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Specificity of Ertapenem Susceptibility Screening for Detection of *Klebsiella pneumoniae* Carbapenemases▽

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Detection of *Klebsiella pneumoniae* carbapenemases (KPCs) can be nonspecific, especially when KPCs are uncommon. We determined the positive predictive value and specificity of ertapenem resistance for KPC detection in 2,696 *Enterobacteriaceae* isolates. The positive predictive value and specificity of ertapenem resistance for KPC detection were 74% and 99.2%, respectively.

Detection of *Klebsiella pneumoniae* carbapenemases (KPCs) can be difficult because carbapenem MICs may be high but still in the susceptible range as defined by Clinical and Laboratory Standards Institute (CLSI) criteria (3), especially when an automated susceptibility testing instrument is used (1). Ertapenem is the least-active carbapenem against KPCs, and the use of this drug in automated or manual susceptibility testing has been found to be a highly sensitive method for the detection of KPCs (1). However, the specificity of ertapenem testing has been questioned because *Enterobacteriaceae* with extended-spectrum β-lactamases and porin mutations may also be ertapenem resistant. One recent European study found that only 2 of 171 ertapenem-resistant *Enterobacteriaceae* isolates produced carbapenemases, neither of which was a KPC (6).

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We evaluated the performance of ertapenem susceptibility screening for KPCs for all mucoid lactose-positive *Enterobacteriaceae* regardless of susceptibility profile and for all broad-spectrum-cephalosporin-resistant *Enterobacteriaceae* isolated in our laboratory during 2007. Mucoid lactose-positive bacteria were selected because it was known in 2006 that KPCs were present only in *Klebsiella pneumoniae* at our institution. In addition, all non-urine source *Enterobacteriaceae* that underwent susceptibility testing in our laboratory from August to December 2007 were included. Prior to August 2007, all ertapenem susceptibility testing was performed by using the disk diffusion test; subsequently, the use of an ertapenem-containing Vitek II GN-20 card (bioMérieux, Inc., Durham, NC) was instituted. Use of the GN-20 card allowed broader testing of ertapenem against all *Enterobacteriaceae* undergoing antimicrobial susceptibility testing. Meropenem susceptibility testing was performed by using Etest methodology (bioMérieux, Inc) for all KPC-positive bacteria to determine if this method was as sensitive as ertapenem screening for detecting KPCs. Interpretive criteria were defined in CLSI M100–S18 (3); these criteria for ertapenem disk diffusion and MICs are specified as resistant (≤15 mm and ≥8 μg/ml, respectively), intermediate (16 to 18 mm and 4 μg/ml, respectively), and susceptible (≥19 mm and ≤2 μg/ml, respectively).

The presence of KPCs was confirmed by both PCR and the modified Hodge test using meropenem as the indicator drug, both performed as previously described (7). The KPC gene was sequenced for selected KPC-positive isolates as previously described (7).

Ertapenem screening was performed with 2,696 *Enterobacteriaceae* isolates, including isolates of *Enterobacter* spp. (564 isolates), *Escherichia coli* (616 isolates), *K. pneumoniae* (1,352 isolates), and *Proteus mirabilis* (164 isolates). Seventy-eight isolates were ertapenem resistant, and seven isolates were ertapenem intermediate (Table 1). Of these 85 ertapenem-intermediate or -resistant isolates, 63 were KPC positive, all of which were *K. pneumoniae* isolates. All 63 KPC-positive bacterial isolates were found to be positive by both the modified Hodge test and KPC PCR. Sequencing of two KPC-positive isolates confirmed their identities as the KPC-positive variants KPC-2 and KPC-3.

The positive predictive value of ertapenem screening for *K. pneumoniae* isolates was 79%, with a slightly higher predictive value for ertapenem-resistant isolates than for intermediate isolates (80% versus 60%; $P = 0.3$ by Fisher's exact test). Ertapenem screening specificity for KPCs was 99.2%, in large part because only 2.3% of all screened bacteria were positive for KPCs. Ertapenem screening was falsely positive for KPCs in all five ertapenem-intermediate or -resistant *Enterobacter* and *E. coli* isolates, none of which was KPC positive. The ertapenem disk zone size of inhibition for the KPC-positive ertapenem-intermediate bacteria was 16 mm for all three isolates, versus 17, 17, 18, and 19 mm for the four non-KPC bacteria, indicating that it might be possible to distinguish KPC-positive from KPC-negative bacteria solely by the size of the ertapenem disk zone of inhibition, using 16 mm as the breakpoint. Roughly half of the isolates screened for ertapenem resistance were tested by Vitek II and half by disk diffusion testing, with equivalent performances for each type of test.

As reported by others, automated imipenem or meropenem susceptibility testing by Vitek II was very insensitive for the detection of KPCs, with 65 and 48% of KPCs reported as

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TABLE 1. Ertapenem-intermediate or -resistant isolate screen results

Organism	No. of KPC-positive isolates/no. of ertapenem-positive isolates (% total)		
	Intermediate	Resistant	Intermediate or resistant
Enterobacter spp.	0/2	0/2	0/4
E. coli	0/0	0/1	0/1
K. pneumoniae	3/5 (60)	60/75 (80)	63/80 (79)
Total	3/7 (43)	60/78 (77)	63/85 (74)

susceptible to imipenem and meropenem, respectively (1, 2, 5). In contrast, only 7 and 12% of KPCs were meropenem susceptible when tested by the Etest and disk diffusion methods, respectively.

These results show that ertapenem screening for KPCs has a moderately high positive predictive value (79%) and a very high specificity (99.2%) in our region. The positive predictive value is high despite a low (2.3%) prevalence of KPC-positive bacteria among the bacteria being screened and is certainly aided by the rarity (0.4%) of non-KPC ertapenem-resistant isolates. Regardless, confirmatory testing for KPC presence is required for all ertapenem-resistant bacteria, as the false-positive rate was 26% for all isolates tested and 100% for all non-*K. pneumoniae* isolates tested. Both the modified Hodge test and the KPC PCR test performed identically, except for the latter's advantages of a more rapid turnaround time and less dependence on experience with reading the Hodge tests, which are sometimes difficult to read. The performance of ertapenem screening is likely to be much different in other regions where KPCs are rare and especially if ertapenem-resistant non-KPC bacteria are common.

New recommendations for screening for KPCs in *Enterobacteriaceae* were published by the CLSI after the completion of this study (4). These recommendations use screening breakpoints currently in the susceptible range, using either ertapenem or meropenem disk diffusion testing or broth dilution susceptibility testing using ertapenem, meropenem, or imipenem. The disk diffusion breakpoints are 19 to 21 mm and 16 to 21 mm for ertapenem and meropenem, respectively. The suggested MIC screening breakpoints are 2 μg/ml and 2 to 4 μg/ml for ertapenem and for both meropenem and imipenem, respectively. Modified Hodge testing with either ertapenem or meropenem is recommended for isolates with positive screening test results. Based on our results, these new screening breakpoints would likely detect a small number of KPCs not detected by the methods we used but with an even lower positive predictive value than we observed. It is important to note that the new CLSI MIC breakpoint criteria are for conventional broth dilution methods and are not applicable to automated susceptibility instruments, based on our results and those of others, especially for meropenem and imipenem testing (1, 2, 5).

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