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Short Communication

Evaluation of the BD Phoenix automated system for determining antimicrobial susceptibility against carbapenem-resistant Enterobacteriaceae compared with broth microdilution

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ABSTRACT

Purpose: Carbapenem-resistant Enterobacteriaceae (CRE) are increasingly widespread in the healthcare system, resulting in infections associated with mortality of up to 50%. Many laboratories use automated systems to identify CRE isolates and determine susceptibility. The aim of this study was to evaluate categorical agreement between the BD Phoenix automated system and the gold standard – broth microdilution – in determining minimum inhibitory concentrations of CRE.

Methodology: The activity of amikacin, aztreonam, cefepime, ceftazidime, ertapenem, gentamicin, levofloxacin, meropenem, nitrofurantoin, piperacillin-tazobactam and tobramycin on 125 CRE isolates collected from an academic medical centre was evaluated. Categorical agreement between BD Phoenix and broth microdilution was determined, as well as minor error rates, major error rates and very major error rates.

Results: BD Phoenix significantly overestimates susceptibility of CRE isolates to amikacin, aztreonam, cefepime, ceftazidime, gentamicin, levofloxacin, meropenem, nitrofurantoin and tobramycin compared with broth microdilution. Overall, categorical agreement of 76% between testing methods indicates the potential diminished ability of BD Phoenix to predict resistance accurately in highly drug-resistant isolates. All tested antimicrobials had higher major error rates compared with previous literature.

Conclusions: BD Phoenix has diminished ability to determine susceptibility of CRE isolates. Further studies are warranted in order to validate BD Phoenix susceptibility testing in highly resistant CRE isolates. The mechanism by which isolates are resistant to carbapenems does not impact the ability of BD Phoenix to determine susceptibility.

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1. Introduction

Carbapenem-resistant Enterobacteriaceae (CRE) infections are associated with a mortality rate of approximately 50% and have become an increasingly prevalent global problem [1]. As carbapenem agents are considered to be among the most potent antimicrobials for combating Gram-negative infections, the emergence and spread of carbapenemases that hydrolyse these agents is a cause for alarm. According to the Centers for Disease Control and Prevention, CRE infections pose an immediate public health threat that requires urgent and aggressive action. Although historically uncommon, CRE have been identified in healthcare facilities in all 50 states of the USA as of 2018 [2]. Nationally, it has been reported that approximately 10% of *Klebsiella* spp. bloodstream infections are caused by CRE [3].

Resistance to carbapenem agents primarily results from the horizontal transfer of plasmid-encoded carbapenem hydrolysing enzymes. Molecular class A, group 2f β-lactamases include *Klebsiella pneumoniae* carbapenemase (KPC), which has been associated with outbreaks of multi-drug-resistant infections worldwide [1,4–6]. Molecular class B, groups 3a and 3b β-lactamases include the metallo-β-lactamases (MBLs) IMP and VIM which are becoming increasingly common in the USA [6]. As of February 2018, New Delhi metallo-β-lactamase-producing (NDM) organisms are the most commonly reported MBL-producing infections [2]. According to Kulengowski et al., 20% of CRE isolated at the UK HealthCare academic medical center are VIM-producing organisms

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[7]. Plasmids which carry the genes containing carbapenem hydrolysing enzymes also carry genes which confer resistance to many other antimicrobial drug classes, including fluoroquinolones and aminoglycosides [8,9].

Many laboratories use automated identification and antimicrobial susceptibility systems for clinical isolates to decrease the time and labour required to determine susceptibility results for hospitalized patients. Inaccuracies in these systems could result in potentially devastating treatment failures for patients with CRE infections. Due to the significant morbidity and mortality associated with CRE infections, it is vital that healthcare practitioners are able to assess which medications are treatment options when patients present with these infections. Previous studies have demonstrated the accuracy of automated systems for identification and susceptibility testing of Gram-negative organisms compared with the agar dilution method, broth microdilution and Etests [10–13]. Conversely, there is little information on the validation of automated systems to appropriately determine susceptibility data for highly resistant CRE isolates. The purpose of this study was to assess the level of categorical agreement between the BD Phoenix automated system (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and broth microdilution in determining minimum inhibitory concentrations (MICs) of CRE at an academic medical centre.

2. Materials and methods

2.1. Bacterial isolates

In total, 125 CRE isolates were obtained from UK HealthCare Albert B. Chandler Hospital from 1 January 2012 to 31 December 2016. Organisms were identified by their resistance to either ertapenem or meropenem on BD Phoenix as part of routine patient care. Isolates were frozen and stored at -80°C for use in the microbiology laboratory.

2.2. Susceptibility testing

MICs were obtained from BD Phoenix automated susceptibility testing (Versions V6.01, V6.01A, and V6.21A) and broth microdilution for all CRE isolates against amikacin, aztreonam, cefepime, ceftazidime, ertapenem, gentamicin, levofloxacin, meropenem, nitrofurantoin, piperacillin-tazobactam and tobramycin. This panel was selected for drugs used at the study institution during routine clinical practice. Stock antimicrobials were prepared for microdilution according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). MICs from broth microdilution were determined in accordance with CLSI standardized laboratory practices using the BioStack Microplate Stacker and Precision Pipetting System (Biotek Instruments, Inc., Winooski, VT, USA) [14]. Dilution of clinical isolates to a standard inoculum of 1.5×10^8 colony-forming units (CFU)/mL was performed via McFarland standard matching. An additional 1:200 dilution was created with cation-adjusted Mueller–Hinton broth to make a final inocula of 6×10^5 CFU/mL. Isolates were cultured and incubated at 37°C overnight. Broth microdilution experiments were performed in duplicate on two separate days; the modal MIC was documented. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as quality control strains. Broth microdilution MIC data for quality control strains are available in Table S8 (see online supplementary material). Susceptibility, intermediate and resistant breakpoints were obtained from the CLSI document, 'Performance Standards for Antimicrobial Susceptibility Testing, 28th Edition' [15]. Breakpoints were used for interpretation of the MIC values obtained from both broth microdilution and BD Phoenix [15]. No correction was necessary for short-calling MIC ranges as BD Phoenix provides an MIC for most organisms at any of the concentrations defined. There exist antimicrobial/organism pairings in which a maximum or minimum MIC is reported by BD Phoenix even if there is a lower or higher concentration on the panel. However, these limited ranges encapsulate the susceptible, intermediate and resistant breakpoints for all tested isolates [16]. Quality control ranges used for BD Phoenix susceptibility testing are available in Table S9 (see online supplementary material).

2.3. Subgroup analysis

Further analysis was performed on subgroups divided according to isolate resistance mechanism: KPC-producing (n=71), MBL-producing (n=21) and non-carbapenemase-producing CRE (n=27). Isolates that expressed both KPC and MBL enzymes were excluded from subgroup analysis (n=5). Resistance phenotypes were determined using a combined disc test with meropenem alone, as well as in combination with phenylboronic acid or ethylenediaminetetraacetic acid. Tsakris et al. evaluated this phenotypic testing method previously for the detection of carbapenemase production and differentiation of KPC and MBL enzymes [17].

2.4. Susceptibility data analysis

All data from broth microdilution and BD Phoenix were reported and transcribed into Excel (Microsoft Corp., Redmond, WA, USA) spreadsheets for analysis. Categorical agreement between the two testing methods was determined. Essential agreement could not be evaluated accurately because BD Phoenix has a limited reportable MIC range for each antimicrobial compared with broth microdilution. However, reportable ranges for BD Phoenix consistently contain the susceptible, intermediate and resistant breakpoint concentrations for tested isolates. Categorical agreement was defined as identical susceptibility testing categories reported for both testing methods. McNemar's test was used to assess statistical significance for paired, binominal data. Two-sided P-values $\leq$0.05 indicated statistical significance.

2.5. Error rates and CLSI acceptable discrepancy rates

Minor error (MiE) rates, major error (ME) rates and very major error (VME) rates between broth microdilution and BD Phoenix for determining categorical data were determined. MiE was when one testing method categorized an isolate as intermediate and the other did not. ME was when BD Phoenix categorized an isolate as resistant and broth microdilution categorized the same isolate as susceptible. VME was when BD Phoenix categorized an isolate as susceptible and broth microdilution categorized the same isolate as resistant. The counts of MiEs, MEs and VMEs were used as numerators in their respective equations to determine the error rates for BD Phoenix. The denominator was established using the appropriate population of isolates outlined in CLSI guidance on susceptibility testing criteria based on the isolates' MIC value [18]. Therefore, isolates for each antimicrobial were divided into three populations: those with MIC values greater than or equal to two dilutions above the intermediate MIC ($\geq$I+2), those with MIC values between one dilution above the intermediate MIC and one dilution below the intermediate MIC (I+1 to I-1), and those with MIC values less than or equal to two dilutions below the intermediate MIC ($\leq$I-2).

3. Results

Of the 125 CRE clinical isolates collected, 14 distinct species were identified by the automated system BD Phoenix: *K. pneumoniae* (n=58), *Enterobacter cloacae* (n=31), *Citrobacter freundii* (n=9), *E. coli* (n=7), *Enterobacter aerogenes* (n=4), *Klebsiella oxytoca* (n=3),

Table 1
Antimicrobial susceptibility for carbapenem-resistant Enterobacteriaceae isolates using broth microdilution and BD Phoenix by drug class (n=125)

Percentage susceptibility (# susceptible/n)[a]				
Antimicrobial	Broth microdilution (n=125)	BD Phoenix automated system (n=125)	P-value	Categorical agreement
Aminoglycosides				
Amikacin	81% (101)	93% (116)	0.021	91%
Gentamicin	35% (44)	42% (53)	0.002	66%
Tobramycin	14% (18)	30% (37)	0.001	71%
β-lactam/β-lactamase inhibitors				
Piperacillin-tazobactam	1% (1)	30% (37)	0.125	95%
Carbapenems				
Ertapenem	2% (3)	1% (1)	1	94%
Meropenem	18% (23)	37% (46)	0.002	50%
Cephalosporins				
Cefepime	5% (6)	20% (25)	0.0001	68%
Ceftazidime	1% (1)	6% (8)	0.03	85%
Fluoroquinolones				
Levofloxacin	15% (19)	33% (41)	0.001	61%
Monobactams				
Aztreonam	2% (3)	12% (15)	0.003	94%
Nitrofurans				
Nitrofurantoin	16% (20)	36% (45)	0.0002	63%

[a] Clinical and Laboratory Standards Institute breakpoints were used to assess susceptibility.

Enterobacter spp. (n=2), *Citrobacter amalonaticus* (n=2), *Citrobacter youngae* (n=2), *Enterobacter hormaechei* (n=2), *Klebsiella ozaenae* (n=2), *Enterobacter gergoviae* (n=1), *Providencia rettgeri* (n=1) and *Serratia marcescens* (n=1). BD Phoenix overestimated susceptibility of CRE isolates for 82% (9/11) of tested antimicrobials – amikacin, gentamicin, tobramycin, levofloxacin, nitrofurantoin, aztreonam, cefepime, ceftazidime and meropenem. High levels of resistance to ertapenem and piperacillin-tazobactam precluded detection of a significant difference between broth microdilution and BD Phoenix – 2% vs. 1% and 1% vs. 3% susceptibility, respectively (Table 1).

Overall, categorical agreement was calculated as 76% for all organisms and antimicrobials tested. Categorical agreement varied greatly, even between antimicrobial agents of the same class, ranging from 50% to 95%. The highest rate of categorical agreement was seen in beta-lactam agents (79%), followed by 76%, 63%, and 61% for aminoglycosides, nitrofurans and fluoroquinolones, respectively. The antimicrobials selected for this study with the highest categorical agreement values (aztreonam, ertapenem and piperacillin-tazobactam) were the agents with the lowest percentage susceptibility by both BD Phoenix and broth microdilution (Table 1). MIC values determined from both testing methods for all isolates and antimicrobials are listed in Tables S1–S7 (see online supplementary material).

The susceptibility results for KPC-producing isolates, MBL-producing isolates and non-carbapenemase-producing CRE isolates are summarized in Tables 2a, 2b, and 2c, respectively. In isolates producing KPC, susceptibility was overestimated by BD Phoenix for cefepime, levofloxacin, aztreonam and nitrofurantoin with an average categorical agreement of 75% [44–99%] (Table 2a). BD Phoenix also significantly overestimated susceptibility for MBL-producing isolates for meropenem, aztreonam and nitrofurantoin (Table 2b). In isolates where carbapenemase enzymes were not detected, a significant overestimation of susceptibility of BD Phoenix was detected for levofloxacin alone (Table 2c).

All tested antimicrobials showed increased error rates in at least one category – VME, ME or MiE. Only ertapenem exhibited VME rates as low as previously observed evaluations of automated susceptibility testing methods. Only amikacin, cefepime, nitrofurantoin and tobramycin exhibited low ME rates. Finally, only four of 11 antimicrobials demonstrated relatively low MiE rates – aztreonam, ertapenem, gentamicin and piperacillin-tazobactam. Table 3 reports the calculated error rates for broth microdilution vs. BD Phoenix. No agent exhibited consistently low error rates for BD Phoenix when compared with broth microdilution for CRE isolates.

4. Discussion

This study demonstrated that BD Phoenix overestimates susceptibility of CRE compared with broth microdilution for nine of 11 (82%) tested antimicrobials. The differences in susceptibility were found to be significant, despite previous literature validating use of BD Phoenix for Gram-negative bacilli by repeated testing [10–13,19,20]. In 2002, Endimiani et al. evaluated BD Phoenix against broth microdilution for the identification and susceptibility testing of 136 non-fermenting Gram-negative isolates. They concluded that the automated system correctly measured the susceptibility of antipseudomonal drugs, with a reported categorical agreement of 93.1% [12]. The present study reports an overall categorical agreement of 76%, but there were antimicrobials with categorical agreement considerably higher than the average: aztreonam, ertapenem and piperacillin-tazobactam. However, this observation may best be explained by high levels of resistance to these drugs (98–99%).

For all drugs tested, increased error rates were observed compared with rates described previously for automated susceptibility testing, with 10 of 11 (91%) drugs yielding an elevated VME rate within at least a single intermediate range-demarcated subpopulation. The error rates found for each antimicrobial agent stratified by population are outlined in Table 3. According to CLSI in-vitro susceptibility testing criteria, of greatest concern are the discrepancies that occur with MICs greater than or equal to two-fold concentrations above ($\geq$I+2) or below ($\leq$I-2) the intermediate MIC [18]. The present study found rates of MiE, ME and VME that were considerably greater than found in previous studies. In 2002, Steward et al. assessed the accuracy of five antimicrobial testing methods – agar dilution, disk diffusion, Etest (AB BIODISK North America, Inc., Piscataway, N.J.), MicroScan WalkAway (Dade MicroScan, Inc., West Sacramento, Calif.) and Vitek (bioMérieux Vitek, Inc., Durham, N.C.) – in Enterobacteriaceae and *Pseudomonas* spp. isolates using imipenem and meropenem. For Enterobacteriaceae, the number of MEs across all testing methods ranged from 0 to 1 for imipenem and from 0 to 2 for meropenem, and an ME rate ranging from 0% to 2.3%. Despite the lower error rates, the authors concluded that testing susceptibilities of carbapenems with automated systems resulted in high rates of ME and variability,

Table 2a
Antimicrobial susceptibility for *Klebsiella pneumoniae* carbapenemase-producing carbapenem-resistant Enterobacteriaceae isolates using broth microdilution and BD Phoenix by drug class (n=71)

Percentage susceptibility (# susceptible/n)[a]				
Antimicrobial	Broth microdilution (n=71)	BD Phoenix automated system (n=71)	P-value	Categorical agreement
Aminoglycosides				
Amikacin	80% (57)	87% (62)	0.1306	89%
Gentamicin	26% (18)	39% (28)	0.5023	56%
Tobramycin	15% (11)	30% (21)	0.9609	73%
β-lactam/β-lactamase inhibitors				
Piperacillin-tazobactam	0% (0)	0% (0)	1	99%
Carbapenems				
Ertapenem	0% (0)	0% (0)	1	94%
Meropenem	6% (4)	46% (33)	0.0817	44%
Cephalosporins				
Cefepime	6% (4)	25% (18)	0.0005	65%
Ceftazidime	0% (0)	0% (0)	0.1310	85%
Fluoroquinolones				
Levofloxacin	6% (4)	32% (23)	0.0002	62%
Monobactams				
Aztreonam	0% (0)	14% (10)	0.0094	96%
Nitrofurans				
Nitrofurantoin	18% (13)	35% (25)	0.0095	61%

[a] Clinical and Laboratory Standards Institute breakpoints were used to assess susceptibility.

Table 2b
Antimicrobial susceptibility for metallo-β-lactamase-producing carbapenem-resistant Enterobacteriaceae isolates using broth microdilution and BD Phoenix by drug class (n=21)

Percentage susceptibility (# susceptible/n)[a]				
Antimicrobial	Broth microdilution (n=21)	BD Phoenix automated system (n=21)	P-value	Categorical agreement
Aminoglycosides				
Amikacin	90% (19)	100% (21)	0.4795	90%
Gentamicin	14% (3)	33% (7)	0.1336	71%
Tobramycin	0% (0)	5% (1)	1	86%
β-lactam/β-lactamase inhibitors				
Piperacillin-tazobactam	0% (0)	0% (0)	1	100%
Carbapenems				
Ertapenem	0% (0)	0% (0)	1	100%
Meropenem	0% (0)	19% (4)	0.0455	71%
Cephalosporins				
Cefepime	0% (0)	0% (0)	1	95%
Ceftazidime	0% (0)	0% (0)	1	100%
Fluoroquinolones				
Levofloxacin	57% (12)	33% (7)	0.1820	48%
Monobactams				
Aztreonam	0% (0)	48% (10)	0.0044	52%
Nitrofurans				
Nitrofurantoin	10% (2)	43% (9)	0.0233	57%

[a] Clinical and Laboratory Standards Institute breakpoints were used to assess susceptibility.

Table 2c
Antimicrobial susceptibility for non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae isolates using broth microdilution and BD Phoenix by drug class (n=27)

Percentage susceptibility (# susceptible/n)[a]				
Antimicrobial	Broth microdilution (n=27)	BD Phoenix automated system (n=27)	P-value	Categorical agreement
Aminoglycosides				
Amikacin	100% (27)	100% (27)	1	100%
Gentamicin	33% (9)	52% (14)	0.0736	81%
Tobramycin	30% (8)	52% (14)	0.0771	56%
β-lactam/β-lactamase inhibitors				
Piperacillin-tazobactam	0% (0)	15% (4)	0.3711	74%
Carbapenems				
Ertapenem	7% (2)	0% (0)	1	85%
Meropenem	67% (18)	22% (6)	0.0817	44%
Cephalosporins				
Cefepime	4% (1)	15% (4)	0.0736	78%
Ceftazidime	4% (1)	7% (2)	0.1336	89%
Fluoroquinolones				
Levofloxacin	7% (2)	33% (9)	0.0455	63%
Monobactams				
Aztreonam	7% (2)	11% (3)	0.2482	81%
Nitrofurans				
Nitrofurantoin	19% (5)	30% (8)	1	81%

[a] Clinical and Laboratory Standards Institute breakpoints were used to assess susceptibility.

Table 3
Calculated very major error (VME), major error (ME) and minor error (MiE) rates for broth microdilution vs. BD Phoenix against carbapenem-resistant Enterobacteriaceae

Antimicrobial	MIC range category	MiE	ME	VME
Amikacin	≥I+2	0% (0/1)		100% (1/1)
	I+1 to I-1	44% (18/41)	2% (1/41)	2% (1/41)
	≤I-2	0% (0/40)	13% (5/40)	
Gentamicin	≥I+2	2% (1/61)		20% (12/61)
	I+1 to I-1	35% (13/37)	11% (4/37)	16% (6/37)
	≤I-2	4% (1/27)	4% (1/27)	
Tobramycin	≥I+2	4% (2/56)		11% (6/56)
	I+1 to I-1	29% (15/51)	0% (0/51)	14% (7/51)
	≤I-2	6% (1/18)	11% (2/18)	
Levofloxacin	≥I+2	4% (3/85)		27% (23/85)
	I+1 to I-1	22% (6/27)	11% (3/27)	30% (8/27)
	≤I-2	15% (2/13)	31% (4/13)	
Nitrofurantoin	≥I+2	13% (7/56)		18% (10/56)
	I+1 to I-1	30% (18/61)	2% (1/61)	13% (8/61)
	≤I-2	13% (1/8)	0% (0/8)	
Aztreonam	≥I+2	1% (1/121)		10% (12/121)
	I+1 to I-1	0% (0/3)	0% (0/3)	67% (2/3)
	≤I-2	0% (0/1)	100% (1/1)	
Cefepime	≥I+2	8% (9/113)		14% (16/113)
	I+1 to I-1	83% (5/6)	0% (0/6)	0% (0/6)
	≤I-2	0% (0/6)	0% (0/6)	
Ceftazidime	≥I+2	6% (7/122)		5% (6/122)
	I+1 to I-1	0% (0/3)	33% (1/3)	33% (1/3)
	≤I-2	0% (0/0)	0% (0/0)	
Piperacillin-tazobactam[a]	≥I_{High}+2	2% (2/121)		2% (3/121)
	I_{High}+1 to I_{Low}-1	25% (1/4)	25% (1/4)	25% (1/4)
	≤I_{Low}-2	0% (0/0)	0% (0/0)	
Ertapenem	≥I+2	3% (3/118)		1% (1/118)
	I+1 to I-1	33% (2/6)	17% (1/6)	0% (0/6)
	≤I-2	0% (0/1)	100% (1/1)	
Meropenem	≥I+2	7% (6/83)		39% (32/83)
	I+1 to I-1	31% (8/26)	12% (3/26)	12% (3/26)
	≤I-2	6% (1/16)	63% (10/16)	

MIC, minimum inhibitory concentation; I, intermediate MIC.
[a] Piperacillin-tazobactam has two-dilution intermediate range.

requiring a second verification testing method for these agents [21]. The present study supports this conclusion.

Data on the validation of automated systems to appropriately determine susceptibility data for highly resistant isolates are lacking. Endimiani et al. included a small number of resistant isolates producing extended-spectrum β-lactamases (n=5) and MBLs (n=4) in their study evaluating BD Phoenix, although separate susceptibility data for resistant isolates were not reported [12]. To the authors' knowledge, the present study is the largest to evaluate the BD Phoenix automated susceptibility testing method for multiple drug classes against highly-resistant CRE isolates. The results are consistent with a previous study by Zhao et al. in 2017 which evaluated three automated susceptibility testing methods against agar dilution. Seventy-five CRE isolates were run against four agents: amikacin, ciprofloxacin, gentamicin and levofloxacin. The resulting rates of MiE, ME and VME were 3.11%, 2.44% and 4.33%, respectively [20]. The error rates found in the present study are markedly greater than those observed by Zhao et al. Even with lower error rates, Zhao et al. arrived at the conclusion that clinical laboratories should seek a second, independent method for determining susceptibility data for CRE isolates when using aminoglycosides and fluoroquinolones. The present study arrives at the same conclusion given the higher rates of MiE, ME and VME.

Subgroup analyses demonstrated similar patterns of elevated error rates associated with BD Phoenix, despite smaller population sizes in the subgroups resulting in fewer significant results (Tables 2 and 3). Furthermore, for some agents, it was not possible to conclude that the results were different due to high levels of resistance or, in the case of amikacin, susceptibility.

Limitations of the present study include the fact that the study isolates were taken from a single referral medical centre. Therefore, data from other centres should be reported to determine external validity. Furthermore, because the susceptibility results from BD Phoenix were provided to the research laboratory independent of this project, there were a small number of isolates and agents for which BD Phoenix susceptibility data did not exist (0.51% of susceptibility data). In the event of these absences, the MIC value was omitted from analysis. Additionally, isolates were tested via BD Phoenix during the course of routine clinical practice. The isolates were then frozen and broth microdilution was performed at a later date, potentially providing an explanation for differentiation in MIC results. A further limitation is the absence of repeat susceptibility testing using BD Phoenix. However, in routine patient care, BD Phoenix susceptibility results are typically not repeated before being reported to clinicians. Repeated testing is performed using Etest if an organism is ertapenem resistant and meropenem susceptible to verify the result, which is then reported. If CREs are identified, all carbapenem susceptibility reporting changes to document resistance. All MIC values are still reported. Therefore, the study design remains applicable to current clinical practice, with repeated testing performed by broth microdilution permitting more accurate reference MIC determination for each organism. As all organisms tested demonstrated carbapenem resistance, these data are not applicable to carbapenem-susceptible isolates for which BD Phoenix received Food and Drug Adminstration approval for antimicrobial susceptibility testing. Lastly, BD Phoenix was used to identify which isolates were carbapenem resistant for study inclusion. This was almost entirely determined by resistance to ertapenem, the most sensitive marker for carbapenemase production.

This may have excluded isolates susceptible to ertapenem by BD Phoenix and resistant by broth microdilution. This may further explain why ertapenem exhibited lower VME rates compared with the other study antimicrobials.

5. Conclusions

BD Phoenix significantly overestimated susceptibility of CRE isolates compared with the gold standard, broth microdilution. This phenomenon was still observed when stratifying isolates by mechanism of carbapenem resistance. When compared with previous evaluations of automated susceptibility testing, BD Phoenix was associated with higher error rates for all tested antimicrobials, and only ertapenem exhibited a lower VME rate. The authors agree with previous conclusions that secondary susceptibility testing methods should be used to verify antimicrobial activity against CRE. Further data are needed to assess the ability of BD Phoenix to accurately determine the MICs of highly resistant CRE isolates.

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Conflict of interest

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Not required.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.05.002.

References

[1] Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. J Infect Dis 2017;215:S28–36.
[2] Centers for Disease Control and Prevention. Tracking CRE. Atlanta, GA: CDC, 2017. Available at: cdc.gov/hai/organisms/cre/trackingcre.html [last accesed May 2019].
[3] Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infect Control Hosp Epidemiol 2013;34:1–14.
[4] Bradford PA, Bratu S, Urban C, Visalli M, Mariano N, Landman D, et al. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. Clin Infect Dis 2004;39:55–60.
[5] Leavitt A, Navon-Venezia S, Chmelnitsky I, Schwaber MJ, Carmeli Y. Emergence of KPC-2 and KPC-3 in carbapenem-resistant *Klebsiella pneumoniae* strains in an Israeli hospital. Antimicrob Agents Chemother 2007;51:3026–9.
[6] Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother 2010;54:969–76.
[7] Kulengowski B, Burgess DS. Ceftazidime-avibactam activity compared to commonly utilized antimicrobials against carbapenem-resistant Enterobacteriaceae from an academic medical center. ASM Microbe 2017, Washington, DC: American Society for Microbiology; 2017. New Orleans, LA.
[8] Baran I, Aksu N. Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. Ann Clin Microbiol Antimicrob 2016;15:20.
[9] van Loon K, Voor AF, Vos MC. A systematic review and meta-analyses of the clinical epidemiology of carbapenem-resistant Enterobacteriaceae. Antimicrob Agents Chemother 2018;62 e01730–17.
[10] Stefaniuk E, Baraniak A, Gniadkowski M, Hryniewicz W. Evaluation of the BD Phoenix automated identification and susceptibility testing system in clinical microbiology laboratory practice. Eur J Clin Microbiol Infect Dis 2003;22:479–85.
[11] Carroll KC, Glanz BD, Borek AP, Burger C, Bhally HS, Henciak S, et al. Evaluation of the BD Phoenix automated microbiology system for identification and antimicrobial susceptibility testing of Enterobacteriaceae. J Clin Microbiol 2006;44:3506–9.
[12] Endimiani A, Luzzaro F, Tamborini A, Lombardi G, Elia V, Belloni R, et al. Identification and antimicrobial susceptibility testing of clinical isolates of nonfermenting Gram-negative bacteria by the Phoenix automated microbiology system. New Microbiol 2002;25:323–9.
[13] Menozzi MG, Eigner U, Covan S, Rossi S, Somenzi P, Dettori G, et al. Two–center collaborative evaluation of performance of the BD Phoenix automated microbiology system for identification and antimicrobial susceptibility testing of Gram-negative bacteria. J Clin Microbiol 2006;44:4085–94.
[14] Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. 10th edition. Wayne, PA: CLSI; 2015. CLSI Document M07-A10.
[15] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 28th edition. Wayne, PA: CLSI; 2018. CLSI Document M100.
[16] Clinical and Laboratory Standards Institute. Laboratory procedure – BD Phoenix™ NMIC/ID Panels BD Technical Center BD Formatted Procedures. Wayne, PA: CLSI; 2006.
[17] Tsakris A, Poulou A, Pournaras S, Voulgari E, Vrioni G, Themeli-Digalaki K, et al. A simple phenotypic method for the differentiation of metallo-beta-lactamases and class A KPC carbapenemases in Enterobacteriaceae clinical isolates. J Antimicrob Chemother 2010;65:1664–71.
[18] Clinical and Laboratory Standards Institute. Development of in vitro susceptibility testing criteria and quality control parameters. Wayne, PA: CLSI; 2001. CLSI Guideline M23 p. 21.
[19] He Q, Chen W, Huang L, Lin Q, Zhang J, Liu R, et al. Performance evaluation of three automated identification systems in detecting carbapenem-resistant Enterobacteriaceae. Ann Clin Microbiol Antimicrob 2016;15:40.
[20] Zhao Z, Lan F, Liu M, Chen W, Huang L, Lin Q, et al. Evaluation of automated systems for aminoglycoside and fluoroquinolone susceptibility testing for carbapenem-resistant Enterobacteriaceae. Antimicrob Resist Infect Control 2017;6:77.
[21] Steward CD, Mohammed JM, Swenson JM, Stocker SA, Williams PP, Gaynes RP, et al. Antimicrobial susceptibility testing of carbapenems: multicenter validity testing and accuracy levels of five antimicrobial test methods for detecting resistance in Enterobacteriaceae and *Pseudomonas aeruginosa* isolates. J Clin Microbiol 2003;41:351–8.