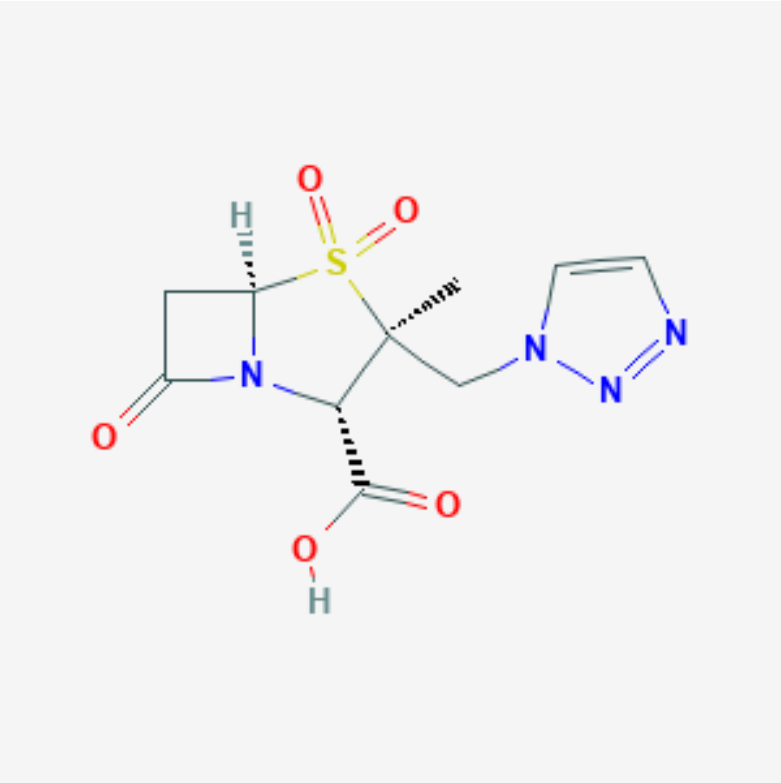

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C/T vs Levo for cUTI

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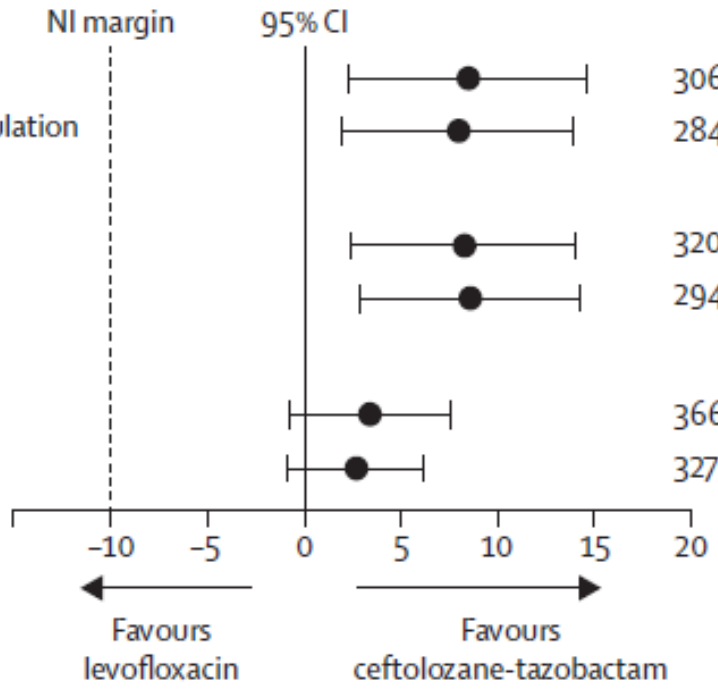

	Difference (%)	Number of patients/total (%)		Percentage difference	
		Ceftolozane-tazobactam	Levofloxacin	(95% CI)	(99% CI)
Composite cure					
Primary endpoint mMITT population		306/398 (76·9%)	275/402 (68·4%)	8·5 (2·3 to 14·6)	8·5 (0·4 to 16·5)
Secondary endpoint per-protocol population		284/341 (83·3%)	266/353 (75·4%)	8·0 (2·0 to 14·0)	8·0 (0·01 to 15·8)
Microbiological eradication					
mMITT population		320/398 (80·4%)	290/402 (72·1%)	8·3 (2·4 to 14·1)	..
Per-protocol population		294/341 (86·2%)	274/353 (77·6%)	8·6 (2·9 to 14·3)	..
Clinical cure					
mMITT population		366/398 (92·0%)	356/402 (88·6%)	3·4 (−0·7 to 7·6)	..
Per-protocol population		327/341 (95·9%)	329/353 (93·2%)	2·7 (−0·8 to 6·2)	..

Wagenlehner *Lancet* 2015 16;385

C/T for CR-*Pseudomonas*

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Table 2. Antimicrobial susceptibility profiles of carbapenem-resistant *P. aeruginosa*, 2007–16 (n=466)

	MIC (mg/L)			MIC breakpoint interpretations[a]		
Antimicrobial agent	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R
Amikacin	8	>64	≤1 to ≥64	80.7	6.9	12.4
Ceftazidime	16	>32	1 to ≥32	41.2	17.2	41.6
Ceftolozane/tazobactam	1	4	0.25 to ≥64	92.5	3	4.5
Ciprofloxacin	2	>16	≤0.06 to ≥16	37.6	15	47.4
Colistin	1	2	0.12 to ≥16	94	0	6
Meropenem	16	32	8 to ≥64	0	0	100
Piperacillin/tazobactam	32	256	≤1 to ≥512	43.1	26	30.9
Tobramycin	1	64	≤0.5 to ≥64	72.7	3.7	23.6

[a]Using CLSI M100 27th edition (2017) MIC interpretative breakpoints.

McCracken *J Antimicrob Chemother* 2019; 74(S4)

Ceftazidime-avibactam (C/A)

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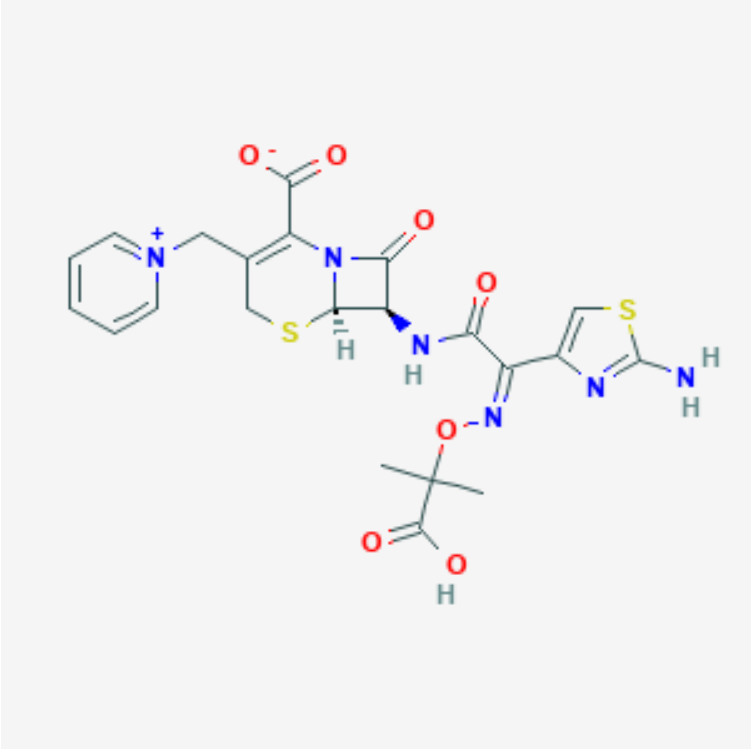

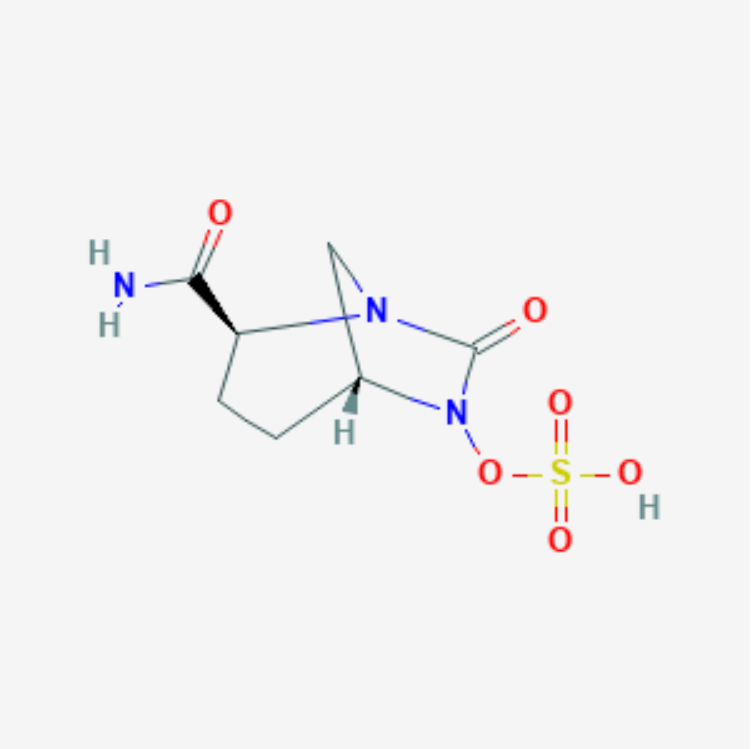

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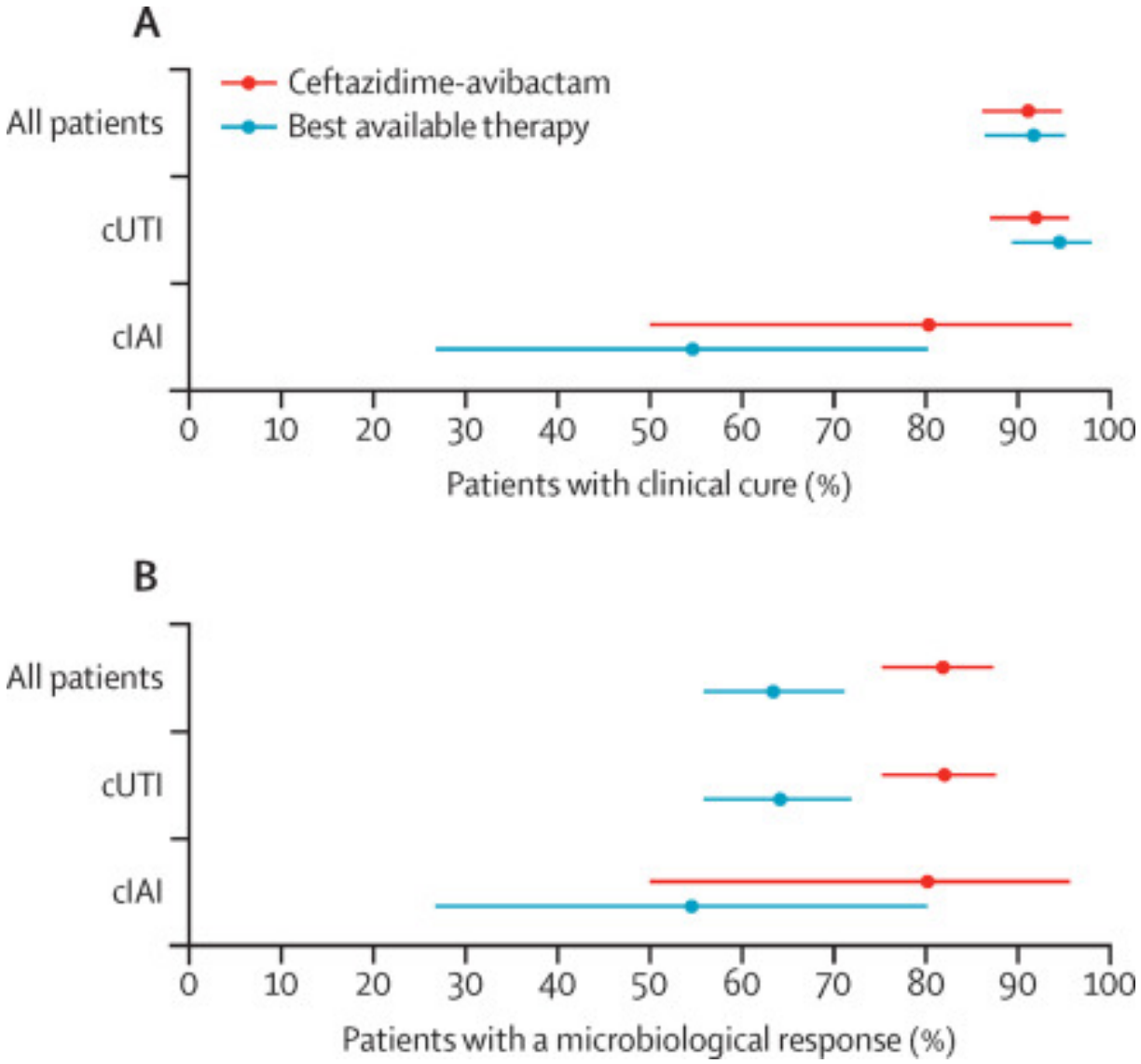
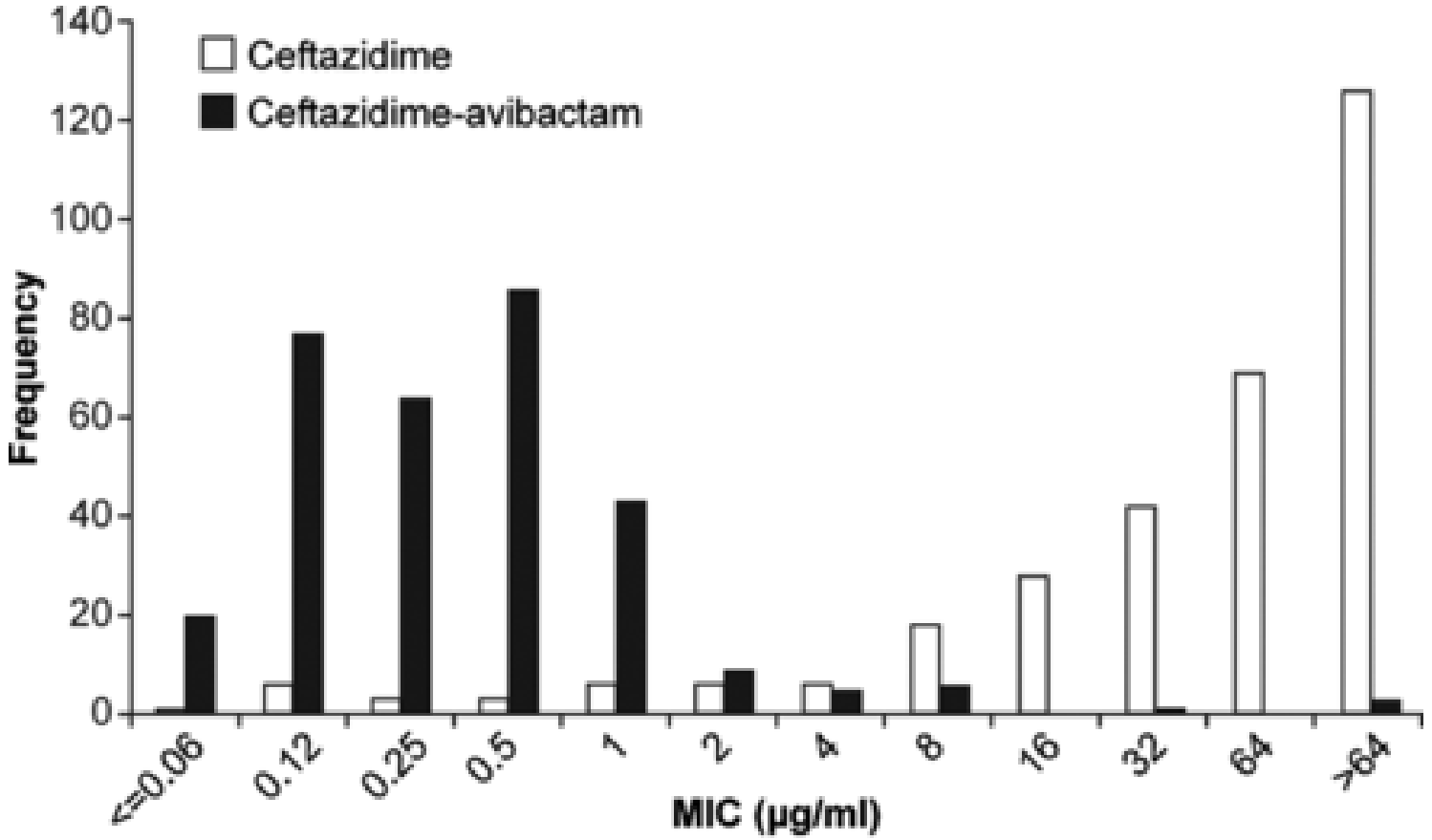
Clinical performance of C/A vs BAT



Carmeli, *Lancet Inf Dis*, 2016. 16(6)

Impact of avibactam on ceftazidime MICs of *Enterobacterales*



Stone, *Antimicrob Agents Chemother*, 2017. 61(2)

Activity of C/A against MDR *Pseudomonas* and *Enterobacterales*

Table 2
Antimicrobial susceptibility of resistant subsets of *Enterobacteriaceae* and *P. aeruginosa* isolates from all infection types combined (2015-2016).

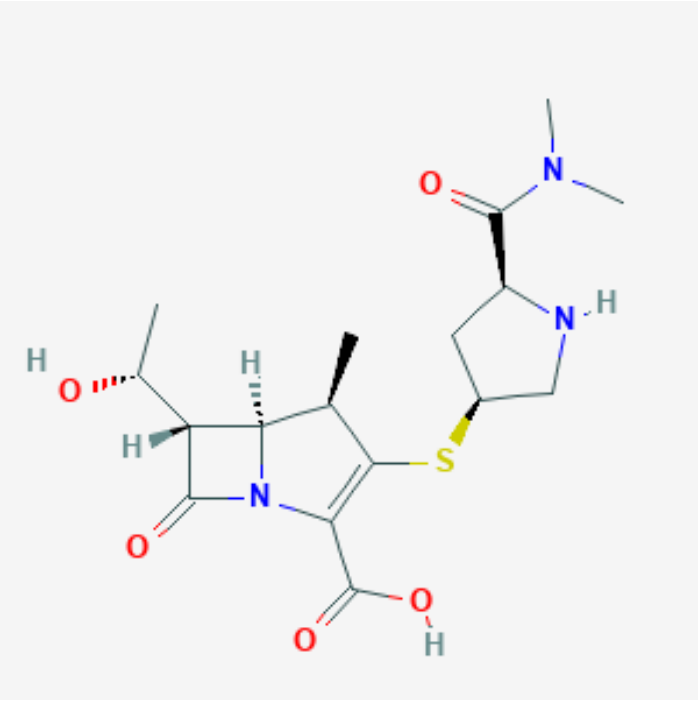
Antimicrobial agent	MIC_{50}	MIC_{90}	CLSI[a]		EUCAST[a]	
			%S	%R	%S	%R
Enterobacteriaceae						
MDR (1,528)[b]						
Ceftazidime-avibactam	0.25	1	99.3[c]	0.7	99.3	0.7
Ceftazidime	32	>32	29.5	65.5	21.7	70.5
Cefepime	16	>16	36.7	51.9	31.4	57.6
Ceftriaxone	>8	>8	19.8	77.0	19.8	77.0
Piperacillin-tazobactam	16	>64	55.7	26.9	48.4	44.3
Meropenem	0.06	8	83.8	13.5	86.5	7.6
Levofloxacin	>4	>4	21.4	69.6	11.9	84.0
Gentamicin	>8	>8	38.5	50.4	34.9	61.5
Amikacin	4	16	92.8	1.7	86.4	7.2
Tigecycline[c]	0.5	4	86.2	0.4	72.3	13.8
Colistin	0.25	>8			61.6	38.4
XDR (212)[d]						
Ceftazidime-avibactam	0.5	4	97.6[c]	2.4	97.6	2.4
Ceftazidime	>32	>32	8.0	89.6	3.3	92.0
Cefepime	>16	>16	10.4	79.7	6.6	85.8
Ceftriaxone	>8	>8	2.4	96.7	2.4	96.7
Piperacillin-tazobactam	>64	>64	10.8	80.2	10.8	89.2
Meropenem	8	>32	24.1	67.9	32.1	43.4
Levofloxacin	>4	>4	9.9	81.6	0.9	97.2
Gentamicin	8	>8	23.1	48.1	21.7	76.9
Amikacin	8	32	65.6	9.4	54.7	34.4
Tigecycline[c]	1	4	85.4	0.5	72.2	14.6
Colistin	0.25	>8			61.4	38.6
CRE (238)[e]						
Ceftazidime-avibactam	0.5	2	97.5	2.5[c]	97.5	2.5
Ceftazidime	>32	>32	4.2	93.3	2.1	95.8
Cefepime	>16	>16	8.8	78.2	3.4	87.0
Ceftriaxone	>8	>8	1.7	97.9	1.7	97.9
Piperacillin-tazobactam	>64	>64	3.0	89.5	3.0	97.0
Meropenem	8	>32	3.4	88.2	11.8	49.6
Levofloxacin	>4	>4	24.8	70.2	14.3	81.5
Gentamicin	8	>8	46.6	30.7	42.9	53.4
Amikacin	8	32	73.1	6.7	58.8	26.9
Tigecycline[c]	0.5	2	98.3	0.0	87.0	1.7
Colistin	0.12	>8			80.5	19.5

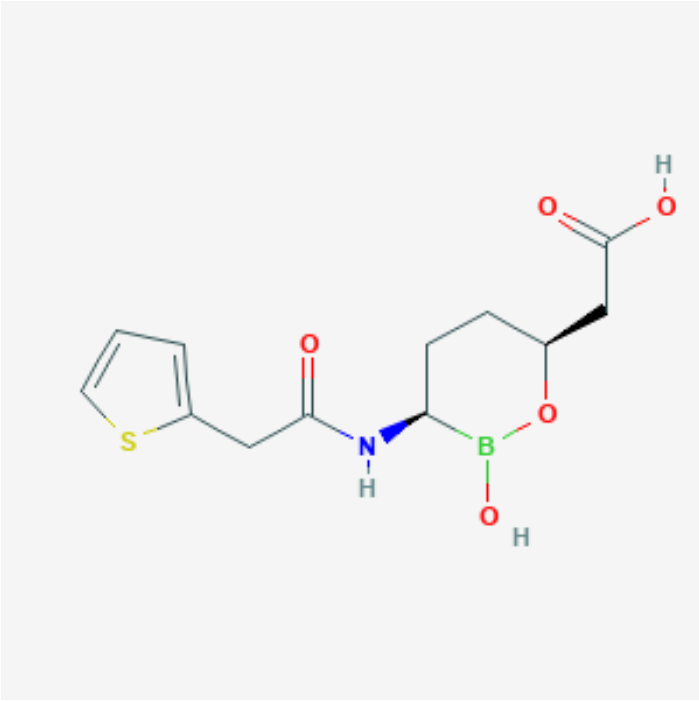
Table 2
Antimicrobial susceptibility of resistant subsets of *Enterobacteriaceae* and *P. aeruginosa* isolates from all infection types combined (2015-2016).

Antimicrobial agent	MIC_{50}	MIC_{90}	CLSI[a]		EUCAST[a]	
			%S	%R	%S	%R
P. aeruginosa						
MDR (879)						
Ceftazidime-avibactam	4	16	89.3[c]	10.7	89.3	10.7
Ceftazidime	16	>32	48.5	36.9	48.5	51.5
Cefepime	16	>16	45.8	16.8	45.8	54.2
Piperacillin-tazobactam	32	>64	34.6	31.9	34.6	65.4
Meropenem	8	32	20.9	59.6	20.9	35.5
Levofloxacin	>4	>4	20.0	62.3	9.1	90.9
Gentamicin	4	>8	53.0	30.8	53.0	47.0
Tobramycin	1	>8	74.7	20.8	74.7	25.3
Amikacin	8	32	85.8	7.6	70.1	14.2
Colistin	1	1	99.1	0.9	99.1	0.9
XDR (393)						
Ceftazidime-avibactam	4	16	80.4[c]	19.6[c]	80.4	19.6
Ceftazidime	32	>32	23.7	54.2	23.7	76.3
Cefepime	16	>16	20.9	30.8	20.9	79.1
Piperacillin-tazobactam	64	>64	7.1	47.8	7.1	92.9
Meropenem	16	32	5.3	80.4	5.3	56.2
Levofloxacin	>4	>4	6.9	75.0	2.3	97.7
Gentamicin	8	>8	37.4	45.3	37.4	62.6
Tobramycin	2	>8	63.3	31.5	63.3	36.7
Amikacin	8	>32	78.6	12.5	59.3	21.4
Colistin	1	1	99.0	1.0	99.0	1.0
β-Lactam-nonsusceptible (329)[f]						
Ceftazidime-avibactam	4	16	76.0[c]	24.0[c]	76.0	24.0
Ceftazidime	32	>32	0.0	70.5	0.0	100.0
Cefepime	16	>16	21.3	33.4	21.3	78.7
Piperacillin-tazobactam	>64	>64	0.0	60.5	0.0	100.0
Meropenem	16	32	0.0	83.9	0.0	55.3
Levofloxacin	>4	>4	22.9	62.8	11.9	88.1
Gentamicin	4	>8	51.7	36.8	51.7	48.3
Tobramycin	1	>8	67.9	28.1	67.9	32.1
Amikacin	8	>32	80.2	11.9	66.9	19.8
Colistin	1	1	99.4	0.6	99.4	0.6

Sadler, Dign Microbiol Infect Dis, 2018. 92(1)

Meropenem-vaborbactam (M/V)





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M/V vs Piperacillin-tazobactam for cUTI

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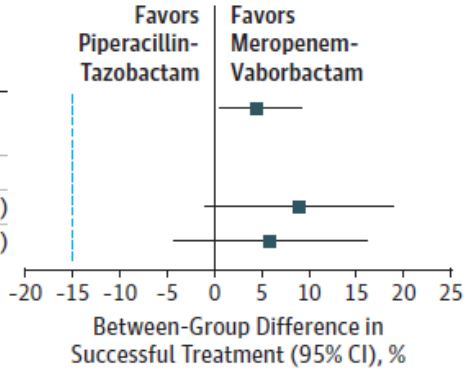
Figure 2. Primary and Secondary Study End Points

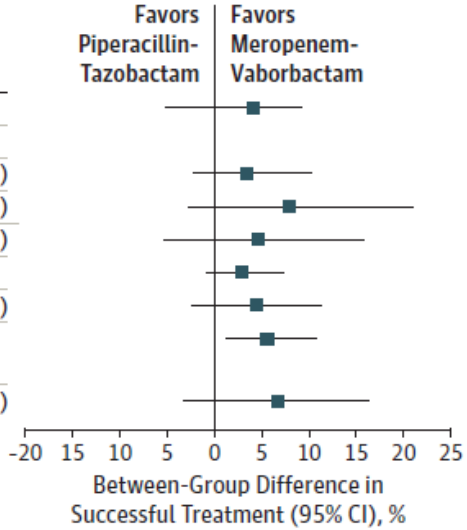
A Primary end points

	No. of Patients Successfully Treated/Total No. (%)		Between-Group Difference (95% CI), %
	Meropenem-Vaborbactam	Piperacillin-Tazobactam	
FDA primary: overall success at end of intravenous treatment (microbiologic MITT analysis)[a,b]	189/192 (98.4)	171/182 (94.0)	4.5 (0.7 to 9.1)
EMA primary: microbial eradication at test of cure			
Microbiologic MITT analysis[b]	128/192 (66.7)	105/182 (57.7)	9.0 (-0.9 to 18.7)
Microbiologic evaluable analysis	118/178 (66.3)	102/169 (60.4)	5.9 (-4.2 to 16.0)



B Secondary end points

	No. of Patients Successfully Treated/Total No. (%)		Between-Group Difference (95% CI), %
	Meropenem-Vaborbactam	Piperacillin-Tazobactam	
Overall success at test of cure[a]	143/192 (74.5)	128/182 (70.3)	4.1 (-4.9 to 9.1)
Overall success at end of intravenous treatment[a]			
Acute pyelonephritis	117/120 (97.5)	95/101 (94.1)	3.4 (-2.0 to 10.2)
Complicated UTI, removable infection source[c]	35/35 (100)	35/38 (92.1)	7.9 (-2.5 to 20.9)
Complicated UTI, nonremovable infection source	37/37 (100)	41/43 (95.3)	4.7 (-5.1 to 15.6)
Clinical cure at end of intravenous treatment[d]	189/192 (98.4)	174/182 (95.6)	2.8 (-0.7 to 7.1)
Clinical cure at test of cure	174/192 (90.6)	157/182 (86.3)	4.4 (-2.2 to 11.1)
Microbial eradication at end of intravenous treatment (FDA criteria)	188/192 (97.9)	168/182 (92.3)	5.6 (1.4 to 10.7)
Microbial eradication at test of cure (FDA criteria)	132/192 (68.8)	113/182 (62.1)	6.7 (-3.0 to 16.2)



Kaye, *JAMA*, 2018. 319(8)

Activity of meropenem-vaborbactam (M/V)

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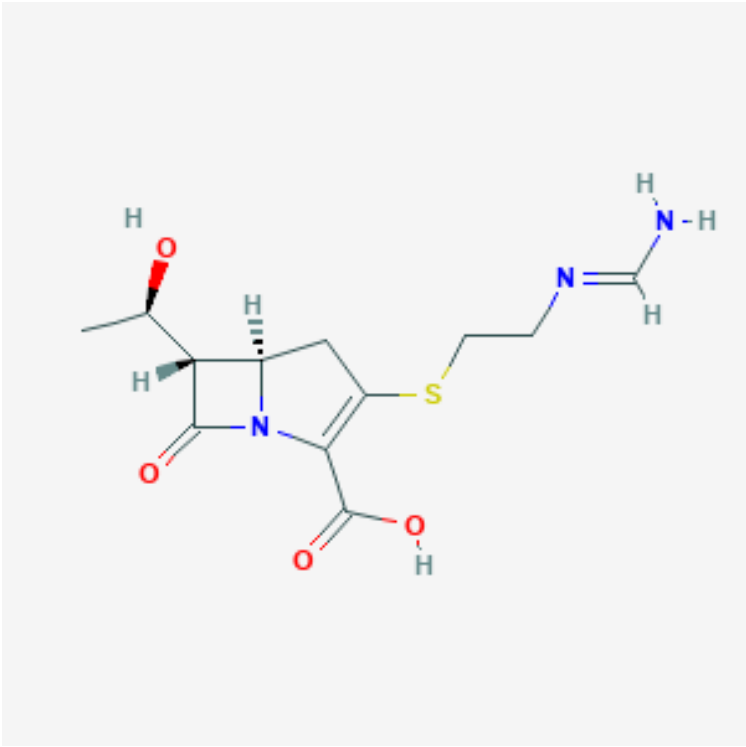
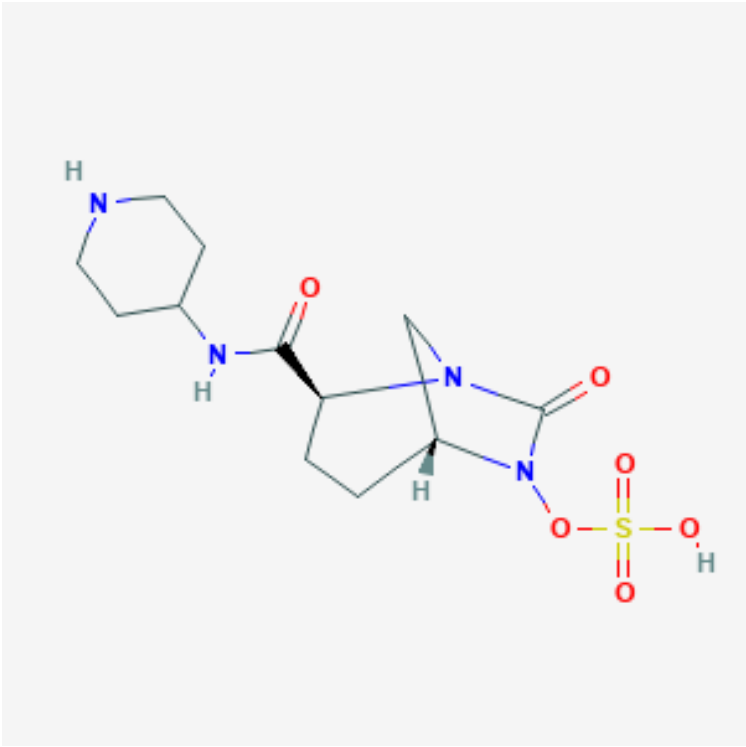
TABLE 1 *In vitro* activities of meropenem-vaborbactam and comparator agents against 991 clinical isolates of KPC-positive *Enterobacteriaceae*

Family, genus, or species[a] (no. of isolates)	Antimicrobial agent(s)	MIC[b] (μg/ml)			% of isolates with the following MIC interpretation[c]:		
		Range	50%	90%	Susceptible	Intermediate	Resistant
All *Enterobacteriaceae*[d] (991)	Meropenem-vaborbactam	≤0.03 to >32	0.06	1	99.0	0.6	0.4
	Meropenem	2 to >32	32	>32	0	4.1	95.9
	Ceftazidime-avibactam	≤0.06 to >64	1	4	98.2		1.8
	Ceftazidime	1 to >64	>64	>64	3.0	2.5	94.5
	Tigecycline	≤0.06 to 8	1	2	95.8	3.6	0.6
	Minocycline	0.5 to >64	8	32	44.5	30.4	25.1
	Gentamicin	≤0.06 to >64	1	>64	63.4	6.3	30.4
	Polymyxin B	0.25 to >16	0.5	16	NA	NA	NA

Hackel, *Antimicrob Agents Chemother*, 2018. 62(1)

Imipenem-relebactam





PubChem

I/R vs imipenem+colistin for imipenem non-susceptible GNRs

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	IMI/REL (n = 21)		Colistin + IMI (n = 10)		Unadjusted Difference	Adjusted Difference[a]	
Endpoint	n	% (95% CI)[b]	n	% (95% CI)[a]	%	%	90% CI
Primary endpoint							
Favorable overall response[c]	15	71.4 (49.8, 86.4)	7	70.0 (39.2, 89.7)	1.4	–7.3	(–27.5, 21.4)
Hospital-acquired bacterial pneumonia/ ventilator-associated bacterial pneumonia	7/8	87.5 (50.8, 99.9)	2/3	66.7		20.8	
Complicated intraabdominal infection	0/2[d]	0.0	0/2[e]	0.0		0.0	
Complicated urinary tract infection	8/11	72.7 (42.9, 90.8)	5/5	100.0 (51.1, 100.0)		–27.3 (–52.8, 12.8)	
Secondary endpoints							
Favorable clinical response (day 28)	15[f]	71.4 (49.8, 86.4)	4[g]	40.0 (16.7, 68.8)	31.4	26.3	(1.3, 51.5)
28-day all-cause mortality	2	9.5 (1.4, 30.1)	3	30.0 (10.3, 60.8)	–20.5	–17.3	(–46.4, 6.7)
Treatment-emergent nephrotoxicity[h]	3/29	10.3 (2.8, 27.2)	9/16	56.3 (33.2, 76.9)		–45.9 (–69.1, –18.4)	

Motsch *Clin Infect Dis* 2019

Activity of I/R against MDR *Pseudomonas*

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Table 2
In vitro activity of imipenem-relebactam against the 10 most common MDR phenotypes of *P. aeruginosa*.

MDR phenotype[a]	*n* (% of all MDR phenotypes)	% of isolates with each specific MDR phenotype that were susceptible to imipenem-relebactam[b]
Aztreonam, cefepime, piperacillin-tazobactam, imipenem, ciprofloxacin	209 (27.9)	67.0
Aztreonam, cefepime, piperacillin-tazobactam	136 (18.1)	100
Aztreonam, cefepime, piperacillin-tazobactam, imipenem	87 (11.6)	75.9
Aztreonam, cefepime, piperacillin-tazobactam, ciprofloxacin	53 (7.1)	100
Aztreonam, piperacillin-tazobactam, imipenem, ciprofloxacin	32 (4.3)	87.5
Aztreonam, piperacillin-tazobactam, imipenem	32 (4.3)	75.0
Aztreonam, cefepime, piperacillin-tazobactam, imipenem, ciprofloxacin, amikacin	31 (4.1)	41.9
Aztreonam, imipenem, ciprofloxacin	31 (4.1)	80.6
Aztreonam, piperacillin-tazobactam, ciprofloxacin	30 (4.0)	100
Aztreonam, cefepime, piperacillin-tazobactam, imipenem, amikacin	9 (1.2)	55.6
Total for 10 most common MDR phenotypes	650 (86.7)[c]	80.0

Karlowsky *Diagn Microbiol Infect Dis* 2019; 95(2)

Learning Assessment #1

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- Which of the following beta-lactam/beta-lactam inhibitor combinations is rarely effective against carbapenem resistant *Pseudomonas aeruginosa*?
- Ceftazidime/Avibactam
- Ceftolozane/Tazobactam
- Meropenem/Vaborbactam
- Imipenem/Relebactam

Learning Assessment #1 (Answer)

UNC SCHOOL OF MEDICINE

- Which of the following beta-lactam/beta-lactam inhibitor combinations is rarely effective against carbapenem resistant *Pseudomonas aeruginosa*?
- Ceftazidime/Avibactam
- Ceftolozane/Tazobactam
- **Meropenem/Vaborbactam**
- Imipenem/Relebactam

Vaborbactam is most useful for inhibiting serine carbapenemases (e.g. KPC) which are rarely found in Pseudomonas as the cause of CRPA. The other inhibitors have good activity against the PDC enzymes that can lead to carbapenem resistance

Determination of Resistance to Antimicrobials

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- Phenotypic Determination
  - Broth dilution
  - Disk Diffusion
  - Gradient Testing
  - Commercial Systems
- Genetic Determination
  - PCR
  - Whole Genome Sequencing (WGS)

Phenotypic Methods

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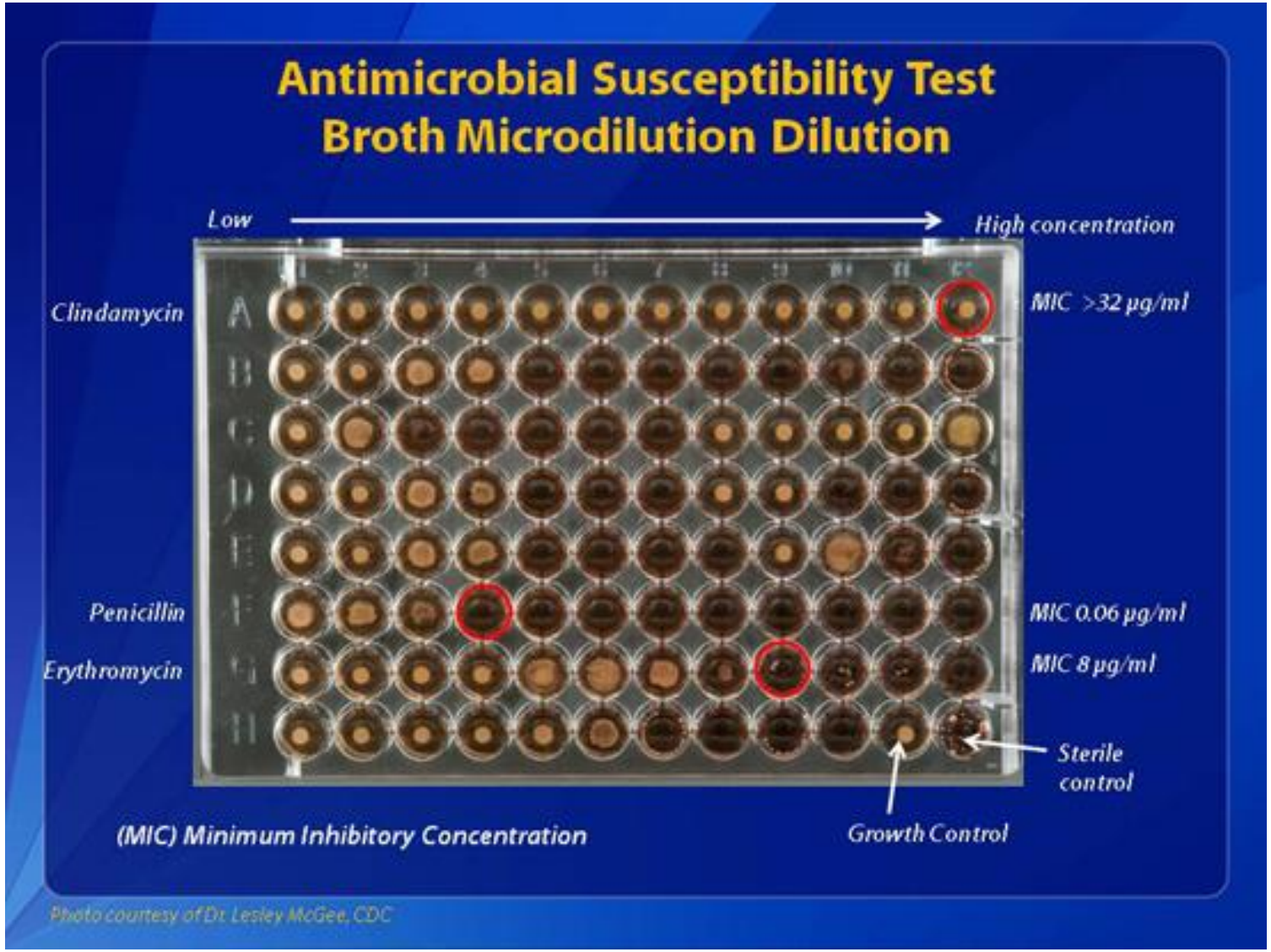
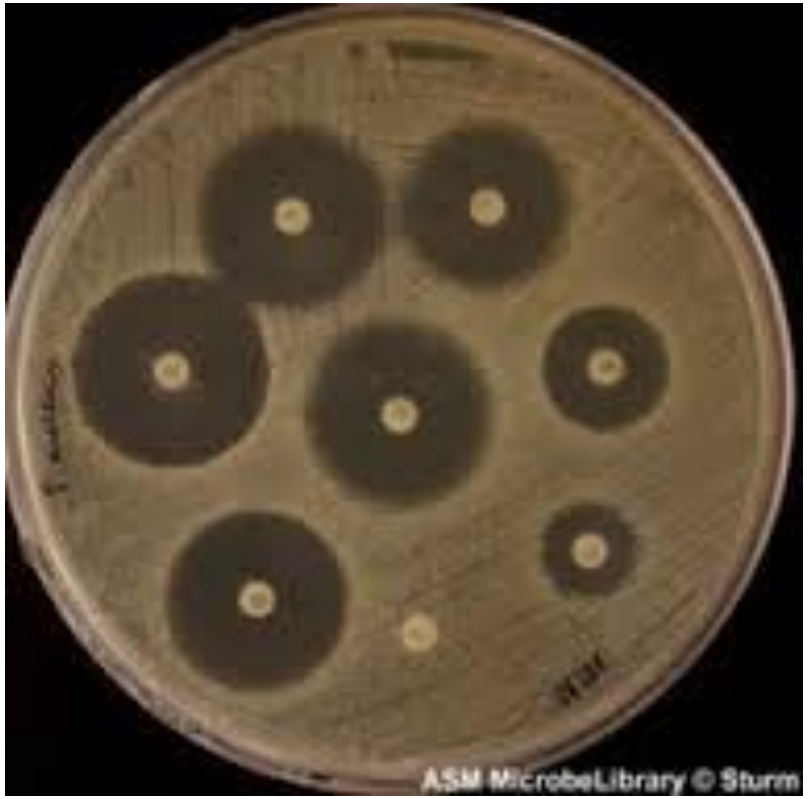
- Most rely on the determination or approximation of a minimum inhibitory concentration or MIC
- Disk diffusion utilizes the zone of inhibition
- Interpretations are performed using established standards or manufacturers recommendations

Broth Microdilution

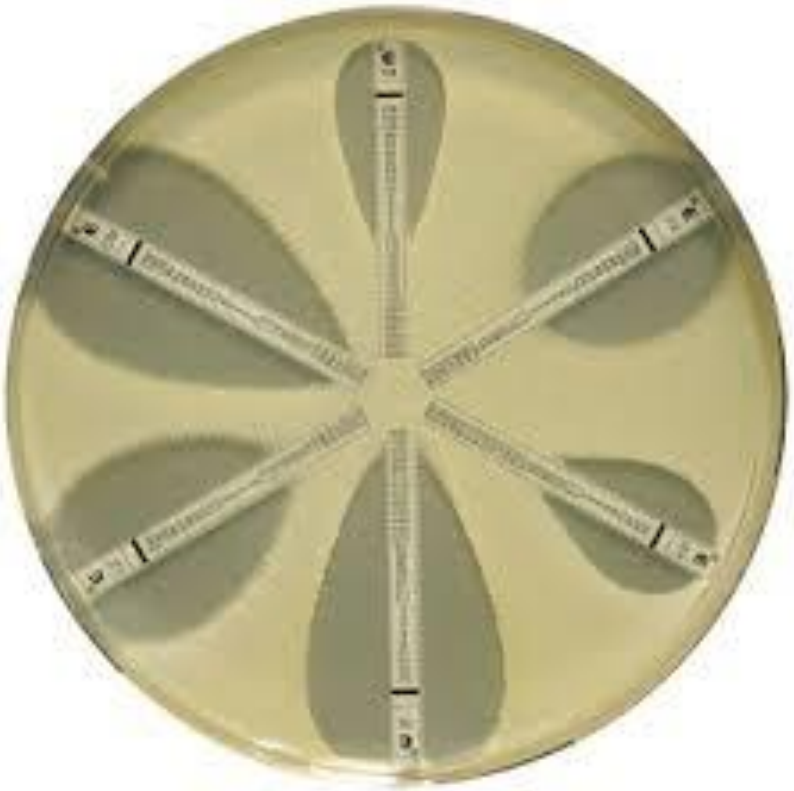


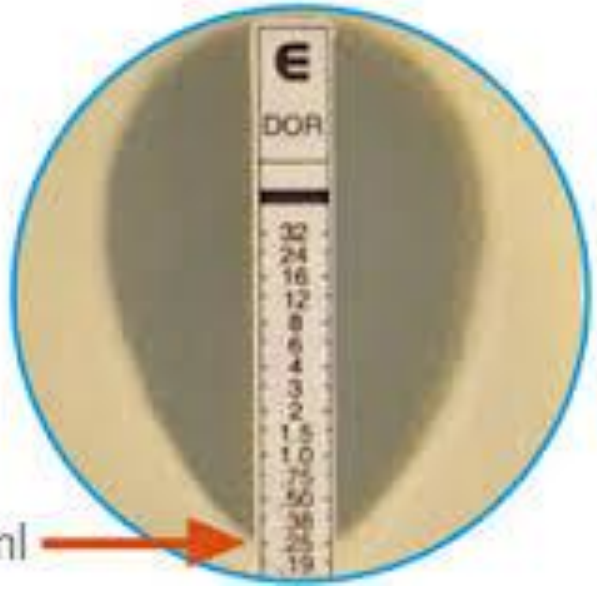
Disk diffusion



Disk diffusion requires the availability of interpretive criteria as zone diameter itself is not readily useful information!

Gradient diffusion





Automated commercial systems

Antimicrobial Testing

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- Not all systems are created equal
- Not all systems/methods have all the new agents available for testing
- A multipronged approach is often needed

Learning Assessment #2

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- Which of the following is true regarding susceptibility testing for antimicrobial resistance?
- Disk diffusion provides an MIC result
- Disk diffusion requires interpretive criteria
- Broth microdilution requires interpretive criteria
- CLSI is the only interpretive criteria used in the US

Learning Assessment #2

UNC SCHOOL OF MEDICINE

- Which of the following is true regarding susceptibility testing for antimicrobial resistance?
- Disk diffusion provides an MIC result
- **Disk diffusion requires interpretive criteria**
- Broth microdilution requires interpretive criteria
- CLSI is the only interpretive criteria used in the US

Zone diameters cannot be used without interpretive criteria

DD provides zone diameters

BMD provides reference MICs which can be used without associated interpretive criteria

CLSI, FDA, and EUCAST all have interpretive criteria that are used in the US

Imipenem-Relebactam a case study

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- Currently only one commercial panel contains imipenem-relebactam
- Gradient strips and disks are available
- Strips may be preferred over disks because of the ability to report an MIC without needed interpretive criteria

Performance of LFC strips vs AR Bank isolates

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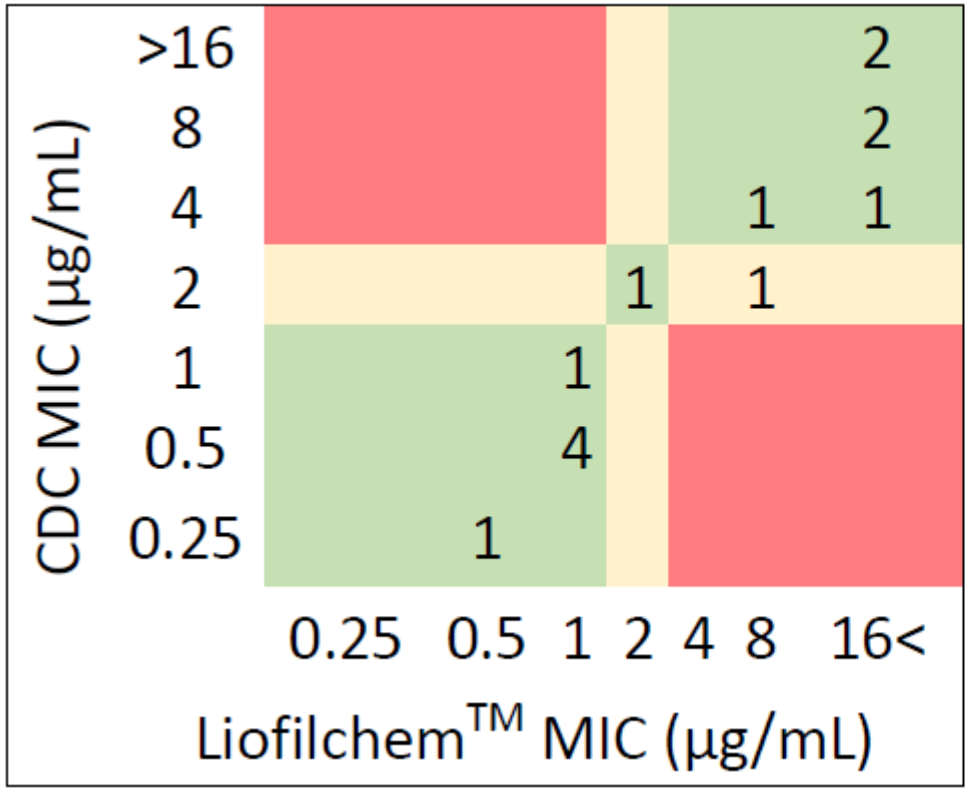
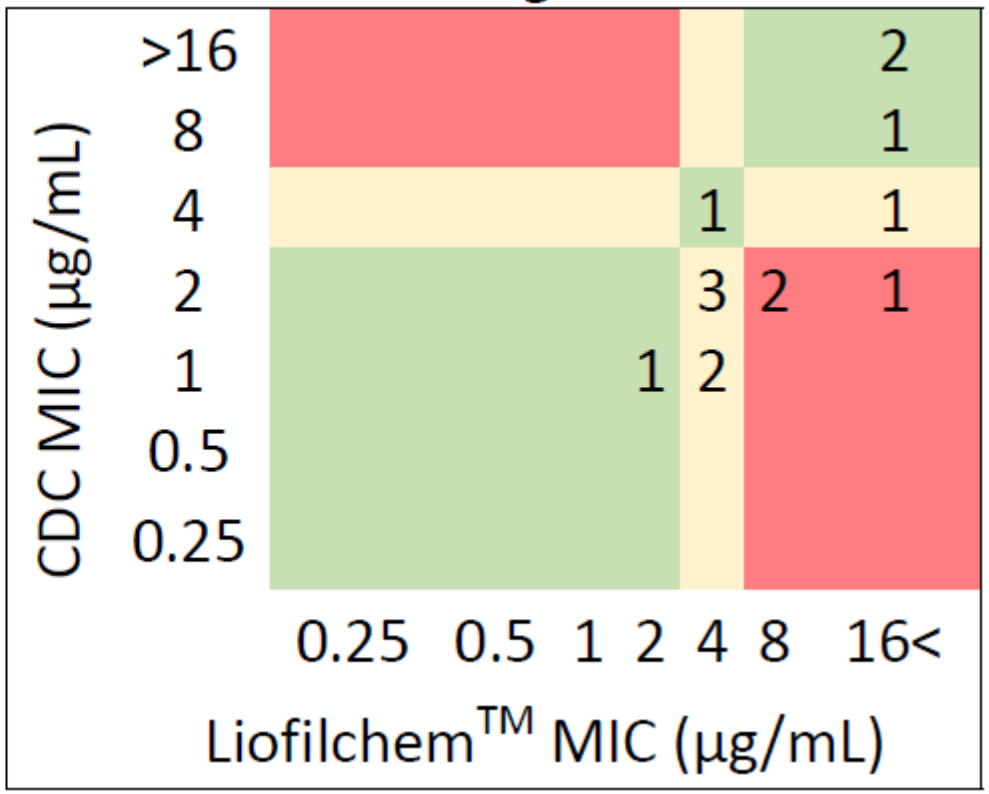
Enterobacterales



Pseudomonas aeruginosa



Performance of bioMerieux Etest® strips against AR Bank isolates

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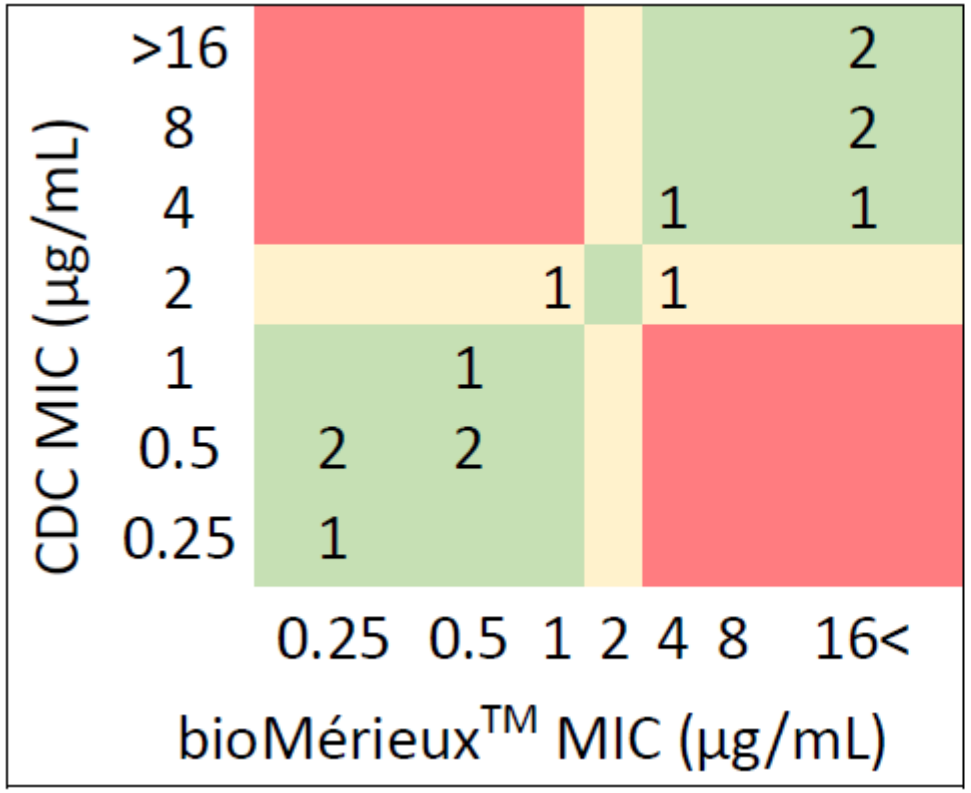
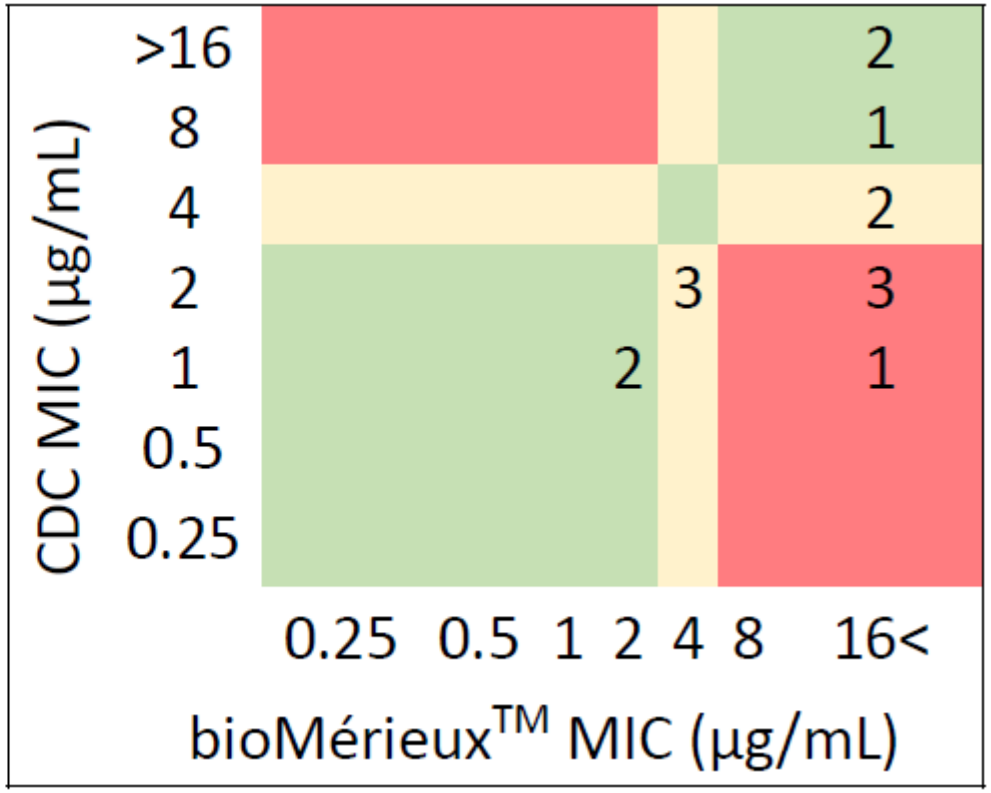
Enterobacterales



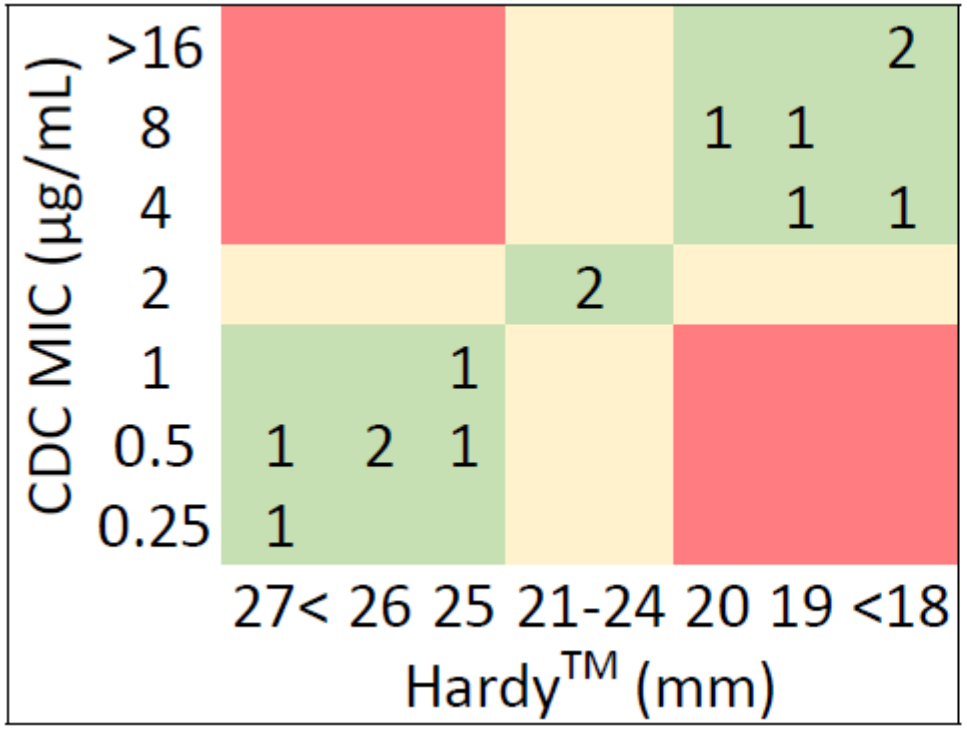
Pseudomonas aeruginosa



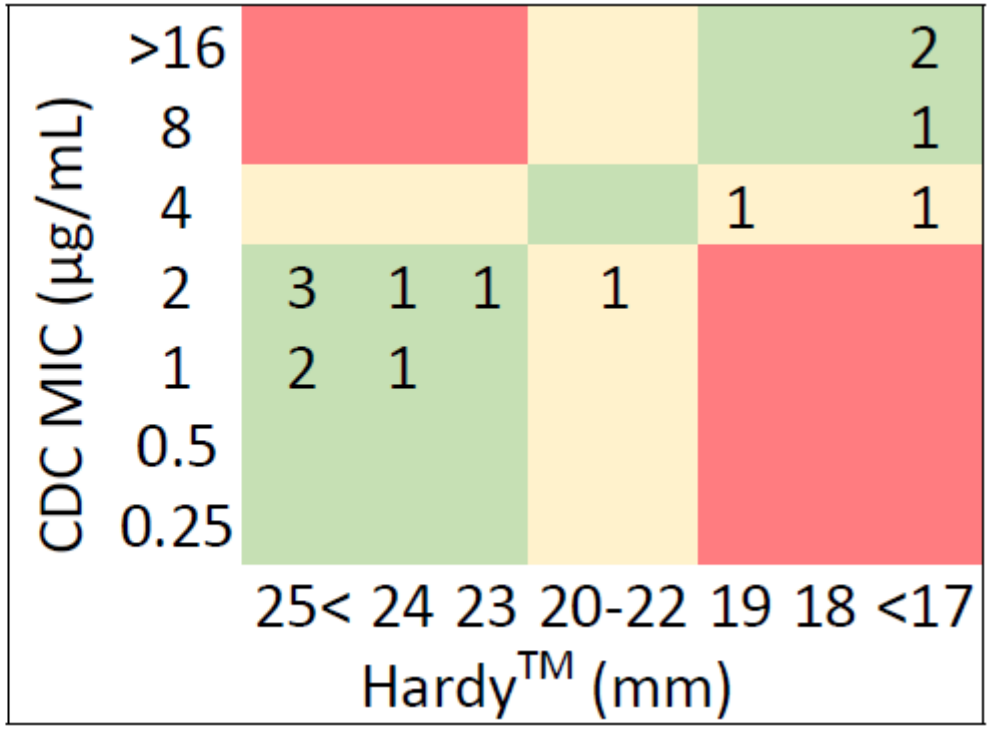
Performance of Hardy disk against AR Bank isolates

UNC SCHOOL OF MEDICINE

Enterobacterales



Pseudomonas aeruginosa



Performance Summary

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- Based on our evaluation we adopted the disk diffusion methodology for testing imipenem-relebactam

Enterobacterales	Categorical Agreement	Minor Error
Liofilchem™	92.9%	7.1%
bioMérieux™	85.7%	14.3%
Hardy™	100.0%	--

P. aeruginosa	Categorical Agreement	Minor Error	Major Error
Liofilchem™	21.4%	42.9%	33.3%
bioMérieux™	21.4%	35.7%	44.4%
Hardy™	78.6%	21.4%	--

What's coming next for CROs in the lab?

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- Determination of the mechanism of resistance in Gram negative bacteria can be challenging
- Increased access to WGS allows for the possibility of a better understanding of the mechanisms of resistance

CRE example 1

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Res	Antibiotic	Value	Unit	Interpretation
1	AMPICILLIN	0		Resistant
1	AMPICILLIN + SULBACTAM	0		Resistant
1	CEFAZOLIN	0		Resistant
1	CEFEPIME	19		Resistant
1	CEFTRIAXONE	12		Resistant
1	GENTAMICIN	23		Susceptible
1	MEROPENEM	18		Resistant
1	LEVOFLOXACIN	0		Resistant
1	ERTAPENEM	15		Resistant
1	PIPERACILLIN + TAZOBACTAM	14		Resistant
1	TOBRAMYCIN	19		Susceptible
1	TRIMETHOPRIM + SULFAMETHOXAZOLE	12		Intermediate

Routine additional testing

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CarbaR PCR

Res	Component	Value	Units	!
1	**Organism**	Klebsiella pneumoniae		
1	**IMP PCR**	Not Detected		✓
1	**KPC PCR**	Detected		!
1	**NDM PCR**	Not Detected		✓
1	**OXA48 PCR**	Not Detected		✓
1	**VIM PCR**	Not Detected		✓

Additional Antimicrobial Agents

Res	Antibiotic	Value	Unit	Interpretation
1	CEFTAZIDIME-AVIBACTAM	1		**Susceptible**
1	MEROPENEM/VABORBACTAM	0.06		**Susceptible**

CRE example 2

UNC SCHOOL OF MEDICINE

Res	Antibiotic	Value	Unit	Interpretation
1	AMPICILLIN	0		**Resistant**
1	AMPICILLIN + SULBACTAM*			
1	CEFAZOLIN	0		**Resistant**
1	CEFAZOLIN (URINE)*	0		
1	CEPHALEXIN			
1	CEFTRIAXONE	0		**Resistant**
1	CIPROFLOXACIN	12		**Resistant**
1	GENTAMICIN	14		**Intermediate**
1	MEROPENEM	0		**Resistant**
1	LEVOFLOXACIN	22		**Susceptible**
1	ERTAPENEM*			
1	NITROFURANTOIN	12		**Resistant**
1	TOBRAMYCIN	0		**Resistant**
1	TRIMETHOPRIM + SULFAMETHOXAZOLE	0		**Resistant**

Routine additional testing

UNC SCHOOL OF MEDICINE

CarbaR PCR

Res	Component	Value	Units	!
1	**Organism**	Klebsiella pneumoniae		
1	**IMP PCR**	Not Detected		✓✓
1	**KPC PCR**	Detected		!
1	**NDM PCR**	Not Detected		✓✓
1	**OXA48 PCR**	Not Detected		✓✓
1	**VIM PCR**	Not Detected		✓✓

Additional Antimicrobial Agents

Res	Antibiotic	Value	Unit	Interpretation
1	CEFTAZIDIME-AVIBACTAM	8		**Susceptible**
1	MEROPENEM/VABORBACTAM	>64		**Resistant**
1	PLAZOMICIN*	0.5		**Susceptible**
1	ERAVACYCLINE*	0.25		**Susceptible**

Enter whole genome sequencing

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Agent	Predicted phenotype	Genotypes
OmpK35/OmpK36	Resistant	OmpK35-0%, OmpK36-0%
Carbapenems	Resistant	KPC-2, KPC-2, KPC-2, KPC-2
ESBL+Inhibitors	Not Found	
ESBLs	Not Found	
Beta-lactams+Inhibitor	Not Found	
Beta-lactams	Resistant	OXA-1, TEM-1D
Trimethoprim	Not Found	
Tigecycline	Not Found	
Tetracycline	Not Found	
Sulfonamides	Resistant	sul1
Rifampicin	Resistant	arr-3
Phenicols	Resistant	catB3
Macrolides	Resistant	mphA
Glycopeptides	Not Found	
Fluoroquinolones	Resistant	qnrB4
Fosfomycin	Not Found	
Colistin	Not Found	
Aminoglycosides	Resistant	aac(6')-Ib' (homolog), aac(6')-Ib-cr, aph3-Ia

OmpK mutations associated with decreased uptake of BLIs, vaborbactam more than avibactam

qnrB is associate with low level FQ resistance (most FQ resistance driven by gyrA mutations)

aac(6')-Ib-cr is associated with low level FQ resistance as well

CRE case 3

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Res	Antibiotic	Value	Unit	Interpretation
1	AMPICILLIN	0		Resistant
1	AMPICILLIN + SULBACTAM	0		Resistant
1	CEFAZOLIN	0		Resistant
1	CEFEPIME	25		Intermediate
1	CEFTRIAXONE	25		Susceptible
1	GENTAMICIN	21		Susceptible
1	MEROPENEM	19		Resistant
1	LEVOFLOXACIN	27		Susceptible
1	ERTAPENEM	17		Resistant
1	PIPERACILLIN + TAZOBACTAM*	21		Susceptible
1	TOBRAMYCIN	22		Susceptible
1	TRIMETHOPRIM + SULFAMETHOXAZOLE	24		Susceptible

Routine additional testing

UNC SCHOOL OF MEDICINE

CarbaR PCR

Res	Component	Value	Units	!
1	**Organism**	Klebsiella pneumoniae		
1	**IMP PCR**	Not Detected		✓✓
1	**KPC PCR**	Not Detected		✓✓
1	**NDM PCR**	Not Detected		✓✓
1	**OXA48 PCR**	Not Detected		✓✓
1	**VIM PCR**	Not Detected		✓✓

Additional Antimicrobial Agents

Res	Antibiotic	Value	Unit	Interpretation
1	CEFTAZIDIME-AVIBACTAM	4		**Susceptible**
1	MEROPENEM/VABORBACTAM	2		**Susceptible**

WGS Case 3

Agent	Predicted phenotype	Genotypes
OmpK35/OmpK36	**Resistant**	**OmpK35-40%**
Carbapenems	Not Found	
ESBL+Inhibitors	Not Found	
ESBLs	Not Found	
Beta-lactams+Inhibitor	Not Found	
Beta-lactams	Not Found	
Trimethoprim	Not Found	
Tigecycline	Not Found	
Tetracycline	Not Found	
Sulfonamides	Not Found	
Rifampicin	Not Found	
Phenicols	Not Found	
Macrolides	Not Found	
Glycopeptides	Not Found	
Fluoroquinolones	Not Found	
Fosfomycin	Not Found	
Colistin	**Resistant**	**PmrB-17%**
Aminoglycosides	Not Found	

Isolate showed elevated but not resistant MICs to ceftazidime-avibactam and meropenem-vaborbactam

WHAT???

An example of NGS for Gram negative resistance

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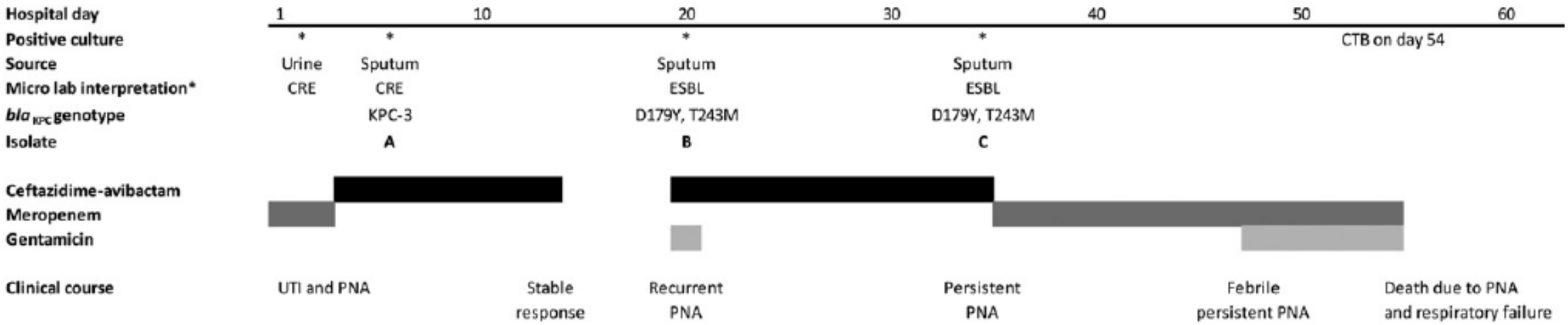
- CRE *K. pneumoniae*, treated with ceftazidime-avibactam for 10 days
- 4 days later patient has recurrence of pneumonia and C/A restarted
- 14 days later meropenem susceptible "ESBL" *K. pneumoniae* recovered and patient transitioned to meropenem
- Both isolates had C/A MICs >256



Shields, *Antimicrob Agents Chemother*, 2017. 61(3

C/A MIC values of various KPC-3 mutants

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Isolate	C/A MIC	Mero MIC	Mutation
1-A	2/4	128	WT
1-B	256/4	0.5	D179Y, T243M
1-C	256/4	0.25	D179Y, T243M
2-A	4/4	32	WT
2-B	32/4	8	V240G
2-C	>256/4	4	D179Y
2-D	4/4	4	T243A
3-A	2/4	32	WT
3-B	128/4	0.25	D179Y
3-C	64/4	0.125	D179Y

Adapted from Shields, *Antimicrob Agents Chemother*, 2017. 61(3)

What's next for sequencing?

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MECHANISMS OF RESISTANCE

Check for updates

Applying Rapid Whole-Genome Sequencing To Predict Phenotypic Antimicrobial Susceptibility Testing Results among Carbapenem-Resistant *Klebsiella pneumoniae* Clinical Isolates

Pranita D. Tamma,[a] Yunfan Fan,[b] Yehudit Bergman,[c] Geo Pertea,[d] Abida Q. Kazmi,[c] Shawna Lewis,[c] Karen C. Carroll,[e] Michael C. Schatz,[d,f] Winston Timp,[b] Patricia J. Simner[e]

[a]Johns Hopkins University School of Medicine, Department of Pediatrics, Division of Pediatric Infectious Diseases, Baltimore, Maryland, USA
[b]Johns Hopkins University, Department of Biomedical Engineering, Baltimore, Maryland, USA
[c]Johns Hopkins University School of Medicine, Department of Pathology, Baltimore, Maryland, USA
[d]Johns Hopkins University, Department of Computer Science, Baltimore, Maryland, USA
[e]Johns Hopkins University School of Medicine, Department of Pathology, Division of Medical Microbiology, Baltimore, Maryland, USA
[f]Johns Hopkins University, Department of Biology, Baltimore, Maryland, USA

What's next for sequencing?

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TABLE 1 Percent agreement between three different sequencing and analysis approaches compared to phenotypic antimicrobial susceptibility testing results for 40 *Klebsiella pneumoniae* clinical isolates

	Phenotypic antimicrobial susceptibility testing results (%)		% agreement with antimicrobial susceptibility testing results		
Antibiotic	Susceptible	Not susceptible	Real-time approach	Assembly approach	Hybrid Nanopore-Illumina assembly
Piperacillin-tazobactam	25	75	80	85	85
Ceftriaxone	25	75	93	95	95
Cefepime	28	72	95	98	98
Ceftazidime-avibactam	93	7	100	100	100
Ertapenem	78	22	83	85	85
Meropenem	40	60	93	95	95
Amikacin	78	22	78	85	85
Gentamicin	60	40	45	93	95
Ciprofloxacin	33	67	30	98	98
Colistin	93	7	93	98	98
Doxycycline	50	50	63	80	80
Trimethoprim-sulfamethoxazole	35	65	68	93	93
Overall agreement			77	92	92

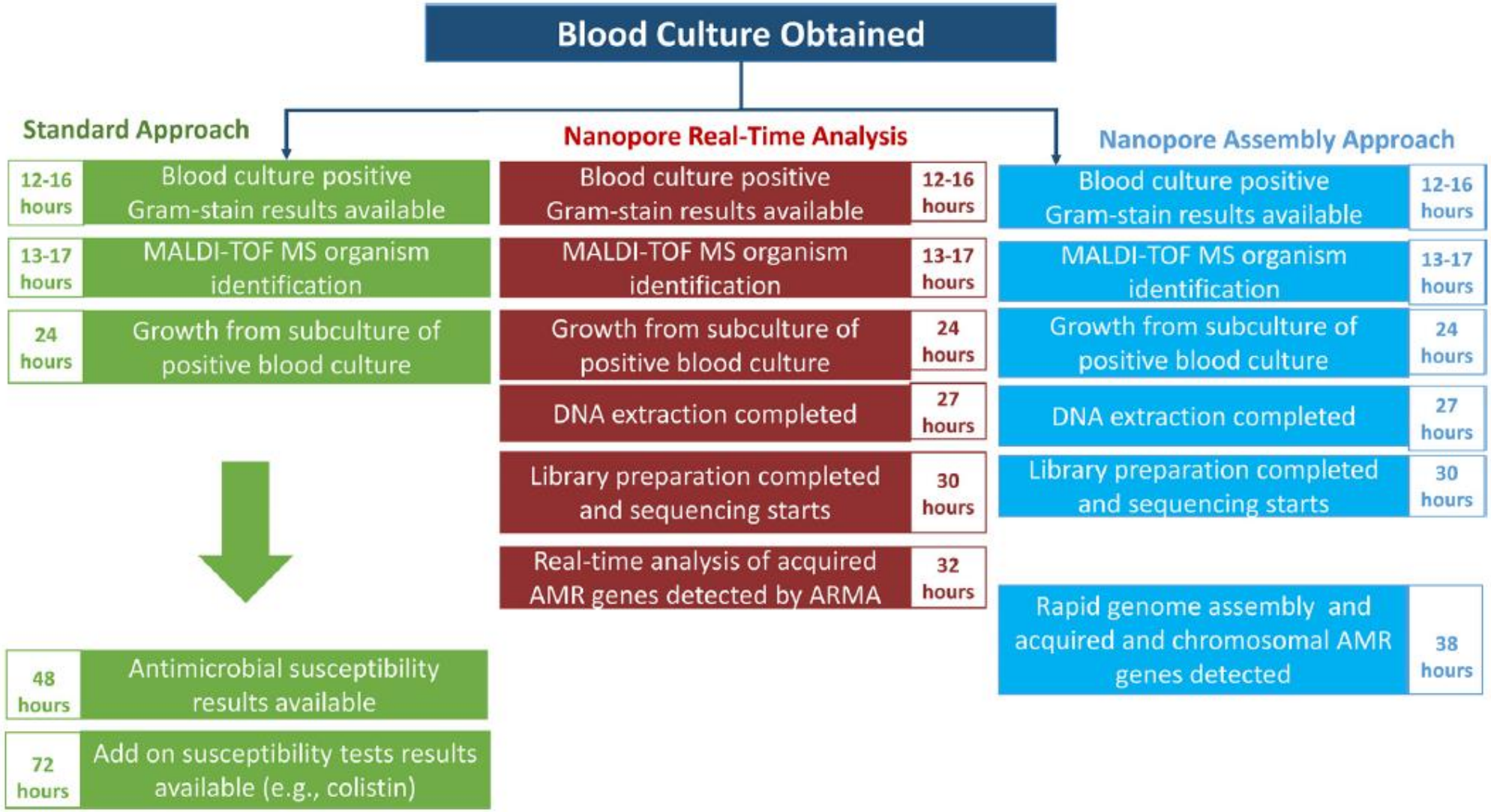

FIG 1 Schematic of Nanopore sequencing with a real-time analysis and assembly-based approach for identifying resistance genes compared to standard of care testing, using an example of a positive blood culture. MALDI-TOF MS, matrix-assisted laser desorption ionization–time of flight mass spectrometry; AMR, antimicrobial resistance; AST, antimicrobial susceptibility testing.

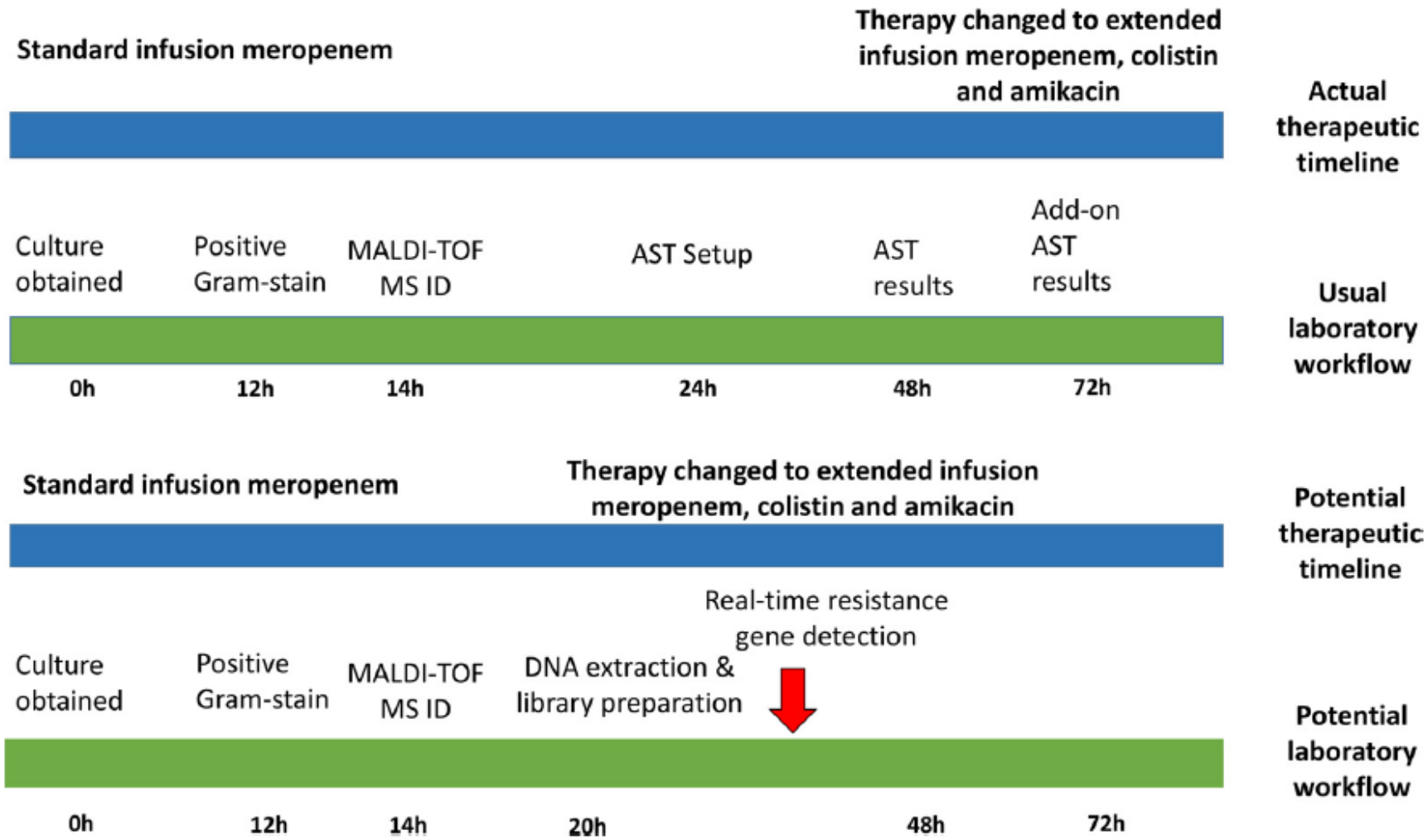

FIG 2 Timeline comparing availability of organism identification and antimicrobial susceptibility testing along with actual and anticipated antibiotic treatment decisions using standard approaches versus live-streaming whole-genome sequencing data generated from Nanopore sequencing with assembly; data generated based on the case of a 64-year-old liver transplant recipient with an NDM-1, CTX-M-15, and CMY-4-producing *Klebsiella pneumoniae* bacteremia.

What are the barriers to real time sequencing for AST?

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- Cost
  - Sequencing costs continue to drop
  - Bioinformatic and computational power needed continue to rise
- Genotype vs phenotype (the mind-body problem of microbiology)
  - Limited data on what is more important in terms of clinical response
  - What to do about novel drugs without known mechanisms of resistance

Learning Assessment #3

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- Which of the following is true regarding antimicrobial susceptibility testing
- Commercial PCR tests identify all common mechanisms of CRE
- New antimicrobials are readily available for testing on commercial AST panels
- Bacterial WGS can easily replace phenotypic AST methods
- There is wide variation in the performance of phenotypic AST methods

Learning Assessment #3

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- Which of the following is true regarding antimicrobial susceptibility testing
- Commercial PCR tests identify all common mechanisms of CRE
- New antimicrobials are readily available for testing on commercial AST panels
- Bacterial WGS can easily replace phenotypic AST methods
- **There is wide variation in the performance of phenotypic AST methods**

Commercial PCRs detect some carbapenemase genes, but many CRE do not carry carbapenemases

It takes many years for new drugs to be available on most commercial panels

Bacterial WGS while exciting is still costly and too time consuming for most indications

Summary

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- Antimicrobial resistance is a issue
- Genome sequencing can be a tool for identifying not only relatedness between strains but novel mechanisms of resistance
- A combination of genetic and phenotypic information is needed to best address treatment options