Accuracy of *in vitro* susceptibility tests for carbapenemase-producing Gram-negative bacteria

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Accurate susceptibility results on antibiotic-resistant bacteria are essential for proper treatment of infections. In this study, 100 metallo-β-lactamase (MBL)-producing strains and 95 isolates with *Klebsiella pneumoniae* carbapenemase (KPC) were tested for carbapenem susceptibility using two automated platforms, the Phoenix and Vitek-2 systems, and a manual Etest. Phoenix showed higher categorical agreements (97 % for imipenem and 94 % for meropenem) compared with those from Vitek-2 (92 and 74 %) and Etest (89 and 96 %), respectively, when testing MBL strains. Categorical agreement for imipenem tests with KPC-producing strains was 88.4 % with the Phoenix system, 83.2 % with the Vitek 2 system and 90.5 % with the Etest. Categorical agreement was 100 % for all tests with ertapenem. In conclusion, the Phoenix system demonstrated a higher accuracy than Vitek-2 in testing carbapenemase-producing strains, particularly in MBL strains.

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INTRODUCTION
Antibiotic-resistant Gram-negative bacteria (e.g. carbapenem-resistant *Acinetobacter*, *Pseudomonas* and *Enterobacteriaceae*) are associated with significant morbidity (Carmeli et al., 2010; Kollef et al., 2008; Sabuda et al., 2008; Souli et al., 2008; Tumbarello et al., 2007; Zavascki et al., 2006) and pose therapeutic challenges for physicians. β-Lactamase-mediated carbapenem resistance is a particular problem because these bacteria are resistant to all penicillins, cephalosporins and carbapenems. The most common type of carbapenem resistance in Gram-negative bacteria is encoded by class A [e.g. with *Klebsiella pneumoniae* carbapenemase (KPC)], class B (e.g. NDM, IMP, VIM and SPF), or class D (e.g. OXA) β-lactamase genes. Reliable antimicrobial susceptibility testing methods are critical to provide clinicians with valuable information that can be translated into positive clinical outcomes. Because the MIC values for many carbapenem-resistant bacteria fall near interpretive breakpoints, the accuracy of some *in vitro* susceptibility test methods has been poor (Bulik et al., 2010), although one study demonstrated improved accuracy for KPC strains using revised Clinical and Laboratory Standards Institute (CLSI) breakpoints (Doern et al., 2011). In the study reported here, we extend this work by evaluating the performance of two commercial automated systems (Phoenix and Vitek-2) and one manual agar diffusion system (Etest) with strains expressing carbapenem resistance mediated by a variety of carbapenemase genes.

METHODS

Clinical isolates. Three clinical microbiology laboratories in China screened their collection of blood culture isolates between January 2010 and December 2011 for carbapenemase-producing Gram-negative bacteria by PCR using previously described primers (Queenan & Bush, 2007). The carbapenemase genes included KPC and the metallo-β-lactamase (MBL) genes VIM, IMP, NDM-1 and SPM. The following strains were selected: 100 *Klebsiella* spp. (79 KPC, 20 IMP and one NDM), 48 *Pseudomonas* spp. (29 VIM, 18 IMP and one SPM), 16 *Acinetobacter* spp. with NDM-1, 15 *Enterobacter* spp. (nine IMP, four KPC, one NMD-1 and one SPM), nine *Escherichia* spp. with KPC, two *Serratia* spp. with KPC, two *Citrobacter* spp. (one KPC and one NDM-1), two *Chryseobacterium* spp. with VIM and one *Providencia* sp. with NDM-1.

1These authors contributed equally to this work.

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; KPC, *Klebsiella pneumoniae* carbapenemase; MBL, metallo-β-lactamase

Two supplementary tables are available with the online Supplementary Material.
Antimicrobial susceptibility tests. All isolates were tested simultaneously following the manufacturers’ instructions using the two automated systems: the Phoenix system with NMIC/ID panels and V6.10/V5.21A software (Becton Dickinson) and the Vitek-2 system with AST-GN24 cards and 04.02 PC software (bioMérieux). The Etest (bioMérieux) was performed according to the manufacturer’s instructions. All breakpoints for imipenem, meropenem and ertapenem for both automated systems were updated to CLSI (2012) before the experiment. MIC interpretive breakpoints for imipenem, ertapenem and meropenem were unchanged from 2012 and 2015 (CLSI, 2015) except for imipenem and meropenem tests with Acinetobacter spp. (Table 1).

Data analysis. Based on the sequencing data, all organisms were considered resistant to the carbapenems. Results that were reported as susceptible by the test systems were considered by definition a very major error; results that were reported as intermediate were considered a minor error. The percentages for each type of errors were calculated.

RESULTS

The percentages of categorical agreements from the three test methods are summarized in Table 2. Imipenem and ertapenem were tested for the 95 KPC-producing strains, and imipenem and meropenem were tested for the 100 MBL-producing strains. Categorical agreement for the KPC strains was similar with Phoenix, Vitek 2 and the Etest for both imipenem (88.4, 83.2 and 90.5 %, respectively) and ertapenem (100 % for all systems). For MBL-producing isolates, categorical agreement with Phoenix was 97 % for imipenem and 94 % with meropenem, with Vitek-2 agreement was 92 % with imipenem and 74 % with meropenem, and with the Etest agreement was 89 % with imipenem and 96 % with meropenem. Errors were broadly distributed, with all strains carrying MBL, but were observed most commonly with strains with low MICs near the interpretive breakpoints as determined by the Etest (Tables S1 and S2, available in the online Supplementary Material).

The messages triggered in the expert systems of the two automated system are summarized in Table 3. For the 100 MBL-producing strains, BDxpert triggered carbapenem resistance messages in 89 isolates, whilst Vitek-2 AES triggered carbapenemase messages in only 57 isolates. For KPC-producing isolates, BDxpert showed carbapenem resistance messages in all the isolates and Vitek-2 AES showed carbapenem resistance messages in 89 isolates with six being missed. Four of the six only indicated ESBL in the triggered messages and two showed no matched phenotype in the system.

DISCUSSION

In this study, the accuracy of two automated susceptibility test systems was compared for carbapenem-resistant strains. Detection of MBL-mediated carbapenem resistance was assessed using imipenem and meropenem. Previously, a high error rate with automated systems was observed

Table 1. CLSI breakpoints shifts from 2012 to 2015

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<tr>
<td></td>
<td>Imipenem</td>
<td>Meropenem</td>
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<tr>
<td>Enterobacteriaceae (S/R)</td>
<td>≤ 1/≥ 4</td>
<td>≤ 1/≥ 4</td>
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<tr>
<td>Pseudomonas (S/R)</td>
<td>≤ 2/≥ 8</td>
<td>≤ 2/≥ 8</td>
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<tr>
<td>Acinetobacter (S/R)</td>
<td>≤ 4/≥ 16</td>
<td>≤ 4/≥ 16</td>
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R, Resistant; S, sensitive.
(Espedido et al., 2007; Giakkoupi et al., 2005); however, CLSI interpretive criteria were changed in 2010. This change improved the in vitro test performance with KPC-producing strains (Doern et al., 2011), but the impact on a broad range of MBL strains is unknown. For the 100 MBL-producing strains tested in this study, the Phoenix system showed a satisfactory performance in MBL testing, whilst Vitek-2 had an unacceptable rate of minor errors, particularly for meropenem. A previous study showed that both Phoenix and Vitek-2 reliably detected MBL using a smaller group of 20 MBL-producing Enterobacteriaceae (Woodford et al., 2010); however, in the study reported here, the 100 MBL-producing strains that were tested included Acinetobacter spp. and Pseudomonas spp., as well as Enterobacteriaceae. The 12 minor errors that occurred only in the Vitek-2 system were in the non-Enterobacteriaceae isolates, indicating potential limitations of the system.

The susceptibility of KPC-producing strains to imipenem and ertapenem was evaluated. Ertapenem showed full categorical agreement for all strains; however, imipenem had agreement rates of 88.4 and 83.2 % for the Phoenix and Vitek-2 systems, respectively. These errors were primarily minor, although very major errors were observed with approximately 6 % of the tests.

In a previous report, BDXpert was found to be sensitive and specific for classifying carbapenemase-producing Gram-negative bacteria, whilst Vitek-2 AES was less sensitive and specific, particularly for MBL-producing strains (Woodford et al., 2010). The data presented here also support the observation that the two systems have different advantages in interpreting and predicating the underlying carbapenem resistance mechanisms.

In conclusion, the Phoenix system had higher categorical agreement compared with the Vitek-2 system in accurately classifying carbapenemase-producing Gram-negative rods. Whereas both systems could accurately identify KPC-producing strains, carbapenem susceptibility should be interpreted cautiously, particularly if an intermediate susceptibility result is reported. The Phoenix expert system can accurately classify all KPC-producing strains and most MBL-producing strains, but the Vitek-2 system does not accurately identify MBL-producing strains. Alternative test methods such as gene sequencing or targeted PCR amplification are required to identify these strains.

REFERENCES


