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ORIGINAL ARTICLE

Breakpoint beware: reliance on historical breakpoints for *Enterobacteriaceae* leads to discrepancies in interpretation of susceptibility testing for carbapenems and cephalosporins and gaps in detection of carbapenem-resistant organisms

Melanie L. Yarbrough[1] · Meghan A. Wallace[1] · Robert F. Potter[2] · Alaric W. D'Souza[2] · Gautam Dantas[1,2,3,4] · Carey-Ann D. Burnham[1,3,5,6]

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Abstract

Carbapenem-resistant *Enterobacteriaceae* (CRE) are an important public health and infection prevention threat. CRE are typically detected via phenotypic antimicrobial susceptibility testing (AST), for which interpretive standards were modified in recent years. Our objective was to measure the impact of breakpoint changes on AST interpretation for CRE. Zone sizes from disk diffusion AST for *Enterobacteriaceae* isolates recovered from clinical cultures over a 1-year period (n = 10,183) and CRE from clinical and environmental sources from the USA and Pakistan (n = 342) were evaluated. Results were interpreted according to historical (CLSI M100-S19) and current (CLSI M100-S29) breakpoints. Interpretive errors were calculated according to the FDA definitions. Using current breakpoints as the reference standard, 56 (17%) very major (false susceptibility) errors occurred for cefepime and 13 (45%) very major errors for meropenem interpretation using historical breakpoints in clinical isolates of *Enterobacteriaceae*, corresponding to 12 carbapenemase-producing CRE that would have been missed during the 1-year period. For confirmed bla_{KPC} CP-CRE clinical and environmental isolates (n = 149), the very major error rate for historic breakpoints was 8%, 30%, 63%, and 0% for cefepime, meropenem, imipenem, and ertapenem, respectively. For bla_{KPC} isolates, the use of historical breakpoints would have led to 42 (28%) reports of false susceptibility to meropenem. Failure to adopt updated AST breakpoints may lead to reports of false susceptibility for antimicrobials commonly used to treat Gram-negative infections and preclude recognition of CRE. Such errors could negatively impact patient care and hamper infection control and public health efforts.

Keywords Carbapenemase-producing CRE · KPC · Breakpoint · *Enterobacteriaceae*

✉ Melanie L. Yarbrough
myarbro@wustl.edu

[1] Department of Pathology & Immunology, Division of Laboratory and Genomic Medicine, Washington University in St. Louis School of Medicine, 660 S. Euclid Ave, Campus Box 8118, St. Louis, MO 63110, USA

[2] The Edison Family Center for Genome Sciences and Systems Biology, Washington University in St. Louis School of Medicine, St. Louis, MO, USA

[3] Department of Molecular Microbiology, Washington University in St. Louis School of Medicine, St. Louis, MO, USA

[4] Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO, USA

[5] Department of Medicine, Washington University in St. Louis School of Medicine, St. Louis, MO, USA

[6] Department of Pediatrics, Washington University in St. Louis School of Medicine, St. Louis, MO, USA

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Introduction

Carbapenem-resistant *Enterobacteriaceae* (CRE) are endemic in many parts of the world and remain a growing concern to public health [1, 2]. CRE are associated with a high mortality rate due to limited treatment options [3–5]. CRE are defined by the Centers for Disease Control and Prevention (CDC) as *Enterobacteriaceae* that are not susceptible to at least one carbapenem antibiotic or that possess a carbapenemase (http://www.cdc.gov/hai/organisms/cre/definition.html#dif). Carbapenemase-producing CRE (CP-CRE) are a subset of CRE that have documented laboratory evidence of carbapenemase production by phenotypic or molecular testing. CP-CRE are of particular concern for infection control, as resistance is mediated by mobile genetic elements that are transmissible between bacteria and patients [6, 7].

The presence of CRE is typically detected using phenotypic antimicrobial susceptibility testing (AST) on isolates recovered in clinical cultures. Thus, correct characterization relies on timely and accurate AST results. The Clinical and Laboratory Standards Institute (CLSI) updated AST interpretive standards for *Enterobacteriaceae* in 2010 for carbapenems (M100-S20) and 2014 for cefepime (M100-S24) [8, 9]. Although these interpretive standards have been modified for several years, it is documented that many laboratories have delayed or even failed to adopt new breakpoints for *Enterobacteriaceae* [10, 11]. In fact, many laboratories continued to use outdated interpretive criteria, even after public health interventions to increase knowledge and overcome barriers to updating breakpoints [11]. Many laboratories may presume that adoption of updated criteria is unnecessary due to a low incidence of CRE in their region. The use of outdated breakpoints may further perpetuate this perception and puts a laboratory at risk of reporting inaccurate AST results and potentially failing to detect CP-CRE, which may have devastating consequences on patient outcomes and hamper infection control and public health measures to limit the spread of these highly resistant organisms [12]. Here, our objective was to measure the impact of the use of historical BPs on AST interpretation of cephalosporin and carbapenem antimicrobials in a large cohort of *Enterobacteriaceae* isolated from routine clinical specimens and in an enriched CRE population of clinical and environmental isolates.

Materials and methods

Clinical isolate population

The laboratory information system (Cerner Millennium, North Kansas City, MO) was queried for antimicrobial susceptibility results from all clinical isolates of *Enterobacteriaceae* tested over a period of 1 year (January–December 2017) in the clinical microbiology laboratory at Barnes-Jewish Hospital, a 1250-bed tertiary care medical center in St. Louis, MO, USA. Organisms were identified using MALDI-TOF MS (Bruker BioTyper). All isolates were tested by Kirby-Bauer disk diffusion according to the CLSI guidelines [13]. For each isolate, disk diffusion zone sizes were recorded, if tested, for ceftazidime, ceftriaxone, cefepime, and meropenem.

CRE population

Disk diffusion zone sizes were recorded, if tested, for cefepime, meropenem, imipenem, and ertapenem from a collection of characterized clinical and environmental CRE isolates. For this study, isolates were included and classified as CRE if AST for meropenem was interpreted as not susceptible by CLSI M100-S29 breakpoints (meropenem zone size < 23 mm) [14]. The CRE strains were recovered from banked isolates obtained from patients and hospital surfaces from tertiary care hospitals in the USA and Pakistan between 2010 and 2017. For CRE isolates, the presence of a carbapenemase was detected by one of several possible methods: (1) a laboratory developed real-time PCR for bla_{KPC} and bla_{NDM}, (2) the modified carbapenem inactivation method [15], (3) the Cepheid Xpert Carba-R assay (Cepheid, Sunnyvale, CA) according to manufacturer's package insert [15], or (4) resistance gene detection via whole-genome sequencing (WGS). Briefly, for WGS, a standardized experimental and computational pipeline was used for analysis, as previously described [16, 17]. Bacterial strains were sequenced using Illumina whole-genome sequencing. De novo assemblies of bacteria had open reading frames annotated with prokka and antibiotic resistance genes identified with ResFinder [18, 19].

AST interpretive analysis

Zone sizes for cephalosporins and carbapenems were interpreted using historical (CLSI M100-S19) and contemporary (CLSI M100-S29) breakpoints for *Enterobacteriaceae* [14, 20]. Very major errors (false susceptibility) were calculated according to the FDA definitions using current breakpoints as the reference method [21]. Very major errors are defined as interpretation of an isolate as susceptible by historical breakpoints but resistant by current breakpoints. Categorical agreement was defined as isolates with the same AST interpretation using historical and current breakpoints. Statistical comparisons were done using Pearson's chi-squared test with Yates' continuity correction using R statistical software (version 3.5.2).

Results

Enterobacteriaceae recovered from routine testing of clinical isolates

Antimicrobial susceptibility testing results for all clinical isolates of *Enterobacteriaceae* (n = 10,183) tested over a period of 1 year were analyzed. Major species or groups represented in the analysis were *Escherichia coli* (6116), *Klebsiella pneumoniae* (1735), *Enterobacter* species (633), *Serratia* species (297), *Proteeae* group (1167), and *Citrobacter* species (235). Using current (2019) CLSI breakpoints as the reference method for interpretation of susceptibility results (Table 1), the frequency of errors in interpretation with the use of historical (2009) breakpoints was calculated (Table 2). For ceftazidime and ceftriaxone, there were no very major errors in interpretation for *Enterobacteriaceae* that tested resistant using current breakpoints. There were 56/339 (17%) very major errors for cefepime and 13/29 (45%) very major errors for meropenem among clinical isolates that tested resistant to these drugs. The overall categorical agreement between historical and current breakpoints for ceftazidime, ceftriaxone, cefepime, and meropenem for all 10,183 clinical isolates tested was 97%, 98%, 98%, and 99.8%, respectively (Table 2). During this time period, 31/10,183 (0.3%) isolates tested as not susceptible to meropenem according to current breakpoints. These CRE included producers of bla_{KPC} (n = 22), CP-CRE of unknown mechanism (n = 2), and non-CP-CRE (n = 7). Using criteria in which isolates were further characterized for carbapenemase production if the strain tested intermediate or resistant to meropenem, 15 out of 31 CRE isolates would have been characterized as falsely susceptible to meropenem using historical breakpoints, of which 12 were identified with further testing as CP-CRE that would have otherwise gone undetected.

Table 2 Frequency of errors and overall categorical agreement of historical and current breakpoints for all *Enterobacteriaceae* clinical isolates tested in 2017 (n = 10,183)

Antimicrobial	Very major errors	Overall categorical agreement
Ceftazidime	0/519 (0%)	9873 /10,183 (97%)
Ceftriaxone	0/800 (0%)	10,015/10,183 (98%)
Cefepime	56/339 (17%)	9983/10,183 (98%)
Meropenem	13/29 (45%)	10,163/10,183 (99.8%)

Analysis of an enriched cohort of clinical and environmental CRE

Given the low incidence of CRE in the population of isolates in this time period, we investigated an extended population of clinical and environmental CRE (n = 342) collected from patients (n = 262) or hospital environmental surfaces (n = 80) over the course of 7 years. Isolates from the USA (n = 277) and Pakistan (n = 65) that were not susceptible to meropenem were included in the dataset. The median (range) zone sizes for cefepime and meropenem from CRE isolates were 14.5 (6–34) mm and 14 (6–22) mm, respectively, compared with a median zone size of 33 (6–47) mm and 32 (6–47) mm for the collection of *Enterobacteriaceae* recovered from routine clinical specimens tested in a 1-year period (Fig. 1). Of the 342 isolates included in the analysis, the overall very major error rate for *Enterobacteriaceae* was 7% for cefepime (n = 272), 30% for meropenem (n = 301), 50% for imipenem (n = 245), and 0% for ertapenem (n = 160) (Table 3, Fig. 2). Interestingly, very major error rates for meropenem were significantly higher for *Enterobacter* spp. ($p < 0.01$) than for other major species of *Enterobacteriaceae* (*K. pneumoniae* and *E. coli*). The overall

Table 1 Historical (CLSI M100-S19) and contemporary (CLSI M100-S29) disk diffusion breakpoints (millimeters) for interpretation of disk diffusion for *Enterobacteriaceae*

	Historical breakpoint (M100-S19)			Current breakpoint (M100-S29)		
Antimicrobial	S[a]	I[a]	R[a]	S	I/SDD[b]	R
Ceftazidime	≥ 18	15–17	≤ 15	≥ 21	18–20	≤ 17
Ceftriaxone	≥ 21	14–20	≤ 13	≥ 23	20–22	≤ 19
Cefepime	≥ 18	15–17	≤ 14	≥ 25	19–24[c]	≤ 18
Meropenem	≥ 16	14–15	≤ 13	≥ 23	20–22	≤ 19
Ertapenem	≥ 19	16–18	≤ 15	≥ 22	19–21	≤ 18
Imipenem	≥ 16	14–15	≤ 13	≥ 23	20–22	≤ 19

[a] S, susceptible; I, intermediate; R, resistant

[b] SDD, susceptible dose-dependent, where indicated

[c] SDD breakpoint

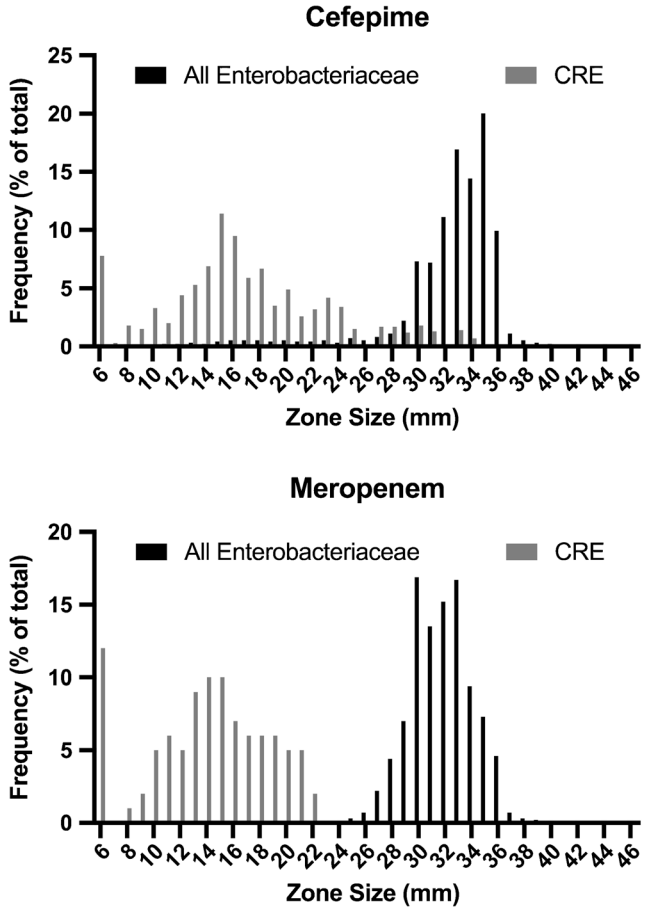

Fig. 1 Comparison of cefepime and meropenem zone sizes among *Enterobacteriaceae*. The zone size distributions for cefepime (top panel) and meropenem (bottom panel) as tested by Kirby Bauer disk diffusion is shown. The frequency of the number of isolates with a particular zone size (mm) is graphed as a percent of the total isolates for all *Enterobacteriaceae* tested in 2017 (n = 10,183; black bars) and clinical and environmental isolates of carbapenem resistant *Enterobacteriaceae* (n = 342; gray bars)

categorical agreement for CRE susceptibility testing between historic and current breakpoints was 70% for cefepime, 43% for meropenem, 40% for imipenem, and 85% for ertapenem (Table 3).

Interpretive error rates for CP-CRE possessing bla_{KPC}

Of the 342 clinical and environmental CRE, 251 isolates were interrogated further for carbapenemase production and mechanism of resistance. Of these strains, 149 were characterized as encoding bla_{KPC}, 21 as bla_{NDM}, 11 as $bla_{OXA\text{-}48\text{-}like}$, 1 as $bla_{OXA\text{-}66}$, 9 as both bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$, 45 as CP-CRE (mechanism not otherwise specified), and 15 as non-CP-CRE (Table 3). For bla_{KPC} isolates, 10/128 (7%) very major errors occurred for cefepime interpretation. The incidence of very major errors for bla_{KPC} isolates was 30% for meropenem (n = 141) and 63% for imipenem (n = 131) (Table 3, Fig. 3). No very major errors were noted for any CP-CRE for ertapenem. The categorical agreement for bla_{KPC} isolates between historical and current breakpoints was 59% for cefepime (n = 149), 36% for meropenem (n = 149), 23% for imipenem (n = 148), and 80% for ertapenem (n = 98) (Table 3). Of the 149 bla_{KPC} isolates, 82 (55%) tested as susceptible or intermediate to cefepime using 2010 breakpoints versus 21 (14%) that tested as susceptible or susceptible dose-dependent using current breakpoints (Fig. 3). Using historical breakpoints, 57 (38%), 39 (26%), and 21 (14%) isolates tested susceptible to meropenem, imipenem, and ertapenem, respectively, while 6 (4%), 1 (1%), and 8 (5%) isolates tested susceptible to carbapenems using current breakpoints (Fig. 3).

Discussion

Detection of CP-CRE in clinical isolates relies on accurate phenotypic AST results. Our retrospective analysis of a large number of AST results for carbapenems illustrated that historical breakpoints for disk diffusion are insensitive for recognition of CP-CRE. Thus, delayed implementation of revised breakpoints for interpretation of AST in *Enterobacteriaceae* has far-reaching implications for patient management, infection prevention, and public health efforts.

Table 3 Frequency of errors with use of historical breakpoints for carbapenem resistant *Enterobacteriaceae* (CRE)

Category	Cefepime		Meropenem		Imipenem		Ertapenem	
	Very major errors, *n* (%)	Categorical agreement, *n* (%)	Very major errors, *n* (%)	Categorical agreement, *n* (%)	Very major errors, *n* (%)	Categorical agreement, *n* (%)	Very major errors, *n* (%)	Categorical agreement, *n* (%)
Enterobacteriaceae	18/272 (7)	241/342 (70%)	87/301 (30)	145/342 (43%)	123/245 (50)	132/325 (40%)	0/160 (0)	144/170 (85%)
Klebsiella pneumoniae	6/166 (4)	126/177 (71%)	32/169 (19)	96/177 (54%)	83/150 (55)	62/173 (36%)	0/77 (0)	75/81 (93%)
Enterobacter spp.	7/55 (13)	64/89 (72%)	31/81 (38)	32/89 (36%)	17/52 (33)	37/79 (47%)	0/55 (0)	45/56 (80%)
Escherichia coli	1/26 (4)	20/28 (71%)	6/21 (29)	12/28 (43%)	6/15 (40)	19/27 (70%)	0/12 (0)	11/13 (85%)
Serratia spp.	1/8 (13)	8/16 (50%)	3/5 (60)	1/16 (6%)	4/9 (44)	6/16 (38%)	0/3 (0)	3/4 (75%)
Proteeae group	0/1 (0)	10/10 (100%)	4/5 (80)	1/10(10%)	3/5 (60)	2/8 (25%)	0/2 (0)	4/4 (100%)
Citrobacter spp.	0/9(0)	5/10 (50%)	3/10 (30)	2/10 (20%)	6/8 (75)	2/10 (20%)	0/2 (0)	2/2 (100%)
Other[a]	3/7 (43)	8/12 (67%)	8/10 (80)	1/12 (8%)	4/6 (67)	3/12 (25%)	0/9 (0)	4/10 (40%)
Carbapenemase								
KPC	10/128 (8)	88/149 (59%)	42/141 (30)	53/149 (36%)	83/131 (63)	33/148 (23%)	0/91 (0)	78/98 (80%)
NDM	0/19 (0)	19/21 (90%)	3/20 (15)	13/21 (58%)	2/5 (40)	5/6 (83%)	0/2 (0)	2/2 (100%)
OXA-48-like	0/11 (0)	11/11 (100%)	0/10 (0)	10/11 (91%)	7/10 (70)	1/11 (9%)	0/2 (0)	2/2 (100%)
OXA-66	1/1 (100)	0/1 (0%)	n/a	0/1 (0%)	n/a	1/1 (100%)	n/a	n/a
NDM/OXA-48 like	0/9 (0)	9/9 (100%)	0/9 (0)	9/9 (100%)	0/9 (0)	9/9 (100%)	n/a	n/a
CP-CRE (Not defined)	0/43 (0)	35/45 (78%)	1/35 (3)	29/45 (64%)	17/34 (50)	18/45 (40%)	0/2 (0)	2/2 (100%)
Non-CP-CRE	1/10 (10)	11/15 (73%)	5/11 (45)	6/15 (40%)	3/10 (30)	11/15 (73%)	0/14 (0%)	14/15 (93%)

[a] Other includes *Klebsiella oxytoca* (8), *Klebsiella variicola* (1), *Hafnia* spp. (1), *Pantoea* spp. (1), and *Escherichia hermannii* (1)

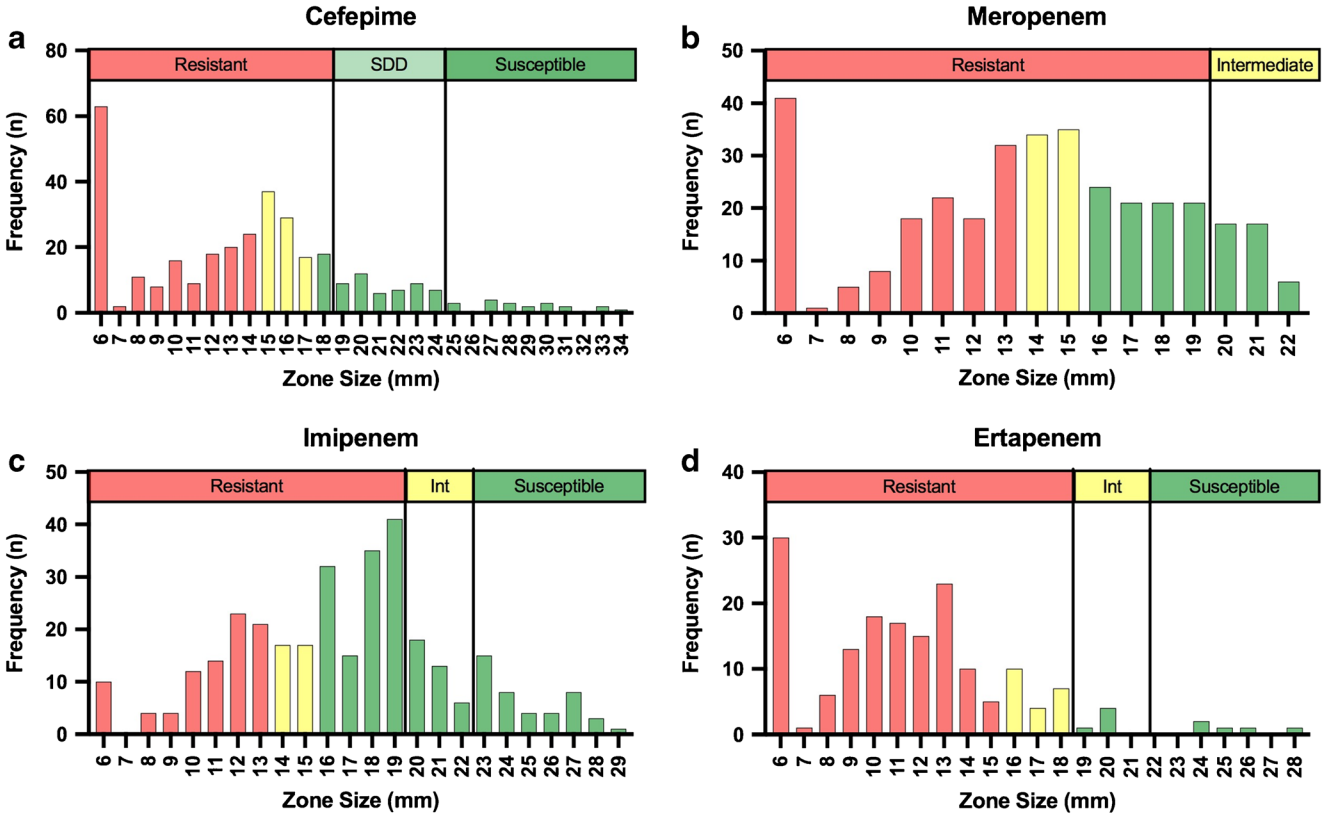

Fig. 2 Differences in antimicrobial susceptibility interpretation using current and historical breakpoints for carbapenem resistant *Enterobacteriaceae*. The number of isolates with the indicated zone size (mm) are shown for cefepime (**a**), meropenem (**b**), imipenem (**c**), and ertapenem (**d**). Current breakpoints are indicated by solid black lines. Susceptibility interpretations using historical breakpoints are indicated as resistant (red bars), intermediate (yellow bars), and susceptible (green bars). Int, intermediate; SDD, susceptible dose-dependent

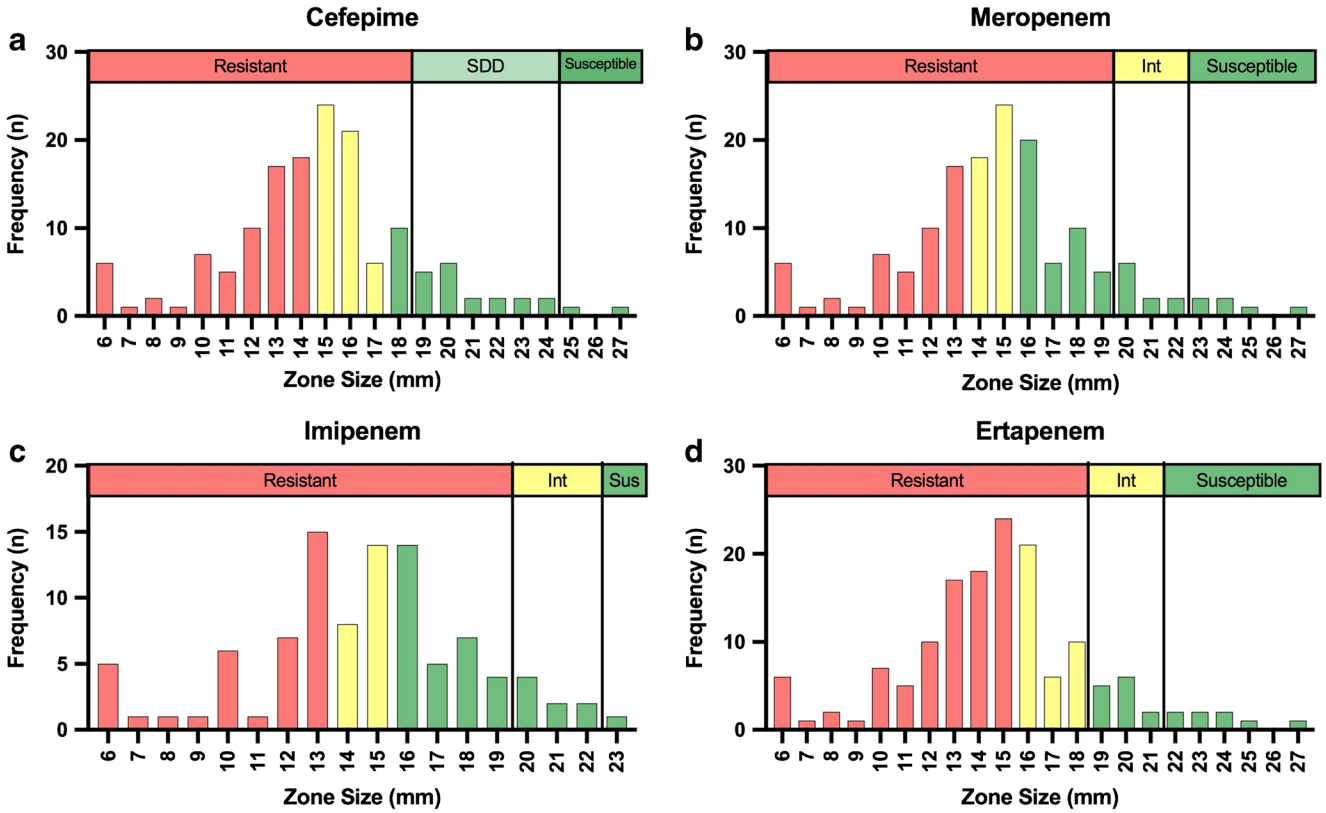

Fig. 3 Differences in antimicrobial susceptibility interpretation using current and historical breakpoints for *Enterobacteriaceae* possessing bla_{KPC}. The number of isolates with the indicated zone size (mm) is shown for cefepime (**a**), meropenem (**b**), imipenem (**c**), and ertapenem (**d**). Current breakpoints are indicated by solid black lines. Susceptibility interpretations using historical breakpoints are indicated as resistant (red bars), intermediate (yellow bars), and susceptible (green bars). Int, intermediate; SDD, susceptible dose-dependent

For *Enterobacteriaceae*, the CLSI modified AST interpretive breakpoints for carbapenems in 2010 and cefepime in 2014 [8, 9]. This was followed by an updated CDC surveillance definition for CRE [22] and endorsement of the new breakpoints by the Infectious Disease Society of America to encourage more reliable detection of CP-CRE [23]. However, there is often a gap between CLSI updates to interpretive criteria and availability of new breakpoints on commercial AST devices [10]. This, in turn, has precluded many clinical laboratories from adopting current standards, as doing so would require an off-label validation of the new breakpoints on the existing device or use of a different method entirely, actions that are not feasible in all clinical settings [11, 24]. Illustrating this, a recent survey of clinical laboratories in CA found that 28% of laboratories were still using outdated breakpoints 5 years after the release of new interpretive criteria [10]. Education around this topic is also important, as many clinical laboratories were unaware that they were using obsolete breakpoints or stated a lack of resources for validation of new breakpoints [11]. Of those using current breakpoints, there was an average lag of 3.4 years between release and implementation of new breakpoints [10]. A recent study used computational modeling to estimate the impact of failure to adopt current interpretive criteria in a timely manner. Bartsch et al. demonstrated that delays of 1 to 5 years could result in hundreds to thousands of additional CRE carriers due to missed detection of CRE that may then spread throughout the community [12].

To quantitate the impact of use of historical breakpoints on detection of CP-CRE in our area, we retrospectively analyzed AST results on all *Enterobacteriaceae* isolated in our clinical microbiology laboratory, which serves a tertiary medical center in the Midwestern United States, over a period of 1 year. While the number of very major errors was low overall, reports of false susceptibility would have occurred in 56 isolates for cefepime and 13 isolates for meropenem. This has immediate implications for patient management, as these antimicrobials are frequently used for treatment of Gram-negative infections in our healthcare setting. Among bla_{KPC} isolates in our study, 55% tested as susceptible or intermediate to cefepime with historical breakpoints. In contrast, only 14% of these isolates tested as susceptible or susceptible dose-dependent using current breakpoints. Given conflicting reports about the utility of cefepime as an agent for use in combination therapy of infections caused by KPC-producing strains, reports of false susceptibility have the potential to lead to inappropriate therapy and also hamper and confound future

studies evaluating this question [25, 26]. Also worrisome is the finding that the use of historical breakpoints would have precluded recognition of 12 of 24 (50%) CP-CRE detected from clinical specimens at our medical center over the span of 1 year. These findings support previous studies that have noted a lack of sensitivity of historical breakpoints for detection of KPC carbapenemases, regardless of AST method used [10, 27]. Humphries et al. found that 20% of KPC producers would have gone undetected in a collection of isolates from CA using historical breakpoints [10]. Notably, many countries in Europe and beyond have adopted clinical breakpoints from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for AST interpretation [28]. Differences in current clinical breakpoints between CLSI and EUCAST for a number of antimicrobial classes within *Enterobacteriaceae* may further compound CRE detection and impact CRE rates. For example, studies have generally noted reduced susceptibility to cefepime but higher susceptibility to carbapenems using EUCAST interpretive criteria compared with CLSI breakpoints for *Enterobacteriaceae* [29–31].

It is important to consider the fact that the categorical agreements between historic and current breakpoints for clinical isolates of *Enterobacteriaceae* in our study were well within the acceptable FDA requirements of > 89.9% for AST devices [21]. However, our analysis of a large population of CRE isolated from both clinical and environmental samples revealed that errors of false susceptibility are far more common among CRE with use of obsolete breakpoints, as the overall categorical agreement for meropenem interpretation in bla_{KPC} CP-CRE was only 36%, compared with 99.8% in the population at large. Additionally, very major error rates for cefepime and meropenem were higher in isolates possessing bla_{KPC}, the most common carbapenemase mechanism in our study and the USA, compared with bla_{NDM} and bla_{OXA}. Our findings are likely generalizable to automated AST instruments and suggest that continued use of outdated breakpoints has the biggest potential for negative impact on the type of CP-CRE that are most frequently encountered in hospitals today.

Our study has several potential limitations. Due to the de-identified nature of the dataset, we are unable to determine the number of unique patients represented by the isolates tested. However, due to the large samples size of over 10,000 clinical isolates, inclusion of a small number of duplicate strains is unlikely to have a significant impact on the overall findings of this investigation. Categorical AST interpretations were based upon zone sizes measured by Kirby Bauer disk diffusion. While this is an AST reference method, it is not commonly used by clinical laboratories in the USA. However, analysis of accuracy of the disk diffusion method is increasingly important, as laboratories have been encouraged to implement alternative methods to automated AST as a means of testing novel antimicrobials and to facilitate more rapid adoption of the latest CLSI interpretation criteria [32]. In addition, many countries in Europe and Latin America in which CRE are endemic are also the heaviest users of disk diffusion [28, 33], and even users of automated AST are adopting the use of selected disk diffusion in order to be aligned with contemporary recommendations and offer testing for new antimicrobials. For these reasons, analysis of the accuracy of the disk diffusion method is increasingly important.

CP-CRE have been identified as an urgent threat to public health [34]. As such, resources such as the Antibiotic Resistance Laboratory Network are available for use by clinical laboratories free of charge for further identification and characterization of CP-CRE isolated from clinical samples [35]. However, these resources are moot if the sentinel clinical laboratory errs at the primary step of detection of carbapenem resistance. Thus, the use of outdated AST interpretive criteria by clinical laboratories represents a major risk point for infection control due to possibility of missing CP-CRE that test falsely susceptible to carbapenems. This has the potential to impact CRE reporting to state public health laboratories for epidemiology purposes and impede recognition of outbreaks. False reports of susceptibility to cephalosporins and carbapenems in patient isolates could also delay proper initiation of contact precautions, leading to increased healthcare worker carriage and nosocomial transmission of multidrug-resistant organisms, further compounding the impact on public health.

Implementation of revised carbapenem breakpoints should be a top priority for clinical laboratories [36]. Several resources are available to facilitate AST updates, including AST interpretive criteria by CLSI and EUCAST available at no cost to users. The CDC-FDA Antimicrobial Resistance Isolate Bank (https://www.cdc.gov/drugresistance/resistance-bank/index.html) will provide sets of organisms that can be used to validate AST methods. Resources such as these are a step in the right direction to facilitate the ability of clinical laboratories to stay abreast of breakpoint changes and implement changes in a timely fashion.

Rates of antimicrobial resistance are predicted to continue to increase at both national and global levels [37, 38]. Our results illustrate that laboratory delays in adoption of the most up-to-date interpretive criteria may not only result in incorrect interpretation of susceptibility profiles for *Enterobacteriaceae* but also lead to missed opportunities for detection of CP-CRE. This has important implications for patient therapy and initiation of appropriate isolation precautions to prevent further spread of these multidrug-resistant organisms.

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Compliance with ethical standards

Conflict of interest M.L.Y., M.A.W., R.F.P., A.W.D., and G.D. have nothing to disclose. C-A.D.B. serves as an advisor on the Clinical and Laboratory Standards Institute subcommittee on Antimicrobial Susceptibility Testing.

Informed consent No informed consent was needed for this study since no personal data were involved.

Ethical approval Approval by the institutional review board was not required for this study.

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References

1. Logan LK, Weinstein RA (2017) The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. J Infect Dis 215(suppl_1):S28–S36
2. Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, Xie L, Yang C, Ma X, Li H, Li W, Zhang X, Liao K, Man S, Wang S, Wen H, Li B, Guo Z, Tian J, Pei F, Liu L, Zhang L, Zou C, Hu T, Cai J, Yang H, Huang J, Jia X, Huang W, Cao B, Wang H (2018) Epidemiology of carbapenem-resistant Enterobacteriaceae infections: report from the China CRE network. Antimicrob Agents Chemother 62(2)
3. Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ (2014) Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. Emerg Infect Dis 20(7):1170–1175
4. Patel TS, Nagel JL (2015) Clinical outcomes of Enterobacteriaceae infections stratified by carbapenem MICs. J Clin Microbiol 53(1): 201–205
5. Centers for Disease Control and Prevention Healthcare-associated Infections: Tracking CRE. http://www.cdc.gov/hai/organisms/cre/TrackingCRE.html. Cited February 16, 2019
6. Lee BY, Bartsch SM, Wong KF, McKinnell JA, Slayton RB, Miller LG, Cao C, Kim DS, Kallen AJ, Jernigan JA, Huang SS (2016) The potential trajectory of carbapenem-resistant Enterobacteriaceae, an emerging threat to health-care facilities, and the impact of the centers for disease control and prevention toolkit. Am J Epidemiol 183(5):471–479
7. Won SY, Munoz-Price LS, Lolans K, Hota B, Weinstein RA, Hayden MK, Centers for Disease C, Prevention Epicenter P (2011) Emergence and rapid regional spread of Klebsiella pneumoniae carbapenemase-producing Enterobacteriaceae. Clin Infect Dis 53(6):532–540
8. CLSI (2010) Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement. CLSI document M100-S20. Clinical and Laboratory Standards Institute, Wayne
9. CLSI (2014) Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. CLSI document M100-S24. Clinical and Laboratory Standards Institute, Wayne
10. Humphries RM, Hindler JA, Epson E, Horwich-Scholefield S, Miller LG, Mendez J, Martinez JB, Sinkowitz J, Sinkowtiz D, Hershey C, Marquez P, Bhaurla S, Moran M, Pandes L, Terashita D, McKinnell JA (2018) Carbapenem-resistant Enterobacteriaceae detection practices in California: what are we missing? Clin Infect Dis 66(7):1061–1067
11. McKinnell JA, Bhaurla S, Marquez-Sung P, Pucci A, Baron M, Kamali T, Bugante J, Schwartz B, Balter S, Terashita D, Butler-Wu S, Gunzenhauser J, Hindler J, Humphries RM (2018) Public health efforts can impact adoption of current susceptibility breakpoints, but closer attention from regulatory bodies is needed. J Clin Microbiol
12. Bartsch SM, Huang SS, Wong KF, Slayton RB, McKinnell JA, Sahm DF, Kazmierczak K, Mueller LE, Jernigan JA, Lee BY (2016) Impact of delays between Clinical and Laboratory Standards Institute and Food and Drug Administration revisions of interpretive criteria for carbapenem-resistant Enterobacteriaceae. J Clin Microbiol 54(11):2757–2762
13. CLSI (2015) Performance standards for antimicrobial disk susceptibility tests; approved standard—twelfth edition. CLSI document M02-A12. Clinical and Laboratory Standards Institute, Wayne
14. CLSI (2019) Performance standards for antimicrobial susceptibility testing; twenty-ninth informational supplement. CLSI document M100-S29. Clinical and Laboratory Standards Institute, Wayne, PA
15. McMullen AR, Yarbrough ML, Wallace MA, Shupe A, Burnham CD (2017) Evaluation of genotypic and phenotypic methods to detect carbapenemase production in gram-negative Bacilli. Clin Chem 63(3):723–730
16. Potter RF, D'Souza AW, Wallace MA, Shupe A, Patel S, Gul D, Kwon JH, Andleeb S, Burnham CA, Dantas G (2017) Draft genome sequence of the blaOXA-436- and blaNDM-1-harboring Shewanella putrefaciens SA70 isolate. Genome Announc 5(29)
17. Potter RF, D'Souza AW, Wallace MA, Shupe A, Patel S, Gul D, Kwon JH, Beatty W, Andleeb S, Burnham CD, Dantas G (2018) Superficieibacter electus gen. nov., sp. nov., an extended-spectrum beta-lactamase possessing member of the Enterobacteriaceae family, isolated from intensive care unit surfaces. Front Microbiol 9: 1629
18. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV (2012) Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67(11):2640–2644
19. Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30(14):2068–2069
20. CLSI (2009) Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement. CLSI document M100-S19. Clinical and Laboratory Standards Institute, Wayne
21. U.S. Food and Drug Administration Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems, https://www.fda.gov/RegulatoryInformation/Guidances/ucm080564.htm. Cited February 16, 2019
22. Chea N, Bulens SN, Kongphet-Tran T, Lynfield R, Shaw KM, Vagnone PS, Kainer MA, Muleta DB, Wilson L, Vaeth E, Dumyati G, Concannon C, Phipps EC, Culbreath K, Janelle SJ, Bamberg WM, Guh AY, Limbago B, Kallen AJ (2015) Improved phenotype-based definition for identifying carbapenemase producers among carbapenem-resistant Enterobacteriaceae. Emerg Infect Dis 21(9):1611–1616
23. Infectious Diseases Society of America (2011) Altert: Antimicrobial Susceptibility Testing. https://wwwidsocietyorg/public-health/antimicrobial-resistance/antimicrobial-resistance/antimicrobial-susceptibility-testing/. Cited February 16 2019
24. CLSI (2016) Verification of commercial microbial identification and antimicrobial susceptibility testing systems. Clinical and Laboratory Standards Institute, Wayne
25. Gomez-Simmonds A, Nelson B, Eiras DP, Loo A, Jenkins SG, Whittier S, Calfee DP, Satlin MJ, Kubin CJ, Furuya EY (2016) Combination regimens for treatment of carbapenem-resistant Klebsiella pneumoniae bloodstream infections. Antimicrob Agents Chemother 60(6):3601–3607
26. Ji S, Lv F, Du X, Wei Z, Fu Y, Mu X, Jiang Y, Yu Y (2015) Cefepime combined with amoxicillin/clavulanic acid: a new choice

for the KPC-producing K. pneumoniae infection. Int J Infect Dis 38:108–114
27. Landman D, Salamera J, Singh M, Quale J (2011) Accuracy of carbapenem nonsusceptibility for identification of KPC-possessing Enterobacteriaceae by use of the revised CLSI breakpoints. J Clin Microbiol 49(11):3931–3933
28. Brown D, Canton R, Dubreuil L, Gatermann S, Giske C, MacGowan A, Martinez-Martinez L, Mouton J, Skov R, Steinbakk M, Walton C, Heuer O, Struelens MJ, Diaz Hogberg L, Kahlmeter G (2015) Widespread implementation of EUCAST breakpoints for antibacterial susceptibility testing in Europe. Euro Surveill 20(2)
29. Bork JT, Heil EL, Leekha S, Fowler RC, Hanson ND, Majumdar A, Johnson JK (2017) Impact of CLSI and EUCAST Cefepime breakpoint changes on the susceptibility reporting for Enterobacteriaceae. Diagn Microbiol Infect Dis 89(4):328–333
30. Cusack TP, Ashley EA, Ling CL, Rattanavong S, Roberts T, Turner P, Wangrangsimakul T, Dance DAB (2019) Impact of CLSI and EUCAST breakpoint discrepancies on reporting of antimicrobial susceptibility and AMR surveillance. Clin Microbiol Infect 25(7): 910–911
31. Sahu C, Jain V, Mishra P, Prasad KN (2018) Clinical and laboratory standards institute versus European committee for antimicrobial susceptibility testing guidelines for interpretation of carbapenem antimicrobial susceptibility results for Escherichia coli in urinary tract infection (UTI). J Lab Phys 10(3):289–293
32. Humphries RM, Kircher S, Ferrell A, Krause KM, Malherbe R, Hsiung A, Burnham CA (2018) The continued value of disk diffusion for assessing antimicrobial susceptibility in clinical laboratories: report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. J Clin Microbiol 56(8)
33. Mendes RE, Reis AO, Gales AC, Jones RN, Sader HS (2003) Ability of Latin America laboratories to detect antimicrobial resistance patterns: experience of the SENTRY Antimicrobial Surveillance Program (1997-2000). Braz J Infect Dis 7(5):282–289
34. Centers for Disease Control and Prevention (2013) Antibiotic resistance threats in the United States, 2013, http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf. Cited March 20 2019
35. Centers for Disease Control and Prevention (2017) Antibiotic resistance lab network. Atlanta, GA: US Department of Health and Human Services. https://wwwcdcgov/drugresistance/solutions-initiative/ar-lab-networkshtml. Cited February 16, 2019
36. Humphries RM, Abbott AN, Hindler JA (2019) Understanding and addressing CLSI breakpoint revisions - a primer for clinical laboratories. J Clin Microbiol 00203-00219
37. O'Neill J (2016) Tackling drug-resistant infections globally: final report and recommendations. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf. Cited March 20 2019
38. World Health Organization (WHO) (2014) Antimicrobial resistance: a global report on surveillance. WHO, Geneva