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KPC Screening by Updated BD Phoenix and Vitek 2 Automated Systems▽

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Current BD Phoenix and Vitek 2 methodologies were assessed as screens for KPC β-lactamases. Using carbapenem MICs or expert system interpretations as screens, both systems exhibited high (97%) sensitivity in tests with 103 well-characterized Gram-negative isolates, 77 of which were KPC producers.

Pathogens producing carbapenemases of the KPC family are increasingly encountered and are typically associated with extensive multidrug resistance, leaving few to no therapeutic options (6, 10, 11). In 2009 the Clinical and Laboratory Standards Institute (CLSI) recommended carbapenemase screening for *Enterobacteriaceae* followed by confirmatory testing of screen-positive isolates (7). Emerging therapeutic outcome data indicate that carbapenem monotherapy for infections by metallo-β-lactamase (MBL) and KPC producers is less reliable than combination therapy, and there is the risk of emergence of increased resistance if therapy is inappropriate (9, 10, 12, 13, 19). This supports the case for performing carbapenemase tests to identify pathogens against which carbapenem monotherapy may be unreliable. Initially, automated instruments were unreliable screens for KPC producers (1, 16), but since 2005 there have been modifications in the software and test panels to improve performance. The current study aimed to evaluate the most recent KPC screens (i.e., available in United States in 2010) of the BD Phoenix (BD Diagnostics, Sparks, MD) and Vitek 2 (bioMérieux, Durham, NC) systems, by using susceptibility panels and software that are commercially available in the United States.

The isolates comprised 77 KPC-producing clinical isolates from hospitals in the United States and Puerto Rico and 26 clinically isolated KPC-negative isolates. β-Lactamases were characterized at Creighton University by previously published methods (14). The KPC producers were *Klebsiella pneumoniae* ($n = 64$), *Klebsiella oxytoca* (2), *Escherichia coli* (3), *Enterobacter cloacae* (4), and *Pseudomonas aeruginosa* (4). The enzymes were KPC-2 (9 isolates), KPC-3 (1), KPC-3-like (1), KPC-4 (3), KPC-8 (1), and KPC-like (62). "Like" indicates that the enzyme was confirmed as a KPC but not sequenced. The KPC-negative isolates included 7 producers of other carbapenemases (4 with class A carbapenemases and 3 with MBLs) and 19 producers of either a K1 β-lactamase, an extended-spectrum β-lactamase (ESBL), and/or an AmpC β-lactamase. These were *K. pneumoniae* ($n = 5$), *K. oxytoca* (2), *E. coli* (5), *E. cloacae* (5), *Enterobacter aerogenes* (1), *Morganella morganii* (1), *Proteus mirabilis* (2), *Serratia marcescens* (3), and *P. aeruginosa* (2). Inocula for both instruments were prepared from the same plate culture. The BD Phoenix panel, NMIC/ID-121, included ertapenem, imipenem, and meropenem. Its software version was V5.75A/V4.75A. The Vitek 2 card, AST-N142, included ertapenem and meropenem. Its software version was 04.02 PC. The reference standard for this study was the characterized β-lactamase status of the isolates. A reference MIC test was not included to assess the accuracy of automated MICs because of the known problem of variable MIC results with KPC producers (2–5, 15, 17). A positive screen comprised either a carbapenem MIC of ≥2 μg/ml or an expert system comment suggesting reduced carbapenem susceptibility or possible carbapenemase production.

Both instruments exhibited the greatest sensitivity by MIC screening, with 75 of the 77 KPC producers (97%) having a carbapenem MIC of ≥2 μg/ml (Table 1). Neither expert system improved KPC detection by yielding additional positive screens.

In BD Phoenix tests, ertapenem was the most sensitive screening agent (94% positive). Imipenem (48%) and meropenem (30%) were much less sensitive. Ertapenem in combination with either meropenem or imipenem provided maximum sensitivity (97%). The two falsely negative MIC screens were due to a KPC-4-producing *E. coli* strain and a *P. aeruginosa* strain that failed to grow in the susceptibility test. The expert system noted an elevated carbapenem MIC for 72 KPC producers (94%) and suggested possible carbapenemase production for 44 isolates (57%). Some expert interpretations could have been clearer. For example, ESBL production was reported as the resistance marker for most KPC producers, with a comment that ESBL production was confirmed and usually followed by a comment that varied from the bland "*Enterobacteriaceae* are usually susceptible to carbapenems…." to unambiguous suggestions of possible carbapenemase production. Since carbapenemases are clinically more important than ESBLs, the possibility of carbapenemase production could have been better emphasized to avoid the impression that ESBLs are more important.

In Vitek 2 tests, meropenem was a more sensitive screening agent (97% positive) than ertapenem (87% for all KPC producers and 92% for all non-*P. aeruginosa* KPC producers). Two KPC-4 producers were MIC screen negative; the *E. coli* isolate missed by the Phoenix and a *K. pneumoniae* isolate. The expert system noted an elevated carbapenem MIC for 68 KPC

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TABLE 1. Detection of KPC-producing strains

Basis of analysis and category	No. (%) positive KPC-producing strains (n = 77) by:	
	BD Phoenix	Vitek 2
MIC analysis		
Any carbapenem MIC ≥2 μg/ml	75 (97)	75 (97)
Ertapenem MIC ≥2 μg/ml	72 (94)	67/75 (92)[a]
Imipenem MIC ≥2 μg/ml	37 (48)	Not tested
Meropenem MIC ≥2 μg/ml	23 (30)	75 (97)
Interpretive comments by experts		
Elevated carbapenem MIC	72 (94)	68 (88)
Possible carbapenemase	44 (57)	63 (82)

[a] The results of 67/75 (92%) apply to *Enterobacteriaceae* only because the Vitek 2 test did not report ertapenem MICs for *P. aeruginosa* (no CLSI breakpoints for this drug/organism combination).

producers (88%) and suggested possible carbapenemase production for 63 (82%). A flaw was that it suggested seven KPC producers were ESBL producers with no comment about reduced carbapenem susceptibility or possible carbapenemase production. This is a potentially harmful interpretation that could lead to a physician inappropriately using carbapenem monotherapy, as already reported (19).

Vitek 2 meropenem MICs were consistently higher than those with the BD Phoenix, which explained the Vitek 2 system's higher positive screening rate with meropenem (97% versus 30%). Unusually, some KPC producers had Vitek 2 ertapenem MICs of ≤0.5 μg/ml with corresponding meropenem MICs of ≥16 μg/ml. This was unexpected, because meropenem is usually more potent and also because ertapenem MICs of ≤0.5 μg/ml are rare for KPC producers (1). Some BD Phoenix imipenem and meropenem MICs were much lower than previous agar dilution MICs for the same isolates. For example, three *K. pneumoniae* isolates (HUH 10, 12, and 13) had agar dilution imipenem MICs of 8 or 16 μg/ml (unpublished data, Creighton University) but BD Phoenix MICs of ≤1 μg/ml, and two had meropenem agar dilution MICs of 16 μg/ml but BD Phoenix MICs of ≤1 μg/ml.

Such methodology-dependent carbapenem susceptibilities for KPC producers are a concern if laboratories implement the most recent CLSI interpretive criteria and report the results in the absence of carbapenemase detection (8). The question arising is, "will the new breakpoints detect all KPCs and eliminate the need for carbapenemase testing?" The answer will vary according to which carbapenems are tested and by which method. Until this issue is resolved, we believe there is potential for therapeutic failures and missed infection control opportunities that could be avoided if appropriate carbapenemase tests were used to identify isolates for which carbapenem susceptibilities may be erroneous (2–5, 15, 17). In general, the carbapenemase tests would initially be comprehensive for all types of carbapenemases but could be more focused (e.g., for metallo-β-lactamase detection) if convenient or if indicated by experience and the types of isolates encountered by the laboratory.

Some carbapenemase-negative isolates had positive MIC screens and/or expert comments, indicating the possibility of carbapenemase production. This is normal for any screening test, and there were no issues of concern with non-carbapenemase producers with either instrument.

In conclusion, in this study the updated systems provided highly sensitive KPC screens. Screening based on a carbapenem MIC of ≥2 μg/ml (meropenem for Vitek 2; ertapenem in combination with either imipenem or meropenem for BD Phoenix) was most sensitive and less prone to misinterpretation than expert system screening. Both systems could be enhanced by improved expert system interpretations and the provision of carbapenemase confirmatory tests (18, 20).

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