Evaluation of Updated Interpretative Criteria for Categorizing *Klebsiella pneumoniae* with Reduced Carbapenem Susceptibility[▽]

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We studied the accuracy of various susceptibility testing methods, including the 2009, 2010, and updated 2010 CLSI recommendations, to identify *Klebsiella pneumoniae* isolates with reduced susceptibility to carbapenems associated with different mechanisms of resistance. Forty-three wild-type (WT) strains, 42 extended-spectrum β -lactamase (ESBL) producers, 18 ESBL producers with outer membrane porin protein loss (ESBL/Omp strains), and 42 *bla*_{KPC}-possessing *K. pneumoniae* (KPC-Kp) isolates were evaluated. Imipenem (IPM), meropenem (MEM), ertapenem (ERT), and doripenem (DOR) were tested by broth microdilution (BMD), Etest, and disk diffusion (DD), and the modified Hodge test (MHT) was performed using IPM and MEM disks. Results were interpreted according to original as well as recently updated interpretative criteria. MHT was positive for all 42 KPC-Kp isolates and 10 of 18 ESBL/Omp strains and therefore had poor specificity in differentiating between KPC-Kp and ESBL/Omp isolates. Based on the updated CLSI standards, phenotypic susceptibility testing by BMD and DD differentiated most carbapenem-susceptible from carbapenem-nonsusceptible *K. pneumoniae* isolates without the need for MHT, while the Etest method characterized many KPC-Kp isolates as susceptible, and breakpoints may need to be lowered for this method. However, both the original and updated CLSI criteria do not adequately differentiate between isolates in the KPC-Kp group, which are unlikely to respond to carbapenem therapy, and those in the ESBL/Omp group, which are likely to respond to carbapenem therapy if MICs are within pharmacokinetic/pharmacodynamic targets. Further studies are required to determine if there is a clinical need to differentiate between KPC-Kp and ESBL/Omp groups.

In the last decade, the frequent occurrence of infections due to resistant Gram-negative organisms, particularly *Klebsiella pneumoniae*, producing a variety of extended-spectrum β -lactamases (ESBLs) has resulted in physicians frequently resorting to the use of carbapenems (35). As a result, an increasing number of *Enterobacteriaceae* with resistance to carbapenems has been observed (19). So far, the most common mechanism of carbapenem resistance detected among members of the *Enterobacteriaceae* in the United States is the production of class A *K. pneumoniae* carbapenemases (KPCs) (8, 9, 33). Although these enzymes have been identified in many species of *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp., *K. pneumoniae* remains the most common organism carrying these worrisome resistance genes (33, 41). However, carbapenem resistance in *K. pneumoniae* may also be due to production of other carbapenemases (e.g., VIM- and IPM-type enzymes and OXA-48) (3, 40) or to changes in outer membrane porin (Omp) proteins, including OmpK35, OmpK36, and OmpK37, often combined with production of an ESBL, AmpC, or both (11, 20, 25, 27).

Determination of carbapenem resistance among members of the *Enterobacteriaceae* is still performed routinely by traditional phenotypic testing. However, detection of *bla*_{KPC}-possessing *K. pneumoniae* (KPC-Kp) isolates presents a significant challenge for clinical laboratories (1, 28). These isolates are difficult to detect because the majority of them do not manifest high-level resistance to carbapenems (e.g., MICs of 1 to 8 μ g/ml for imipenem [IPM] and meropenem [MEM]), based on susceptibility breakpoints currently in use (2, 15). A phenotypic test using boronic acid recently demonstrated an excellent ability in detecting KPC-Kp isolates (10, 34, 42). However, this method is not commercially available and requires an additional day before results are available.

Since clinical failures have been reported for patients infected with KPC-Kp isolates that appeared to be susceptible to imipenem or meropenem by routine susceptibility testing (43), detection and accurate reporting of these strains are major concerns. To address this problem, the Clinical and Laboratory Standards Institute (CLSI) issued recommendations in 2009, reaffirmed in January 2010, to improve the detection of carbapenemases among *Enterobacteriaceae* (5, 6). These recommendations include performing a modified Hodge test (MHT) on isolates resistant to at least one extended-spectrum cephalosporin and with carbapenem MICs or zone diameters at the upper end of the susceptible range (MICs of 2 to 4 μ g/ml for imipenem or meropenem or 2 μ g/ml for ertapenem [ERT] or

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TABLE 1. Original 2009 and 2010 CLSI and FDA criteria for interpretation of susceptibility testing of carbapenems and for detection of carbapenemase production among *Enterobacteriaceae*^a

Agent	MIC breakpoint ($\mu\text{g/ml}$)				Disk diffusion breakpoint (mm)			
	S	MHT ^b	I	R	S	MHT ^b	I	R
IPM	≤ 1	2–4	8	≥ 16	≥ 16	NA	14–15	≤ 13
MEM	≤ 1	2–4	8	≥ 16	≥ 22	16–21	14–15	≤ 13
ERT	≤ 1	2	4	≥ 8	≥ 22	19–21	16–18	≤ 15
DOR ^c	≤ 0.5	NA	NA	NA	≥ 23	NA	NA	NA

^a See references 5 and 6. S, susceptible; I, intermediate; R, resistant; MHT, modified Hodge test; NA, not applicable.

^b MHT is required to detect carbapenemase production. If MHT is positive, report MICs with no interpretation and with the following comment. “This isolate demonstrates carbapenemase production. The clinical efficacy of the carbapenems has not been established for treating infections caused by *Enterobacteriaceae* that test carbapenem susceptible but demonstrate carbapenemase production in vitro.” If MHT is negative, report carbapenems as susceptible (5, 6).

^c Food and Drug Administration criteria are given for DOR.

meropenem or ertapenem zone diameters of 16 to 21 or 19 to 21 mm, respectively), as they may produce carbapenemases such as KPC (Table 1) (5, 6). If the MHT is negative, carbapenem MICs are to be reported as indicating susceptibility; if the MHT is positive, carbapenem MICs are to be reported without an interpretation and with the following comment. “This isolate demonstrates carbapenemase production. The clinical efficacy of the carbapenems has not been established for treating infections caused by *Enterobacteriaceae* that test carbapenem susceptible but demonstrate carbapenemase production *in vitro*.” Performing the MHT also delays the availability of results for a day. Interpretative MIC breakpoints for doripenem (DOR) for use against *Enterobacteriaceae* were set by the U.S. Food and Drug Administration (FDA) at $\leq 0.5 \mu\text{g/ml}$ for susceptibility and $\geq 1 \mu\text{g/ml}$ for nonsusceptibility. The CLSI subsequently issued an update in June 2010, lowering the susceptibility breakpoints to $\leq 1 \mu\text{g/ml}$ for imipenem and meropenem and $\leq 0.25 \mu\text{g/ml}$ for ertapenem and adding a susceptibility breakpoint of $\leq 1 \mu\text{g/ml}$ for doripenem (Table 2) (7).

In the present work, we investigate the accuracy of various standard phenotypic methods for testing four commercially available carbapenems, IPM, MEM, ERT, and DOR, to determine the ability of the 2009 and updated 2010 CLSI criteria to detect KPC-Kp isolates.

MATERIALS AND METHODS

Clinical isolates. One hundred forty-five clinical isolates of *K. pneumoniae* were chosen for the study. The collection included 43 wild-type (WT) isolates that were isolated at University Hospitals Case Medical Center (Cleveland, OH) during 2009 and 42 well-characterized KPC-Kp isolates collected in 2006 and 2007 in the eastern United States (12, 13, 15, 16). The remaining 60 *K. pneumoniae* isolates showed an ESBL phenotype. Twenty of these 60 ESBL-producing isolates were part of a previous group of isolates collected from different

countries in 1996 and 1997 (35). The remaining 40 ESBL producers were collected at the University of Pittsburgh Medical Center (Pittsburgh, PA) in 2005 and 2006 and at the Cleveland Clinic Foundation (Cleveland, OH) in 2007 and were characterized genetically in the present work.

Molecular characterization. The last group of 40 ESBL-producing *K. pneumoniae* isolates was analyzed by PCR amplification and DNA sequencing for *bla*_{KPC}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, *bla*_{PER}, *bla*_{AmpC}, *bla*_{VIM}, and *bla*_{IMP} genes, as previously reported (15). If present, *bla*_{AmpC} genes were additionally characterized as previously described (15).

For selected *bla*_{KPC}-negative isolates in the overall collection with raised carbapenem MICs compared to the WT group, PCR and DNA sequencing of three *Omp* protein genes (*ompK35*, *ompK36*, and *ompK37*) and nine carbapenemase genes (*bla*_{SPM}, *bla*_{SME}, *bla*_{OXA-48}, *bla*_{GIM}, *bla*_{SIM}, *bla*_{IMI}, *bla*_{NMC}, and *bla*_{GES}) were performed as previously described (14, 15, 40). DNA sequences were analyzed using Lasergene 7.2 (DNASTAR, Madison, WI). The amino acid sequences were deduced using the ExPASy Proteomics Server (<http://ca.expasy.org>) and compared with those previously described (GenBank accession numbers are as follows: for *OmpK35*, AJ303057; for *OmpK36*, AJ344089; and for *OmpK37*, AJ011502) (www.lahey.org/Studies/).

Phenotypic characterization. All isolates were initially characterized by use of a MicroScan system (Siemens, Healthcare Diagnostics) using Gram-negative Combo NBPC34 trays, which include IPM, MEM, ERT, and screening for ESBL production with cefotaxime and ceftazidime alone and combined with clavulanate. All isolates were tested against IPM, MEM, ERT, and DOR by reference CLSI broth microdilution (BMD; Trek Diagnostics), disk diffusion (DD) (6), and Etest (bioMérieux) on cation-adjusted Mueller-Hinton (MH) agar or broth. MHT was performed and interpreted according to the current CLSI guidelines, using IPM and MEM disks, for all 145 isolates (6).

For selected *K. pneumoniae* isolates with raised carbapenem MICs, a disk enzymatic assay was performed to detect carbapenemase production. Briefly, a crude extract of β -lactamases was obtained as previously reported (36). Ten microliters of crude extract was placed on a disk of IPM (10 μg), with 10 μl of sterile water placed on another IPM disk as a control. Following 24 h of incubation at room temperature, the two IPM disks were placed on an MH agar plate inoculated with a 0.5 McFarland inoculum of *Escherichia coli* ATCC 25922 and incubated at 35°C overnight. After incubation, the inhibition zone diameter was measured and interpreted as suspicious for carbapenemase production if a ≥ 2 -mm decrease in the zone of inhibition of IPM plus crude β -lactamase extract was detected compared to that of IPM alone.

Data analysis. All phenotypic results were interpreted according to FDA and updated CLSI criteria for DOR and original and updated CLSI criteria for the other carbapenems, including results of MHT when required (Tables 1 and 2) (6, 7). To assess the ability to detect carbapenemase-producing *K. pneumoniae* isolates, sensitivity and specificity were calculated for each carbapenem tested by different methods. Data were also analyzed to determine the methods that best distinguish carbapenem-susceptible groups (WT strains and ESBL producers) from carbapenem-nonsusceptible groups (ESBL producers with changes in *Omp* proteins and KPC-Kp isolates).

RESULTS

Molecular characterization. The 40 previously uncharacterized *K. pneumoniae* isolates were confirmed as *bla*_{ESBL}-containing isolates. Different families of ESBL genes were de-

TABLE 2. Updated CLSI criteria for interpretation of susceptibility testing of carbapenems^a

Agent	MIC breakpoint ($\mu\text{g/ml}$)			Disk diffusion breakpoint (mm)		
	S	I	R	S	I	R
IPM	≤ 1	2	≥ 4	≥ 23	20–22	≤ 19
MEM	≤ 1	2	≥ 4	≥ 23	20–22	≤ 19
ERT	≤ 0.25	0.5	≥ 1	≥ 23	20–22	≤ 19
DOR	≤ 1	2	≥ 4	≥ 23	20–22	≤ 19

^a See reference 7. S, susceptible; I, intermediate; R, resistant.

TABLE 3. Characteristics of *K. pneumoniae* isolates used in this study

<i>K. pneumoniae</i> group	No. of isolates	Area (yr) of detection	Note ^a	Reference or source
WT	43	Cleveland, OH (2009)	Isolates were resistant to ampicillin only; molecular characterization was not performed	This study
ESBL producers	20	Intercontinental distribution (1996-1997)	The following <i>bla</i> _{ESBL} genes were detected: <i>bla</i> _{TEM-10} (<i>n</i> = 3), <i>bla</i> _{TEM-63} (<i>n</i> = 1), <i>bla</i> _{SHV-2} (<i>n</i> = 5), <i>bla</i> _{SHV-5} (<i>n</i> = 12), <i>bla</i> _{CTX-M-2} (<i>n</i> = 1), and <i>bla</i> _{PER-1-like} (<i>n</i> = 2)	35
	22	Pittsburgh, PA (2005-2006), Cleveland, OH (2007)	The following <i>bla</i> _{ESBL} genes were detected: <i>bla</i> _{SHV-2} (<i>n</i> = 2), <i>bla</i> _{SHV-5} (<i>n</i> = 3), <i>bla</i> _{SHV-5-like} (<i>n</i> = 9), <i>bla</i> _{SHV-7} (<i>n</i> = 2), <i>bla</i> _{SHV-12} (<i>n</i> = 2), <i>bla</i> _{TEM-10-like} (<i>n</i> = 3), and <i>bla</i> _{CTX-M-2-like} (<i>n</i> = 3)	This study
ESBL producers with OmpK loss	18	Pittsburgh, PA (2005-2006), Cleveland, OH (2007)	The following <i>bla</i> _{ESBL} or <i>bla</i> _{AmpC} genes were detected: <i>bla</i> _{SHV-2} (<i>n</i> = 1), <i>bla</i> _{SHV-5} (<i>n</i> = 6), <i>bla</i> _{SHV-5-like} (<i>n</i> = 5), <i>bla</i> _{SHV-12} (<i>n</i> = 5), <i>bla</i> _{SHV-75} (<i>n</i> = 1), <i>bla</i> _{CTX-M-2-like} (<i>n</i> = 4), and <i>bla</i> _{CMY-2-like} (<i>n</i> = 1); 10 isolates did not possess complete <i>ompK36</i> and <i>ompK37</i> genes, and 8 isolates did not possess a complete <i>ompK35</i> gene	This study
KPC producers	42	Eastern USA (2006-2007)	The following <i>bla</i> genes were detected: <i>bla</i> _{KPC-2} (<i>n</i> = 25), <i>bla</i> _{KPC-3} (<i>n</i> = 17), <i>bla</i> _{SHV-5} (<i>n</i> = 1), <i>bla</i> _{SHV-11} (<i>n</i> = 40), <i>bla</i> _{SHV-12} (<i>n</i> = 21), <i>bla</i> _{SHV-14} (<i>n</i> = 1), <i>bla</i> _{SHV-26} (<i>n</i> = 1), <i>bla</i> _{SHV-68} (<i>n</i> = 1), and <i>bla</i> _{TEM-1} (<i>n</i> = 38)	12-16

^a Some isolates possess more than one *bla*_{ESBL} gene.

tected, including *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}, whereas *bla*_{KPC}, *bla*_{VIM}, and *bla*_{IPM} were not found among these isolates; only one isolate possessed a *bla*_{AmpC} gene (*bla*_{CMY-2-like}) (Table 3). Of the 60 confirmed ESBL-producing carbapenemase-negative isolates included in the present study (40 characterized above and 20 previously characterized isolates), 18 were nonsusceptible to ERT (1 intermediate and 17 resistant) but susceptible to IPM or MEM on initial MicroScan screening. This subgroup of isolates was categorized as ESBL producers with Omp changes (ESBL/Omp strains). Ten of the ESBL/Omp isolates were analyzed by PCR and DNA sequencing for the *ompK35*, -36, and -37 genes. Disrupted porin coding sequences in *ompK36* and -37 genes were found in all 10 strains, and disrupted sequences in the *ompK35* gene were found in 8 strains. On the basis of the above molecular characterization, we categorized the 145 *K. pneumoniae* isolates studied into four groups: WT (*n* = 43), ESBL producers (*n* = 42), ESBL/Omp strains (*n* = 18), and KPC-Kp isolates (*n* = 42) (Table 3).

Phenotypic characterization. Histograms of MIC distributions of the four carbapenems by BMD and Etest and of zone diameters by DD are shown in Fig. 1, 2, and 3. These show bimodal distributions for all four carbapenems, with the more-susceptible peak including the WT and ESBL groups and the less-susceptible peak including the ESBL/Omp and KPC-Kp groups, with some overlap between the two distributions that varied by method and carbapenem.

Application of previous interpretative criteria showed that all WT isolates were categorized as susceptible to IPM, MEM, ERT, and DOR, regardless of the phenotypic test used. In contrast, differences among methodologies were observed for the remaining three groups of *K. pneumoniae* isolates. By BMD, all ESBL-positive isolates were susceptible to IPM and MEM, whereas two strains (4.8%) were nonsusceptible to ERT and DOR (Fig. 1). Six (33.3%) ESBL/Omp isolates were nonsusceptible to IPM, and 10 (55.6%) were nonsusceptible to MEM; all were resistant to ERT and nonsusceptible to DOR. One (2.4%) KPC-Kp isolate was susceptible to IPM, and two (4.8%) were nonsusceptible to MEM, whereas all isolates were nonsusceptible to ERT and DOR. By Etest, all ESBL isolates

were susceptible to IPM, MEM, and DOR, whereas one (2.4%) strain was intermediate for ERT (Fig. 2). Two (11.1%) ESBL/Omp isolates were nonsusceptible to IPM, and seven (38.9%) were nonsusceptible to MEM; all were resistant to ERT and nonsusceptible to DOR. Twenty-nine (69.0%) KPC-Kp isolates were susceptible to IPM, 26 (61.9%) were susceptible to MEM, 5 (11.9%) were susceptible to ERT, and 1 (2.4%) was susceptible to DOR. By DD, all ESBL isolates were susceptible to IPM and MEM, whereas four (9.5%) isolates were nonsusceptible to ERT and two (4.8%) were nonsusceptible to DOR (Fig. 3). Two (11.1%) ESBL/Omp isolates were nonsusceptible to IPM, 11 (61.1%) were nonsusceptible to MEM, all were nonsusceptible to ERT, and 1 (5.6%) was nonsusceptible to DOR. Twenty-seven (62.3%) KPC-Kp isolates were susceptible to IPM, nine (21.4%) were susceptible to MEM, and none were susceptible to ERT or DOR.

The MHT performed with IPM was positive for all KPC-Kp isolates and for 14 (9.7%) non-KPC-Kp isolates (11 ESBL/Omp strains and 3 ESBL-positive strains). The MHT with MEM was positive for all KPC-Kp isolates and for 10 (6.9%) non-KPC-Kp isolates (9 ESBL/Omp strains and 1 ESBL-positive strain). Examples of MHT results are shown in Fig. 4. Further testing to detect other carbapenemases was then performed on the 10 *bla*_{KPC}-negative strains with positive MHT results for both IPM and MEM; all were negative for *bla*_{VIM}, *bla*_{IPM}, *bla*_{SPM}, *bla*_{SME}, *bla*_{OXA-48}, *bla*_{GIM}, *bla*_{SIM}, *bla*_{IMI}, *bla*_{NMC}, and *bla*_{GES} genes by PCR. Furthermore, none of them showed a decrease in the zone of inhibition of IPM plus crude β-lactamase extract compared to that of IPM alone.

Sensitivities and specificities of original and updated criteria in differentiating KPC-Kp isolates from the other three groups. The ability of the 2009, 2010, and updated 2010 CLSI criteria to differentiate between the KPC-Kp group and the other three groups was evaluated with the 145 study isolates. For these analyses, the strains in the KPC-Kp group were classified as true positive strains, whereas strains in the other three groups were classified as true negative strains if characterized as carbapenem susceptible and as false-negative strains if not. Results of these analyses are presented in Table 4. When the original CLSI/FDA criteria were used, testing by the BMD

FIG. 1. Histograms of broth microdilution MICs ($\mu\text{g/ml}$) of the four carbapenems studied against the 145 *K. pneumoniae* isolates. MIC₅₀ and MIC₉₀ values ($\mu\text{g/ml}$) are also shown. The original (black) and updated (red) susceptibility breakpoints for imipenem, meropenem, doripenem, and ertapenem are indicated (6, 7). S, susceptible; I, intermediate; R, resistant; NS, nonsusceptible; MHT, modified Hodge test. Arrows labeled MHT indicate when the MHT should be performed. WT *K. pneumoniae* isolates are shown in green, ESBL-positive (ESBL) isolates are shown in yellow, ESBL-positive isolates with Omp loss (ESBL/Omp) are shown in lavender, and *bla*_{KPC}-possessing (KPC-Kp) isolates are shown in red. All isolates that tested positive with the MHT are designated by diagonal hatching.

method showed good sensitivity (100%) but limited specificity (83 to 92%), regardless of the carbapenem tested. Similar results were obtained by the Etest and DD methods. Applying the updated 2010 CLSI criteria resulted in 100% sensitivity for all four carbapenems by BMD and DD but in a lower sensitivity by Etest (84 to 90%); specificities for all three methods ranged from 84 to 90% for IPM, MEM, and DOR and from 76 to 77% for ERT. The sensitivity and specificity of MHT alone, performed with IPM and MEM, were 100% and 88 to 91%, respectively.

Differentiation of carbapenem-susceptible from carbapenem-nonsusceptible groups. The clinical significance of the above sensitivity and specificity analyses, which address differentiation of the KPC-Kp group from the other three groups, is confounded by the carbapenem nonsusceptibility of the ESBL/Omp group. A more clinically relevant analysis is differentia-

tion of the two carbapenem-susceptible groups (WT and ESBL-producing strains) from the two carbapenem-nonsusceptible groups (ESBL/Omp and KPC-Kp isolates). Application of updated CLSI criteria accurately characterized all WT isolates by all three methods (Table 5). The ESBL group was also accurately characterized by all three methods with IPM, MEM, and DOR, but not with ERT, where one-half to one-third of isolates were characterized as nonsusceptible. All ESBL/Omp strains were characterized as nonsusceptible to ERT by all three methods and as nonsusceptible to DOR by BMD; up to 7 of the 18 strains in this group were susceptible with other combinations. All KPC isolates were categorized as nonsusceptible by BMD and DD. However, this was not the case by Etest, where between 1 and 10 strains were classified as susceptible, with most of the misclassified strains falling at the upper limit of the susceptible range.

FIG. 2. Histograms of Etest MICs (μg/ml) of the four carbapenems studied against the 145 *K. pneumoniae* isolates. Abbreviations and colors used are the same as those in Fig. 1.

DISCUSSION

Production of carbapenemases is an increasing problem among clinical isolates of Gram-negative bacilli (40). In particular, the spread of KPC-Kp isolates represents a serious threat to our therapeutic armamentarium, as these isolates are resistant to all currently available β-lactams and β-lactam-β-lactamase inhibitor combinations. Prevalence rates of KPC-Kp isolates of >30% have been recorded in some institutions in the eastern United States, in association with nosocomial outbreaks (24, 33). Detection and reporting of KPC-Kp isolates pose a significant challenge to clinical microbiologists, since these organisms may be difficult to detect using standard methods (33, 39). Endimiani et al. reported that approximately 60% of KPC-Kp isolates have IPM or MEM MICs in the susceptible range (15). This low-level expression of the resistance mechanism is the main reason that automated and nonautomated phenotypic tests have difficulty in detecting KPC-Kp isolates (1, 28). In con-

trast to the case for IPM and MEM, KPC-Kp strains frequently have ERT MICs in the resistant range (2, 15). Thus, the use of ERT has been suggested to screen for KPC production among *Enterobacteriaceae* (1, 2, 33). Nevertheless, recent papers have reported increased numbers of ERT-resistant but IPM- or MEM-susceptible *K. pneumoniae* isolates that are not carbapenemase producers (22, 36). These isolates are associated with loss or decreased expression of outer membrane porins combined with production of ESBLs (11, 25). These strains are responsible for the low specificity recorded for ERT and the MHT in detecting KPC-Kp isolates (4, 28).

CLSI modified the carbapenem interpretations for *Enterobacteriaceae* in 2009, recommending performance of the MHT, using either MEM or ERT disks, for strains with susceptibilities near susceptibility breakpoints (Table 1) (5). However, performing the MHT is time-consuming and delays the availability of results by at least 24 h. Additionally,

FIG. 3. Histograms of disk diffusion inhibitory zones (IZ) (mm) of the four carbapenems studied against the 145 *K. pneumoniae* isolates. IZ₅₀ and IZ₉₀ values (mm) are also shown. Abbreviations and colors used are the same as those in Fig. 1.

the MHT is sometimes difficult to interpret, and false-positive results have been reported (4, 26, 34). Use of a phenotypic test using boronic acid distinguished between KPC-Kp isolates and strains not harboring KPC enzymes (10, 34, 42). Boronic acid may offer some advantage in accuracy over the MHT but offers no time advantage, as an additional day is needed before results are available. CLSI updated the carbapenem breakpoints for *Enterobacteriaceae*

FIG. 4. Representative pictures of MHT performed with MEM. (A) Strongly positive MHT for a *bla*_{KPC}-possessing isolate. (B) Negative MHT for a WT isolate. (C) Weakly positive MHT for an isolate producing ESBL and showing Omp loss.

TABLE 4. Statistical analysis of the different phenotypic tests used to differentiate carbapenemase-producing *K. pneumoniae* isolates ($n = 42$) from non-carbapenemase producers ($n = 103$)^a

Method	Interpretative criteria	Sensitivity (%) ^b				Specificity (%) ^b			
		IPM	MEM	ERT	DOR	IPM	MEM	ERT	DOR
BMD	Original CLSI/FDA criteria ^c	100	100	100	100	92.0	89.6	83.1	83.7
	Updated 2010 CLSI criteria ^d	100	100	100	100	86.6	86.6	76.3	85.1
Etest	Original CLSI/FDA criteria ^c	100	100	100	97.7	95.4	92.8	84.4	85.1
	Updated 2010 CLSI criteria ^d	80.8	91.3	97.7	95.5	88.8	85.8	77.4	88.8
DD	Original CLSI/FDA criteria ^c	NA	100	100	100	NA	87.3	82.4	84.4
	Updated 2010 CLSI criteria ^d	100	100	100	100	90.4	84.4	77.4	84.4
MHT alone ^e	NA	100	100	NA	NA	88.0	91.2	NA	NA

^a BMD, broth microdilution; MHT, modified Hodge test; DD, disk diffusion; IPM, imipenem; MEM, meropenem; ERT, ertapenem; DOR, doripenem.

^b Sensitivity = TP/(TP + FN); specificity = TN/(TN + FP). TP, number of carbapenemase producers ($n = 42$); TN, number of non-carbapenemase producers ($n = 103$); TP, number of true positive results; TN, number of true negative results; FP, number of false-positive results; FN, number of false-negative results.

^c Based on original CLSI criteria for IPM, MEM, and ERT, with MHT performed with meropenem, and on FDA criteria for DOR (see Table 1).

^d Based on updated CLSI criteria (see Table 2).

^e Ability of MHT performed on all strains ($n = 145$), using IPM and MEM disks, to identify carbapenemase-producing isolates correctly.

in June 2010, eliminating the need for the MHT and further lowering the ERT breakpoints (7).

In the present work, we evaluated the original and updated criteria for determining carbapenem susceptibility, testing a large collection of well-characterized *K. pneumoniae* isolates. Along with WT strains, KPC-Kp isolates, and ESBL producers, a fourth group of *K. pneumoniae* isolates was investigated. These isolates were categorized as ESBL/Omp strains on the basis of their carbapenem-resistant phenotype and molecular analysis of *ompK* genes.

Based on our results, standard phenotypic susceptibility testing by BMD and DD differentiated most carbapenem-susceptible from carbapenem-nonsusceptible *K. pneumoniae* isolates, based on the updated CLSI criteria (Fig. 1 to 3 and Table 5). However, the Etest method characterized many

KPC-Kp isolates as susceptible, and breakpoints may need to be lowered for this method.

However, several issues are presented by these findings. First, if susceptibility testing is performed by currently available breakpoint MIC methods, including automated systems, such as MicroScan and Vitek methods, many KPC-Kp isolates will be misclassified as susceptible and additional testing will continue to be needed, significantly increasing the turnaround time. Second, many KPC-Kp and ESBL/Omp strains are not distinguished from each other, which may unnecessarily limit the use of carbapenems with ESBL/Omp strains with MICs within pharmacokinetic targets. Third, the MHT does not accurately distinguish between KPC-Kp and ESBL/Omp strains, as many in the latter group are MHT positive. In our study, half of the ESBL/Omp isolates were MHT positive when MEM was employed as suggested by the CLSI. These strains were confirmed as non-carbapenemase producers on the basis of molecular and disk enzymatic assay analyses. This finding has been reported previously, but a clear explanation for this phenomenon is still lacking (4, 34).

The importance of these issues depends on the clinical utility of carbapenems in treating infections due to KPC-Kp and ESBL/Omp isolates. Based upon our findings, an understanding of the enzymatic mechanism of resistance mediated by KPC, and the initial limited expression of KPC by many strains, resulting in MICs that are raised but still in the susceptible range, patients infected with KPC-producing *K. pneumoniae* are unlikely to respond to carbapenems, even if these antibiotics are dosed according to the level of susceptibility manifested by the pathogen and appropriate pharmacokinetic/pharmacodynamic targets are achieved (37, 43). On the other hand, infections due to isolates with Omp loss and coproduction of ESBLs might be treatable with carbapenems if MICs are in the susceptible range based on pharmacokinetic/pharmacodynamic targets (17, 18, 21, 23, 29–32, 38). Further studies are needed to define these relationships and to determine if there is a clinical need to differentiate between the KPC-Kp and ESBL/Omp groups.

TABLE 5. Accuracy of updated 2010 susceptibility breakpoints in differentiating between carbapenem-susceptible groups (WT strains and ESBL producers) and groups with decreased carbapenem susceptibility (ESBL/Omp and KPC-Kp isolates)

Group (n)	Method	No. of susceptible isolates/no. of resistant isolates			
		Imipenem	Meropenem	Ertapenem	Doripenem
WT (43)	BMD ^a	43/0	43/0	43/0	43/0
	Etest ^b	43/0	43/0	43/0	43/0
	DD ^c	43/0	43/0	43/0	43/0
ESBL producers (42)	BMD ^a	40/2	40/2	28/14	40/2
	Etest ^b	40/2	40/2	30/12	42/0
	DD ^c	42/0	40/2	30/12	40/2
ESBL/Omp strains (18)	BMD ^a	4/14	2/16	0/18	0/18
	Etest ^b	5/13	1/17	0/18	5/13
	DD ^c	7/11	1/17	0/18	1/17
KPC-Kp isolates (42)	BMD ^a	0/42	0/42	0/42	0/42
	Etest ^b	10/32	4/38	1/41	2/40
	DD ^c	0/42	0/42	0/42	0/42

^a Based on MIC breakpoints of ≤ 1 μ g/ml (susceptible) and ≥ 2 μ g/ml (non-susceptible) for IPM, MEM, and DOR and ≤ 0.25 μ g/ml (susceptible) and ≥ 0.5 μ g/ml (non-susceptible) for ERT.

^b Based on MIC breakpoints of ≤ 1 μ g/ml (susceptible) and ≥ 1.5 μ g/ml (non-susceptible) for IPM, MEM, and DOR and ≤ 0.25 μ g/ml (susceptible) and ≥ 0.38 μ g/ml (non-susceptible) for ERT.

^c Based on disk diffusion breakpoints of ≥ 23 mm (susceptible) and ≤ 22 mm (non-susceptible) for IPM, MEM, ERT, and DOR.

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