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Detection of *Klebsiella pneumoniae* Carbapenemase (KPC) Production in Non-*Klebsiella pneumoniae* Enterobacteriaceae Isolates by Use of the Phoenix, Vitek 2, and Disk Diffusion Methods[∇]

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In this study, we tested the abilities of the Vitek 2, BD Phoenix, and Kirby Bauer disk diffusion tests to detect carbapenemase production in a collection of 14 *Klebsiella pneumoniae* carbapenemase (KPC)-producing non-*Klebsiella pneumoniae* isolates. In addition, we evaluated 13 KPC-positive *K. pneumoniae* isolates by using each of these methods and applied both 2009 and 2010 CLSI carbapenem interpretive guidelines.

Klebsiella pneumoniae carbapenemases (KPC) are Ambler class A plasmid-encoded enzymes that are capable of hydrolyzing all beta-lactam antibiotics, including monobactams, extended-spectrum cephalosporins, and carbapenems. The KPC enzyme was originally described in 2001 in a *Klebsiella pneumoniae* isolate from North Carolina (18). Since then, KPC has disseminated worldwide and, although harbored predominantly by *Klebsiella pneumoniae*, has also been identified in numerous other genera of the *Enterobacteriaceae* (1, 8, 15, 17).

Many clinical microbiology laboratories utilize automated *in vitro* susceptibility testing systems, but some of these have difficulty detecting carbapenemase production (2, 17). Due to these limitations, current guidelines recommend that KPC production be confirmed by an alternative method (5). However, confirmatory testing options are limited to the modified Hodge test (MHT), which is subjective and suffers from false-positive results, and PCR-based methods, which may not be available in all labs (3). The issue of confirmation has led others to evaluate boronic acid inhibition for KPC confirmation. However, these methods also inhibit AmpC activity and are, therefore, not specific to KPC (11, 13, 14).

While the performance of automated systems for the detection of KPC-producing *K. pneumoniae* has been evaluated, little is known about the ability of these systems to detect KPC production in other genera of the *Enterobacteriaceae*. The purpose of this study was to evaluate KPC detection in this population of organisms. A collection of KPC-positive and -negative organisms isolated from patient specimens at Barnes Jewish Hospital and St. Louis Children's Hospital in St. Louis, MO, were evaluated in this study. Fourteen KPC-producing non-*K. pneumoniae* (non-KPNE⁺) isolates of members of the *Enterobacteriaceae* and 13 KPC-positive *K. pneumoniae* (KPNE⁺) isolates were analyzed using the Phoenix and Vitek 2 instruments. KPC-positive strains included *Citrobacter freundii* ($n = 2$), *Proteus mirabilis* ($n = 2$), *Klebsiella pneumoniae*

($n = 13$), *Escherichia coli* ($n = 3$), *Enterobacter aerogenes* ($n = 2$), *Enterobacter cloacae* ($n = 4$), and *Klebsiella oxytoca* ($n = 1$).

The Clinical and Laboratory Standards Institute (CLSI) recently published a revision to the carbapenem breakpoints (Table 1) (5, 6), resulting in a reduction of the MIC by two doubling dilutions per interpretive category for doripenem, meropenem, and imipenem and by three doubling dilutions for ertapenem. For ertapenem, disk diffusion zone sizes were increased by 4 mm per interpretive category. Imipenem and meropenem zone sizes were increased by 7 mm for the susceptible category and 6 mm for the resistant category. Interpretive criteria for doripenem were also included in the updated guidelines. Our results were analyzed using both 2009 and 2010 CLSI carbapenem breakpoints to understand the impact of these changes on KPC detection and interpretation of carbapenem resistance among genera of the *Enterobacteriaceae* (6).

KPC-producing organisms were identified and confirmed via a two-step process. Gram-negative organisms for which susceptibilities were deemed necessary were tested using a 12-drug disk diffusion panel, which included meropenem (4). Organisms having a reduced meropenem zone size (defined as ≤ 25 mm) were analyzed for *bla*_{KPC} by PCR. The TaqMan real-time KPC PCR assay uses primers and probes which detect all currently described KPC variants. The sequences were as follows: for the KPC forward primer, 5'-GCG GAA CCA TTC GCT AAA CTC GAA-3'; for the KPC reverse primer, 5'-AGA AAG CCC TTG AAT GAG CTG CAC-3'; and for the KPC probe, 5'-/6-FAM/ATA CCG GCT CAG GCG CAA CTG TAA GTT A/6-TAMSp/-3' (where 6-FAM represents 6-carboxyfluorescein and 6-TAM represents 6-carboxytetramethylrhodamine). DNA was purified using a bead-beating extraction process (BD GeneOhm lysis kit; BD, Franklin Lakes, NJ) and analyzed on a Cepheid SmartCycler (Sunnyvale, CA). PCR-positive isolates with reduced meropenem zone sizes were considered to be KPC positive.

Susceptibility testing for the test strains was performed using three different methods; the BD Phoenix (Becton Dickinson, Franklin Lakes, NJ) was used in conjunction with the NMIC/ID-134 panel, the Vitek 2 (bioMérieux, Marcy l'Etoile, France)

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TABLE 1. 2009 and 2010 CLSI carbapenem interpretive guidelines^a

Drug	MIC (mg/liter)						Zone size (mm)					
	S		I		R		S		I		R	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Doripenem	≤0.5 ^b	≤1	4	2	≥8	≥4	≥19	≥23	16–18	20–22	≤15	≤19
Ertapenem	≤2	≤0.25	8	0.5	≥16	≥4	≥16	≥23	14–15	20–22	≤13	≤19
Meropenem	≤4	≤1	8	2	≥16	≥4	≥16	≥23	14–15	20–22	≤13	≤19
Imipenem	≤4	≤1	8	2	≥16	≥4	≥16	≥23	14–15	20–22	≤13	≤19

^a 2009 CLSI breakpoints and 2010 proposed CLSI breakpoints are given. S, susceptible; I, intermediate; R, resistant.

^b 2009 FDA breakpoint for doripenem.

was used in conjunction with the EXN9 and GN24 cards, and disk diffusion susceptibilities for ertapenem, meropenem, and imipenem were determined using 2009 and 2010 CLSI guidelines.

The Vitek 2 system uses two cards in tandem to test four carbapenems (ertapenem, meropenem, doripenem, and imipenem). The GN24 card can be used alone and includes ertapenem and imipenem. The “extension” EXN9 card cannot

be used alone and must be coupled with the GN24 card. By using the 2010 CLSI guidelines for carbapenem interpretation, the Vitek 2 predicted 92.9 and 100% of KPC-positive non-*K. pneumoniae* (non-KPNE⁺) and *K. pneumoniae* (KPNE⁺) isolates, respectively, as nonsusceptible (i.e., intermediate or resistant) to doripenem (Fig. 1). It performed less well for meropenem and imipenem, where only 64.3 and 84.6% of non-KPNE⁺ and KPNE⁺ isolates, respectively, were nonsus-

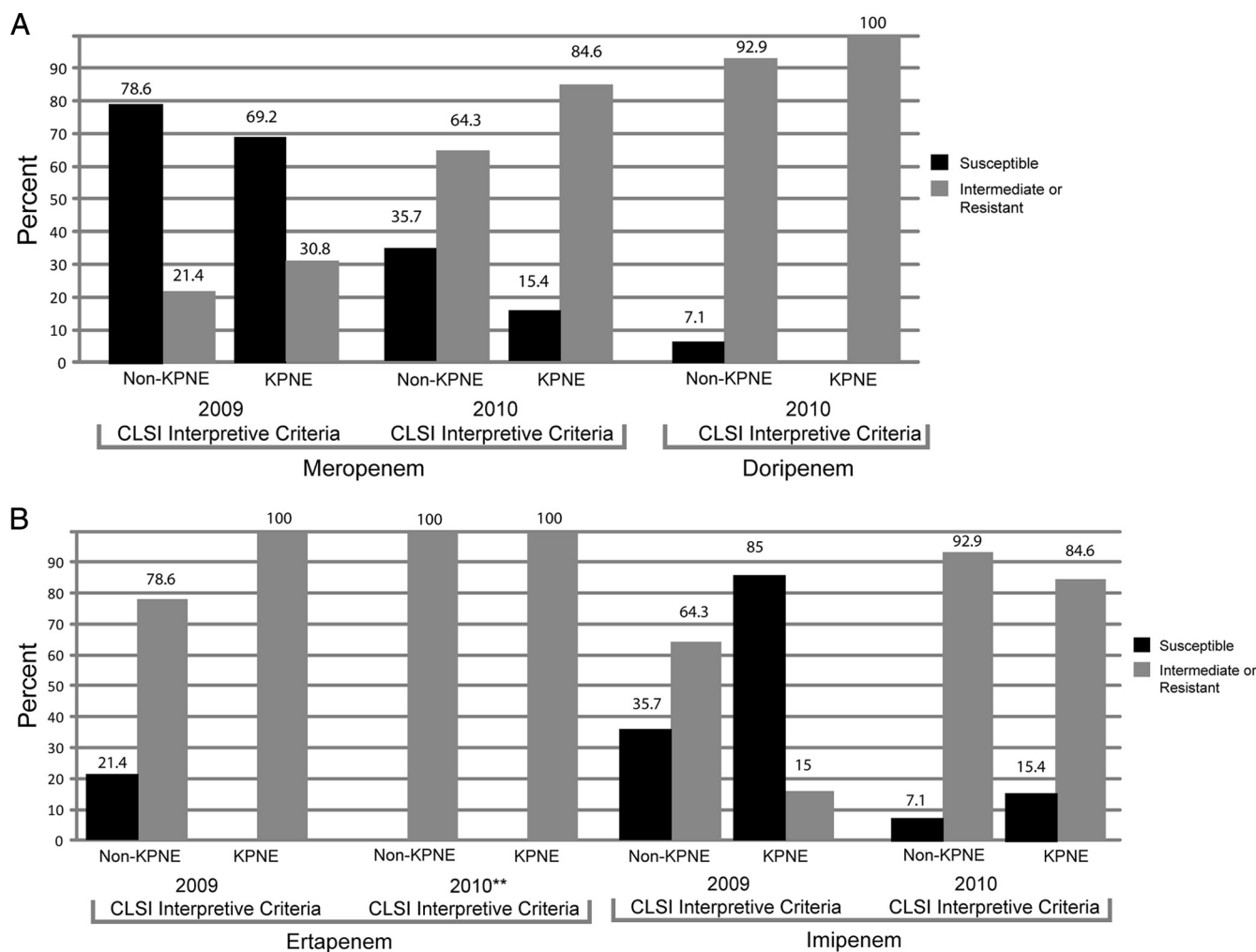


FIG. 1. Percent susceptible and nonsusceptible KPC-producing *Klebsiella pneumoniae* and non-*Klebsiella pneumoniae* isolates, as tested on Vitek 2. (A) EXN9 card. (B) GN24 card. **, the GN24 card does not possess low enough dilutions to categorize isolates as susceptible to ertapenem by the 2010 guidelines.

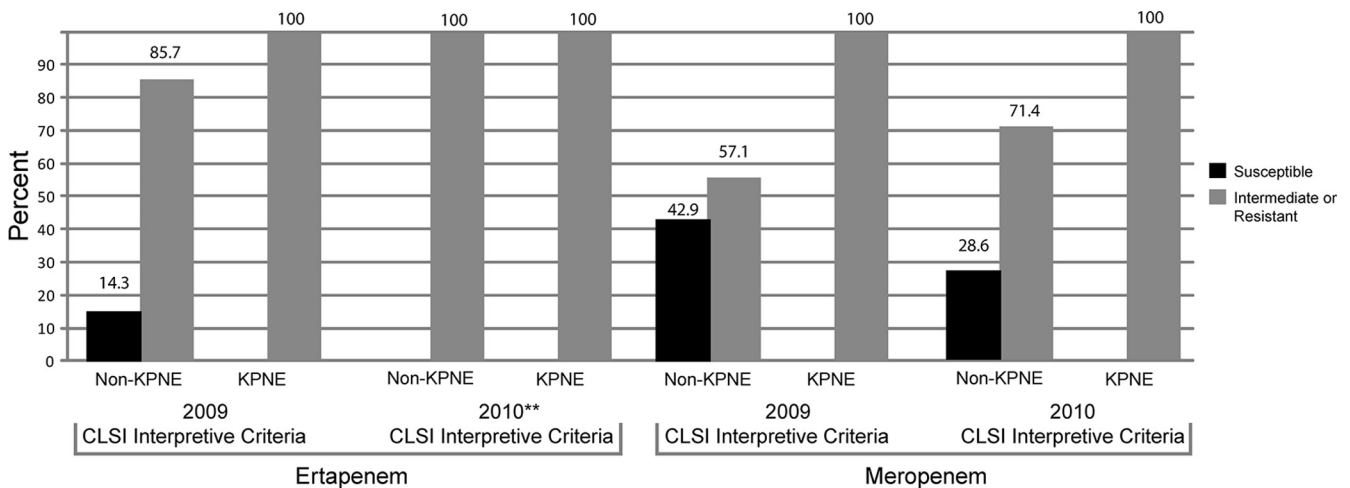


FIG. 2. Percent susceptible and nonsusceptible KPC-producing *Klebsiella pneumoniae* and non-*Klebsiella pneumoniae* isolates, as tested on the BD Phoenix NMIC/ID-134 panel. **, the NMIC/ID-134 panel does not possess low enough dilutions to categorize isolates as susceptible to ertapenem by the 2010 guidelines.

ceptible to meropenem. For imipenem, 92.9% of non-KPNE⁺ isolates and 84.6% of KPNE⁺ isolates were reported as nonsusceptible (Fig. 1). All KPC-producing isolates were classified as nonsusceptible to ertapenem. However, with the implementation of the new CLSI breakpoints, the Vitek cards lack dilutions less than 0.5 mg/liter for ertapenem and as a result cannot classify organisms as susceptible (Fig. 1).

When 2009 CLSI guidelines were applied, the Vitek 2 classified only 21.4 and 30.8% of non-KPNE⁺ and KPNE⁺ isolates, respectively, as nonsusceptible to meropenem (Fig. 1). For imipenem, only 64.3 and 15% of non-KPNE⁺ and KPNE⁺ isolates were classified as nonsusceptible, respectively. As expected, ertapenem demonstrated superior sensitivity for KPC detection, with 78.6 and 100% of non-KPNE⁺ and KPNE⁺ isolates testing as nonsusceptible, respectively.

The problem with ertapenem is not unique to the Vitek 2, as the BD Phoenix NMIC/ID-134 panel also lacks the dilutions necessary to classify isolates as susceptible with the revised (2010) breakpoints. Without that function, the BD Phoenix NMIC/ID-134 is left to rely on meropenem as the sole carbapenem representative in the “susceptible” category. For meropenem, 71.4 and 100% of non-KPNE⁺ and KPNE⁺ isolates, respectively, were classified as nonsusceptible (Fig. 2).

When 2009 breakpoints were used, ertapenem interpretations classified 85.7 and 100% of non-KPNE⁺ and KPNE⁺ isolates as nonsusceptible, respectively. For meropenem, 57.1% of non-KPNE⁺ isolates were classified as nonsusceptible, while 100% of KPNE⁺ isolates were reported as nonsusceptible (Fig. 2).

We also evaluated disk diffusion using ertapenem, meropenem, and imipenem and CLSI 2010 zone size criteria. For ertapenem and meropenem, all KPC-positive isolates were classified as nonsusceptible. Imipenem classified 100% of KPNE⁺ isolates and 90.9% of non-KPNE⁺ isolates as nonsusceptible.

No differences in categorization were observed for ertapenem by using 2009 CLSI interpretations, as 100% of isolates were categorized as intermediate or resistant. A single KPC-

positive isolate each of *E. coli* and *K. pneumoniae* was misclassified as meropenem susceptible by using the old breakpoints. The CLSI breakpoint revisions had the greatest impact on classification of KPC-positive isolates when imipenem was used. With the 2009 criteria, 36.4% of non-KPNE⁺ isolates and 25% of KPNE⁺ isolates were categorized as susceptible to imipenem (Fig. 3).

Although revision to MIC and zone size interpretive guidelines should improve the ability of these methodologies to detect KPC-producing *Enterobacteriaceae*, the improved sensitivity may come with decreased specificity. To better understand how these changes will impact the classification of non-KPC-producing *Enterobacteriaceae*, we evaluated 22 KPC-negative isolates (6 *K. pneumoniae* isolates and 16 isolates of other genera of the *Enterobacteriaceae*). Because both the Vitek 2 and the BD Phoenix are currently unable to classify organisms as susceptible to ertapenem, ertapenem was excluded from these analyses. For this set of organisms, the Vitek 2 reported 3 (14%) isolates as “intermediate” to at least one carbapenem. Two of these were *Enterobacter cloacae* isolates and were also resistant to extended-spectrum cephalosporins. We believe that these strains combined a derepressed *ampC* along with a porin mutation to generate increased resistance. The BD Phoenix categorized one *Enterobacter aerogenes* isolate as resistant to meropenem and one *E. cloacae* isolate as intermediate (9.1%). Based on disk diffusion, 8 isolates (36%) were interpreted as resistant to ertapenem and 1 (4.5%) was interpreted as intermediate. Fifteen isolates (5 *K. pneumoniae* isolates and 10 isolates of other genera) were available for meropenem and imipenem disk diffusion testing. For meropenem, 2 (13%) of the *E. cloacae* isolates were intermediate, and 1 (6.5%) *E. aerogenes* isolate was resistant. The same *E. aerogenes* isolate that was interpreted as resistant to meropenem was also resistant to imipenem (6.5%) (data not shown).

2009 CLSI disk diffusion zone sizes classified 100% of *K. pneumoniae* isolates as susceptible to ertapenem, meropenem, and imipenem. For the other genera, nearly half were catego-

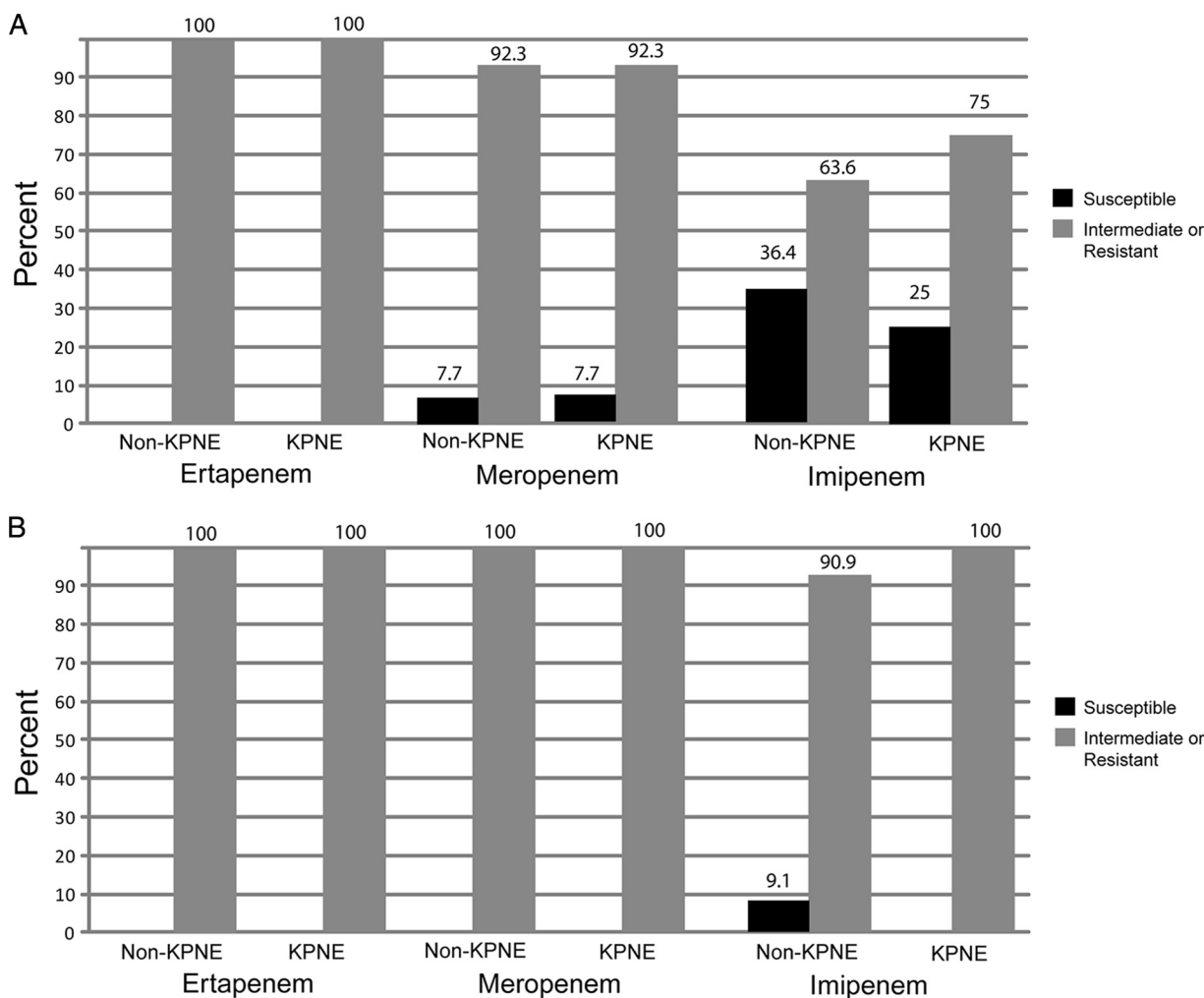


FIG. 3. Percent susceptible and nonsusceptible KPC-producing *Klebsiella pneumoniae* and non-*Klebsiella pneumoniae* isolates, as tested by disk diffusion. (A) 2009 CLSI zone sizes. (B) 2010 CLSI zone sizes.

ized as intermediate or resistant to ertapenem, while for imipenem and meropenem, 88.9% of isolates were susceptible. Only 11.1% were resistant to imipenem and meropenem, which represented a single isolate of *Enterobacter aerogenes* (data not shown).

While the KPC enzyme is still found most commonly in association with *K. pneumoniae*, the *bla*_{KPC} gene has now been identified in numerous other members of the *Enterobacteriaceae* (7, 9, 12). Given the infection control implications and the high morbidity and mortality associated with infections caused by these organisms, it is essential that susceptibility methods identify carbapenem resistance rapidly and reliably (10, 16, 19). Our data show that the new CLSI breakpoints have increased the likelihood that these systems will classify a KPC-producing organism as nonsusceptible to carbapenem antibiotics.

For disk diffusion, our data show that the 2010 breakpoints will have the greatest impact on imipenem categorical interpretation, as 35% of all isolates were classified as susceptible by 2009 criteria whereas all but one were reported as resistant by 2010 criteria (Fig. 3).

Although each system misclassified several KPC-positive strains as susceptible to a carbapenem, most KPC-positive strains were reported as having an unusual susceptibility pattern. In addition, most KPC-positive isolates tested resistant to extended-spectrum cephalosporins and cefepime. One concern is the performance of the Phoenix for KPC-positive *Proteus mirabilis* isolates. Both strains (confirmed as KPC positive by PCR) were reported to be susceptible to meropenem, ceftazidime, cefotaxime, and cefoxitin, with no result generated for cefepime. None of our other KPC-positive study isolates demonstrated across-the-board susceptibility to the carbapenems and extended-spectrum cephalosporins and cefepime with either the Phoenix or the Vitek 2.

One limitation of the 2010 breakpoints for automated systems such as Phoenix and Vitek 2 is that the lowest ertapenem dilution currently available on either panel is 0.5 mg/liter. With the new breakpoints, ertapenem susceptibility is defined as ≤ 0.25 mg/liter, and both systems fail to include dilutions within the actual susceptible range.

In conclusion, accurate and rapid detection of *Enterobacteriaceae* harboring the KPC enzyme is of clinical importance

in ensuring that the correct antimicrobial therapy is given to patients infected with these organisms and that appropriate infection control measures are initiated. Historically, automated systems have struggled to accurately identify KPC-producing organisms. Our analysis indicates that while the non-KPNE⁺ *Enterobacteriaceae* isolates are more likely to be falsely classified as susceptible, this is largely rectified by the 2010 CLSI update to the carbapenem breakpoints.

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