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Comparison of four phenotypic testing methods and a novel genotypic polymerase chain reaction test for the determination of carbapenem-resistant *Enterobacteriaceae* resistance mechanisms at an academic medical center

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ABSTRACT

Carbapenem-resistant *Enterobacteriaceae* (CRE) represent a clinically important group of organisms with limited treatment options that contribute to infections with high mortality rates. In this study, we utilized several different methods to determine resistance mechanisms in CRE to elucidate the mode of resistance and compare results among these methods. Forty-one unique ertapenem-resistant patient isolates obtained over a 4-year period were tested utilizing 4 phenotypic testing methods: Modified Hodge test (MHT), Modified Carbapenem Inactivation Method (mCIM), KPC/Metallo-β-Lactamase (MBL) Confirm Kit™, and Neo-Rapid CARB Kit™. Genotypic testing *via* Xpert Carba-R (Cepheid, Sunnyvale, USA) was considered the benchmark to which phenotypic testing was compared. Presence of a carbapenemase was detected by all phenotypic tests in 48.7% (n = 20/41) of isolates. Genotypic testing indicated that the majority of isolates produced *Klebsiella pneumoniae* carbapenemase (KPC) (n = 26/41, 63.4%); no other carbapenemases were identified. These results may aid in informing optimal empiric antimicrobial therapy within the institution when carbapenem resistance is suspected or proven.

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KEYWORDS: *Enterobacteriaceae*, carbapenem, carbapenemase, KPC, resistance mechanisms.

INTRODUCTION

In an age of increasing antimicrobial resistance, infections resulting from carbapenem-resistant *Enterobacteriaceae* (CRE) demonstrate significant morbidity and have been associated with mortality rates of up to 50% in bloodstream infections due to severely limited treatment options [1]. The Centers for Disease Control and Prevention (CDC) have classified CRE as an urgent antibiotic resistance threat requiring immediate public health attention, with an estimated 9,300 infections and 610 deaths occurring annually as a result of these multidrug-resistant (MDR) pathogens [1]. The primary mechanisms of resistance that have been described in CRE include production of a carbapenemase, deficiency or modification of porin expression coupled with overexpression of β-lactamases, and production of efflux pumps [2]. *Klebsiella pneumoniae* carbapenemase (KPC) is the most common carbapenemase reported in the United States, with other carbapenemases including metallo-β-lactamases (MBL) and oxacillinase (OXA)-48 displaying endemicity to other parts of the world [3]. Infections resulting from carbapenemase-producing CRE (CP-CRE) have been associated with higher severity and an approximate four-fold elevation in risk of mortality when compared to

non-CP-CRE [4]. It is important to understand the various mechanisms of resistance that may occur in CRE as this may affect the success of the treatment regimen utilized.

The Diagnostic Microbiology laboratory at the Medical University of South Carolina (MUSC) utilizes rapid diagnostic testing (FilmArray® BCID Panel) to identify KPC-producing organisms in blood cultures. In the case of blood culture CRE isolates that are not identified as producing KPC or non-blood culture CRE isolates, further testing to determine mechanisms of resistance is not routinely performed. To further elucidate mechanisms of resistance in our population, we conducted a study with the primary objective of characterizing resistance mechanisms in CRE isolates recovered from patients at our institution. We utilized multiple phenotypic testing methods and a genotypic polymerase chain reaction (PCR) test to determine if such testing is routinely warranted as this information could potentially influence empiric antibiotic selection.

MATERIALS AND METHODS

CRE isolates were included in the study if they were obtained from a patient-specific culture at our institution from July 1, 2013 to March 31, 2017. In agreement with the 2015 CDC definition of CRE (defined as resistance to imipenem, meropenem, doripenem, or ertapenem, or documentation of carbapenemase production), we classified ertapenem resistant isolates as CRE for the purposes of this study [5]. Duplicate isolates, defined as 2 or more isolates of the same species from the same patient, were excluded. Isolates had been stored at -70 °C and were recovered from frozen storage and subcultured twice onto blood agar plates (BBL; BD, Sparks, MD) prior to testing. Following overnight incubation, phenotypic tests were performed including Modified Hodge test (MHT), Modified Carbapenem Inactivation Method (mCIM), KPC/Metallo-β-Lactamase (MBL) Confirm Kit™, and Neo-Rapid CARB Kit™. Testing of isolates was performed in batches following Clinical and Laboratory Standards Institute (CLSI) procedures and package insert instructions for commercial assays. All phenotypic tests were performed by the same two investigators on the same day for each batch. In addition, meropenem minimum inhibitory concentration (MIC) was determined *via* Etest® for all isolates. Following completion of all 4 phenotypic tests, isolates were shipped to The Medicines Company laboratories for genotypic testing utilizing the Cepheid Xpert® Carba-R (Cepheid, Sunnyvale, USA) which provides rapid detection of carbapenemases including KPC, New Delhi Metallo-β-Lactamase (NDM), Verona integron-encoded Metallo-β-Lactamase (VIM), OXA-48, and imipenemase (IMP). Data from phenotypic and genotypic testing were analyzed to determine the most prevalent resistance mechanisms identified. Our Institutional Review Board (IRB) deemed this study exempt as it was classified as a quality improvement project aimed at improving practices and enhancing knowledge of these MDR organisms within our institution.

RESULTS

The study population included a total of 41 ertapenem-resistant unique patient isolates arising over a 4-year period. The majority of these organisms were recovered from urine specimens (n = 14/41, 34.1%). *Klebsiella pneumoniae* was the most commonly identified organism (n = 15/41, 36.6%), followed by *Escherichia coli* (n = 9/41, 22.0%), *Enterobacter cloacae* (n = 9/41, 22.0%), *Enterobacter aerogenes* (n = 3/41, 7.3%), *Citrobacter freundii* (n = 3/41, 7.3%), *Raoultella ornithinolytica* (n = 1/41, 2.4%), and *Serratia marcescens* (n = 1/41, 2.4%). In addition, the majority of isolates were obtained from patients located in an intensive care unit (ICU). Most isolates were resistant to both ertapenem and meropenem when routine susceptibility testing was previously performed utilizing disk diffusion (n = 39/41, 95.1%), with the remaining isolates being intermediate to meropenem (n = 1/41, 2.4%) or susceptible to meropenem (n = 1/41, 2.4%). Upon re-testing meropenem MIC by Etest®, the majority of isolates had an MIC ≥ 4 mcg/mL (n = 23/41, 56.1%), indicating meropenem resistance per CLSI standards for antimicrobial susceptibility testing [6] (Figure 1).

Presence of a carbapenemase was detected by all phenotypic testing methods in 48.7% (n = 20/41) of isolates. When analyzing results of individual phenotypic tests, MHT identified carbapenemase production in 68.2% (n = 28/41) of isolates, whereas mCIM identified 65.8% (n = 27/41) of isolates as expressing carbapenemases (Figure 2).

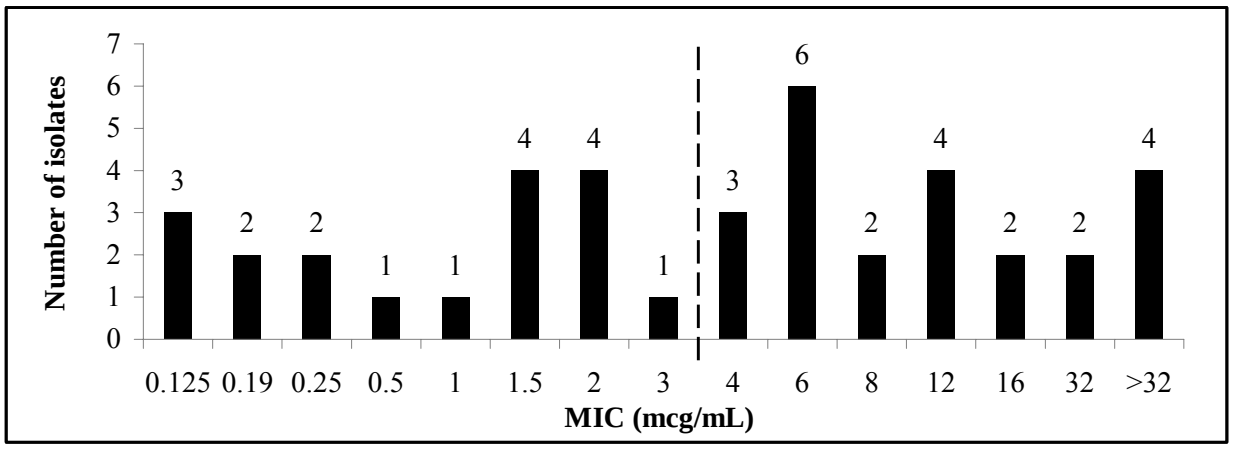

Figure 1. Meropenem MIC distribution by Etest®. Dotted line indicates CLSI MIC breakpoint of ≥ 4 mcg/mL for meropenem resistance in *Enterobacteriaceae*.

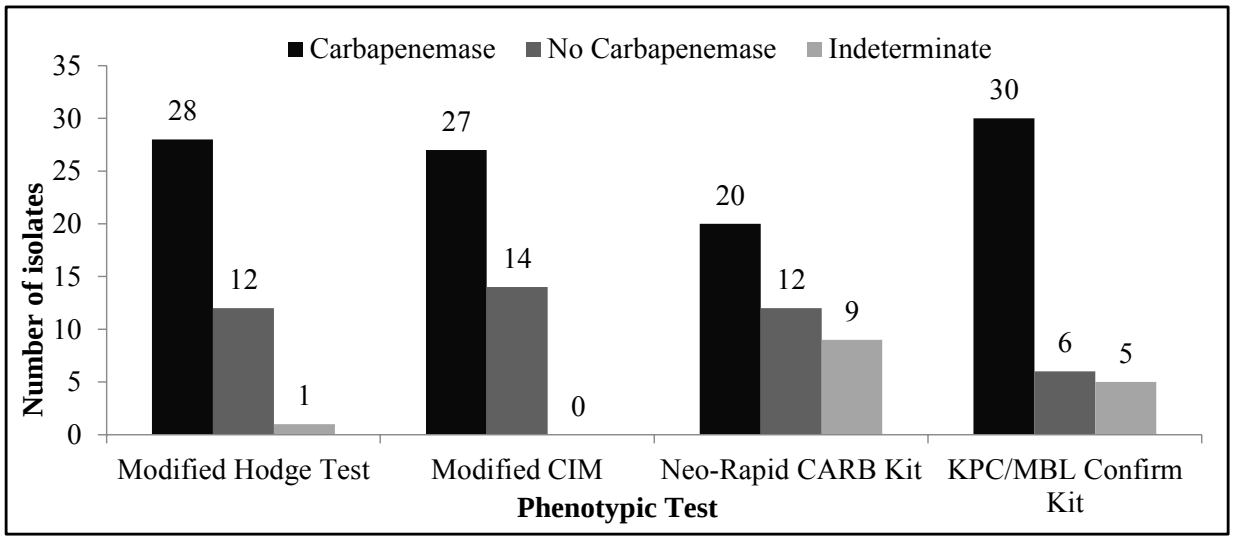

Figure 2. Results of detection of carbapenemase by phenotypic testing method.

Neo-Rapid CARB Kit™ detected carbapenemase production in 48.7% (n = 20/41) of isolates but was associated with the most indeterminate results of all the phenotypic tests with 9 results classified as indeterminate (Figure 2). The majority of isolates identified as carbapenemase-producing by Neo-Rapid CARB Kit™ (n = 18/20, 90%) became positive within 15 minutes, indicating high level carbapenemase expression. Lastly, KPC/MBL Confirm Kit™ identified production of KPC in 73.1% (n = 30/41) of isolates and no isolates were classified as producing MBL (Figure 2). In addition, 3 isolates were identified as producing AmpC in combination with porin loss as determined by KPC/MBL Confirm Kit™, and 2 of these isolates also produced KPC. All phenotypic tests agreed 70.7% (n = 29/41) of the time for either presence or absence of carbapenemase production. Indeterminate results for phenotypic tests were classified as negatives for purposes of calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), as well as for determining agreement between phenotypic tests.

Results of genotypic testing indicated that the majority of isolates expressed KPC (n = 26/41, 63.4%) and no other carbapenemases were produced.

The FilmArray® BCID Panel identified KPC in all CRE blood culture isolates confirmed as producing KPC by Xpert® Carba-R (n = 7/7, 100%), as well as absence of KPC in CRE blood culture isolates that were identified as non-carbapenemase producing (n = 2/2, 100%).

Analyzing results of phenotypic and genotypic tests by organism found that the majority of phenotypic tests were able to detect KPC production when present, regardless of organism (Table 1). Neo-Rapid CARB Kit™ was the only phenotypic test unable to consistently detect the presence of KPC; however it does not appear that this differed significantly by organism (Table 1). As indeterminate results were classified as negative for carbapenemase production, this may have affected the findings for Neo-Rapid CARB Kit™ as it was associated with the most indeterminate results of all of the phenotypic tests. In addition, 3 of the 4 phenotypic tests detected carbapenemase production in *E. cloacae* when it was not present, although this organism made up only 4 of the 15 isolates that did not produce carbapenemase by Xpert® Carba-R (Table 1).

When comparing findings of phenotypic methods to those of genotypic testing, sensitivity to detect carbapenemase was calculated as 100% among all phenotypic tests, with the exception of Neo-Rapid CARB Kit™ (76.9%) (Table 2). Specificity for

Table 1. Comparison of genotypic and phenotypic testing methods for detection of carbapenemase by organism.

Xpert® Carba-R	Organism (No. of isolates)	No. (%) of isolates positive for carbapenemase			
		MHT	mCIM	Neo-Rapid CARB Kit™	KPC/MBL Confirm Kit™, [a]
KPC	*K. pneumoniae* (13)	13 (100)	13 (100)	11 (84.6)	13 (100)
	E. cloacae (5)	5 (100)	5 (100)	3 (60)	5 (100)
	E. coli (4)	4 (100)	4 (100)	3 (75)	4 (100)
	C. freundii (2)	2 (100)	2 (100)	1 (50)	2 (100)
	E. aerogenes (1)	1 (100)	1 (100)	1 (100)	1 (100)[b]
	R. ornithinolytica (1)	1 (100)	1 (100)	1 (100)	1 (100)
None	*E. coli* (5)	0 (0)	0 (0)	0 (0)	0 (0)
	E. cloacae (4)	2 (50)	2 (50)	0 (0)	1 (25)[b]
	E. aerogenes (2)	0 (0)	0 (0)	0 (0)	1 (50)
	K. pneumoniae (2)	0 (0)	0 (0)	0 (0)	2 (100)
	C. freundii (1)	0 (0)	0 (0)	0 (0)	0 (0)
	S. marcescens (1)	0 (0)	0 (0)	0 (0)	0 (0)

[a]KPC/MBL Confirm Kit™ detected only KPC (i.e., no MBL detected)
[b]AmpC and porin loss also detected

Table 2. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of phenotypic tests.

Phenotypic test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Modified Hodge Test	100	86.7	92.8	100
Modified CIM	100	93.3	96.3	100
Neo-Rapid CARB Kit™	76.9	100	100	71.4
KPC/MBL Confirm Kit™	100	73.3	86.7	100

Indeterminate results were classified as negatives for purposes of calculations above.

absence of carbapenemase was greater than 86% among all tests, excluding KPC/MBL Confirm Kit™ (73.3%) (Table 2). PPV was highest for Neo-Rapid CARB Kit™ at 100%, and NPV was 100% for MHT, mCIM, and KPC/MBL Confirm Kit™ (Table 2).

DISCUSSION

Studies similar to ours have been performed to investigate and characterize mechanisms of carbapenem resistance in CRE, as well as to determine the reliability of various phenotypic testing methods for carbapenemase detection. Recently, Tamma and colleagues published a study that included 236 CRE isolates, obtained both retrospectively and prospectively, to compare 11 phenotypic tests for the detection of CP-CRE. Phenotypic tests that were similar to those used in our study included multiple variations of Carba NP tests, MHT, and mCIM. The investigators reported a specificity above 99% for detection of any carbapenemase enzyme by all phenotypic testing methods, with the exception of MHT (91%) and manual Blue Carba assay (96%) [7]. Specificity for carbapenemase detection in our study ranged from 73.3% to 100% across all 4 phenotypic tests, and our smaller sample size may have been partly responsible for our differing findings.

Doyle *et al*. evaluated multiple phenotypic confirmatory tests and PCR testing for the presence of carbapenemases in 142 CP-CRE. Sensitivity and specificity varied between the phenotypic testing methods used; however PCR testing was associated with 100% sensitivity and specificity. In addition, MHT performed best for detection of KPC and OXA-48 enzymes [8]. Birgy *et al*. utilized phenotypic testing methods for the detection of MBL- or KPC-producing *Enterobacteriaceae* and associated resistance mechanisms among 30 genotypically characterized carbapenem-resistant isolates. Isolates included produced MBL, KPC, and OXA-48 carbapenemases, as well as extended-spectrum β-lactamases (ESBL) or AmpC β-lactamases in conjunction with reduced permeability. Phenotypic tests included MHT and use of carbapenemase inhibitor-impregnated agar, specifically ethylenediamine tetraacetic acid (EDTA) or phenylboronic acid with or without cloxacillin, with comparison of measurements of zones of inhibition. MHT identified 95% of carbapenemase-producing strains and carbapenemase inhibitor-impregnated agar identified all MBL- and KPC-producing *Enterobacteriaceae* [9]. Sahin and colleagues evaluated the characteristics of 43 CRE strains at their institution through phenotypic and genotypic testing. MHT and MBL antimicrobial gradient test were performed for the identification of resistance phenotype. Seven strains produced OXA-48 and 1 strain produced NDM-1, while the remainder of isolates did not have resistance genes detected. MHT identified 85% of carbapenemase-positive isolates, whereas MBL identified only 4.7% of positive strains. Results supported a significant relationship between OXA-48 detection and MHT positivity ($p = 0.004$), as well as NDM-1 detection and MBL positivity ($p = 0.000$) [10].

Findings of our study differed from some of the studies discussed above, including that MHT was not the best test for detection of KPC as PPV was lower than that of mCIM and Neo-Rapid CARB Kit™. In addition, we did not identify any isolates producing carbapenemases other than KPC. This may affect empiric prescribing practices when CRE is identified within our institution as certain antimicrobials provide improved activity against different CP-CRE. For example, ceftazidime/ avibactam displays activity against KPC and OXA-48-producing CRE, but not MBL carbapenemases [11]. While other resistance mechanisms may have been present in the isolates identified as non-CP-CRE (i.e., overexpression of β-lactamases, porin mutations, and/or production of efflux pumps), this could not be determined with the phenotypic and genotypic tests conducted in the study. The phenotypic tests performed in the study are utilized to determine the *in vitro* presence of carbapenemase through slightly different methods. MHT utilizes principles of disk diffusion for detection of carbapenemase production. It is a rather simple and inexpensive test to complete; however MHT has been associated with poor sensitivity for detection of carbapenemases other than KPC, as well as false positive results, especially in *Enterobacter* spp. [5]. Specifically, in our study, 50% of *E. cloacae* isolates that did not produce carbapenemase by genotypic testing were classified as CP-CRE by MHT. mCIM utilizes principles of enzymatic hydrolysis to determine the presence of carbapenemase production in bacterial isolates and has been associated with high sensitivity and specificity for many carbapenemases, including KPC, MBL, and OXA-type carbapenemases [12].

Neo-Rapid CARB Kit™ utilizes a colorimetric microtube assay to identify hydrolysis of the carbapenem β-lactam ring in the presence of an indicator [13]. KPC/MBL Confirm Kit™ utilizes multiple carbapenemase inhibitors (cloxacillin, boronic acid, and dipicolinic acid) and is the only phenotypic test that was able to differentiate between KPC and MBL carbapenemases, as well as isolates producing AmpC β-lactamase in conjunction with porin loss as a mechanism of resistance [14].

Limitations of our study include single-center design, which may limit applicability of results to other institutions, and small sample size, with only 41 isolates identified over a 4-year period. In addition, we were unable to assess the performance of phenotypic tests for detection of carbapenemases other than KPC, as no other carbapenemases were evident. Lastly, Neo-Rapid CARB Kit™ and KPC/MBL Confirm Kit™ were associated with a moderate amount of indeterminate results (n = 9 and 5 isolates, respectively), and this may affect calculations of sensitivity, specificity, PPV, and NPV for these tests as indeterminate results were considered to be negative results for the purpose of these calculations.

CONCLUSION

In conclusion, our study identified the majority of CRE isolates within our institution as CP-CRE, specifically KPC. Most phenotypic tests utilized displayed moderately high rates for sensitivity, specificity, PPV, and NPV, although this varied across testing methods. Based on our findings, mCIM appeared to be the most reliable phenotypic test for detection of carbapenemase production given its high sensitivity and PPV (100% and 96.3%, respectively), as well as reliable confirmation of absence of carbapenemase based on high specificity and NPV (93.3% and 100%, respectively). In addition, mCIM was simple to perform, relatively inexpensive, and results were easily interpretable.

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CONFLICT OF INTEREST STATEMENT

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