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Accuracy of Carbapenem Nonsusceptibility for Identification of KPC-Possessing *Enterobacteriaceae* by Use of the Revised CLSI Breakpoints[▽]

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Using the updated 2010 CLSI carbapenem breakpoints for the *Enterobacteriaceae*, nonsusceptibility to ertapenem and imipenem predicted the presence of bla_{KPC} poorly, especially among *Escherichia coli* and *Enterobacter* species. In regions where KPC-producing bacteria are endemic, testing for nonsusceptibility to meropenem may provide improved accuracy in identifying these isolates.

The worldwide spread of the carbapenemase KPC has been unrelenting, in part because of difficulty in its detection. Using breakpoints defined prior to 2010, it was well documented that many KPC-possessing isolates had imipenem and meropenem MICs reported in the susceptible range (1, 2, 19). Approximately 75% of isolates of *Escherichia coli* (13), 60% of isolates of *Klebsiella pneumoniae* (8), and 25% of *Enterobacter* species isolates (14) that possess KPC were found to be susceptible to imipenem and/or meropenem. In particular, isolates of *K. pneumoniae* with underexpression of bla_{KPC} or with the presence of functional OmpK36 have lower carbapenem MICs (3, 10, 12). Most isolates, including 50% of *E. coli* (13), 95% −100% of *K. pneumoniae* (8, 16), and 100% of *Enterobacter* species isolates, have been found to be resistant to ertapenem. Therefore, ertapenem has been suggested as the agent of choice for screening of *Enterobacteriaceae* for KPC β-lactamases (1, 2, 16).

However, ertapenem resistance has been found in *K. pneumoniae* and *Enterobacter* species lacking a carbapenem-hydrolyzing β-lactamase (7, 16, 20). The combination of a class C cephalosporinase and porin deficiency contributes to ertapenem resistance; many of these isolates remain susceptible to the other carbapenems (9). Therefore, using ertapenem alone as the class agent for carbapenem susceptibility testing, particularly in regions where carbapenemases are infrequent (15), may inappropriately eliminate carbapenems as therapeutic agents against these isolates.

In 2010, the CLSI issued new breakpoints for carbapenems and several cephalosporins (5). The performance of these guidelines and the optimal strategy for detecting KPC-possessing isolates in regions of endemicity have not been determined. In this report, we evaluate the updated guidelines for detecting KPC-possessing members of the *Enterobacteriaceae* and provide recommendations for clinical laboratories when these guidelines are implemented.

Single patient isolates of *E. coli*, *K. pneumoniae*, and *Enterobacter* species were collected during borough-wide surveillance studies performed in 2006 and 2009, as previously described (11, 13). The overall susceptibilities for some of the isolates have been previously reported (11, 13). Susceptibilities to piperacillin-tazobactam, ceftazidime, ertapenem, imipenem, and meropenem were tested by the agar dilution method according to CLSI standards (4). All cephalosporin-resistant isolates were screened for the presence of bla_{KPC}, using previously reported primers and PCR conditions (2). Select isolates resistant to ertapenem but lacking bla_{KPC} were screened for the presence of bla_{CMY} and $bla_{CTX\text{-}M}$ using previously described primers and conditions (18, 21). The sensitivity, specificity, and positive predictive value of nonsusceptibility to each β-lactam in the detection of KPC-producing isolates were determined. The study was approved by the Institutional Review Board at SUNY-Downstate Medical Center.

A total of 4,466 isolates of *E. coli*, 2,173 isolates of *K. pneumoniae*, and 418 isolates of *Enterobacter* species were collected during the two surveillance studies. KPC was detected in 14 (0.3%) isolates of *E. coli*, 721 (33.2%) isolates of *K. pneumoniae*, and 23 (5.5%) isolates of *Enterobacter* species. The sensitivity, specificity, and positive predictive value of nonsusceptibility to ertapenem, imipenem, and meropenem in the detection of KPC-possessing isolates are listed in Table 1. Using the updated breakpoints (5), the sensitivity, specificity, and positive predictive value of nonsusceptibility to ceftazidime for *E. coli* were 92.8%, 92.2%, and 3.6%, respectively; for *K. pneumoniae*, they were 99.2%, 67.8%, and 60.3%; and for *Enterobacter* species, they were 100%, 73.9%, and 18.3%, respectively. In addition, the sensitivity, specificity, and positive predictive value of nonsusceptibility to piperacillin-tazobactam for *E. coli* (only the 2009 surveillance isolates were tested) were 100%, 98.6%, and 18%; for *K. pneumoniae*, they were 97.6%, 79.3%, and 70%; and for *Enterobacter* species, they were 95.7%, 79.5%, and 21.4%, respectively.

As noted in Table 1, virtually all KPC producers were nonsusceptible to ertapenem with the updated breakpoints. However, the positive predictive value was inferior to that for imipenem and meropenem, particularly for *E. coli* and *Enterobacter* species (Table 1). Further evaluation of 111 ertapenem-nonsusceptible, KPC-negative isolates was undertaken. Nine of the 111 isolates (8%) were nonsusceptible to another

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TABLE 1. Performance of nonsusceptibility to three carbapenems in the detection of KPC-producing *Enterobacteriaceae*

Bacterium	Statistic	Value for:					
		Updated breakpoint[a]			Previous breakpoint[b]		
		Ertapenem	Imipenem	Meropenem	Ertapenem	Imipenem	Meropenem
E. coli	% sensitivity	100	71.4	100	57.1	21.4	21.4
	% specificity	99.8	99.9	100	100	100	100
	% positive predictive value	60.9	76.9	87.5	88.9	100	100
K. pneumoniae	% sensitivity	100	91.5	97.8	97.6	66.7	56.5
	% specificity	96.8	99	97.8	98.9	100	99.9
	% positive predictive value	93.9	97.8	95.6	97.8	100	99.8
Enterobacter sp.	% sensitivity	90.9	81.8	95.5	81.8	23.8	27.3
	% specificity	85.6	97.7	100	98.2	100	100
	% positive predictive value	26.3	66.7	100	72	100	100

[a] Updated (2010) CLSI breakpoints for susceptibility: <0.25 μg/ml for ertapenem and <1 μg/ml for imipenem and meropenem.
[b] Previous CLSI breakpoints for susceptibility: <2 μg/ml for ertapenem and <4 μg/ml for imipenem and meropenem.

carbapenem. Of 58 isolates screened, 4 (all *E. coli* isolates) produced bla_{CMY} and none produced $bla_{CTX\text{-}M}$. Of nine isolates of *E. coli*, the MIC_{50}/MIC_{90} values of ertapenem, imipenem, and meropenem were 1/2 μg/ml, ≤0.25/≤0.25 μg/ml, and ≤0.25/0.5 μg/ml, respectively. For 46 isolates of *K. pneumoniae*, the MIC_{50}s/MIC_{90}s of ertapenem, imipenem, and meropenem were 1/8 μg/ml, ≤0.25/1 μg/ml, and ≤0.25/2 μg/ml, respectively. Similarly, for 56 isolates of *Enterobacter* species, the MIC_{50}/MIC_{90} values of ertapenem, imipenem, and meropenem were 1/4 μg/ml, ≤0.25/1 μg/ml, and ≤0.25/0.5 μg/ml, respectively. Therefore, although all of these isolates were nonsusceptible to ertapenem by the updated breakpoints, the great majority remained susceptible to imipenem and meropenem.

Identification of KPC-producing members of the *Enterobacteriaceae* is the first step in controlling their spread. Using the previous breakpoints, nonsusceptibility to imipenem and meropenem was clearly an insensitive method for detecting KPC-producing bacteria (1, 2, 19). Since the great majority of KPC-producing *K. pneumoniae* isolates had ertapenem MICs of >2 μg/ml (the previous breakpoint for susceptibility), this agent had been suggested for use in screening isolates (1, 2, 16). It is clearly evident that the updated CLSI breakpoints will increase the sensitivity of identifying KPC-positive isolates. However, it is also becoming increasingly apparent that once these new breakpoints are implemented, there will be problems if ertapenem is used as the screening agent (1, 2, 16). While virtually all of the KPC-producing isolates in this report were nonsusceptible to ertapenem, the increased sensitivity came at the expense of a lower positive predictive value (particularly among *Enterobacter* species). It has been recognized that some members of the *Enterobacteriaceae* are able to become nonsusceptible to ertapenem through mechanisms other than carbapenemase activity (7, 9, 16, 20). As evidenced in this report, these isolates often remain susceptible to other carbapenems. Therefore, continued use of ertapenem as the class agent for carbapenem susceptibility testing, without distinguishing KPC producers from non-KPC producers, would effectively preclude a potentially useful and less toxic therapy for some patients.

The optimal strategy to be used by clinical laboratories for detecting KPC-producing members of the *Enterobacteriaceae* needs to be defined and will likely be influenced by the regional endemicity of β-lactamases. One method (16) using ertapenem as the screening agent supplemented with a confirmatory test (i.e., the modified Hodge test) may be applicable for centers where isolates with bla_{KPC} are infrequent. In regions such as New York City, where isolates with bla_{KPC} are frequent, performing confirmatory tests on a large number of ertapenem-nonsusceptible isolates is impractical. As evidenced in this report, using the updated breakpoints, KPC producers that are not susceptible to ertapenem are typically resistant to at least one other carbapenem. Conversely, only 8% of ertapenem-nonsusceptible isolates lacking bla_{KPC} were nonsusceptible to another carbapenem. Therefore, ertapenem-nonsusceptible isolates concomitantly nonsusceptible to another carbapenem could be reported as resistant to all carbapenems, and confirmatory testing could be restricted to ertapenem-nonsusceptible isolates that retain susceptibility to the other carbapenems. For the latter isolates, a negative confirmatory test would also provide reassurance to clinicians regarding the susceptibility results of the other carbapenems. In regions where KPC producers are commonplace, the use of a carbapenem other than ertapenem as the screening agent also appears feasible. One algorithm, based on isolates with a variety of carbapenemases and other β-lactamases, involved MIC cutoffs for both imipenem and meropenem (17). Our results suggest that testing nonsusceptibility to meropenem by itself may be an efficient method to identify KPC producers, with sensitivities of 95.5 to 100% and positive predictive values of 87.5 to 100%. Testing nonsusceptibility to doripenem, using the updated CLSI breakpoints, has also been reported to be a sensitive method for detecting KPC-producing members of the *Enterobacteriaceae* (6). Susceptibility testing of doripenem against our isolates collected in 2009 showed lower sensitivities than those of meropenem testing for detecting KPC-producing *E. coli* and *Enterobacter* species (data not shown). However, the doripenem results need to be confirmed with a greater number of isolates. Finally, screening methods need to be validated in automated systems, where problems still linger in some systems even with implementation of the updated breakpoints (3).

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