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Comparison of clinical methods for detecting carbapenem-resistant *Enterobacteriaceae*

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ABSTRACT

We evaluated detection of carbapenem-resistant *Enterobacteriaceae* (CRE) by routine minimal inhibitory concentration (MIC) testing, polymerase chain reaction (PCR) using Xpert® Carba-R assay, hydrolysis of ertapenem and imipenem detected by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS), and hydrolysis by colorimetry using the EPI-CRE assay.

Ninety-six *Enterobacteriaceae* isolates possessing carbapenemase genes and 29 carbapenem-susceptible *Enterobacteriaceae* were available for testing. The sensitivity and specificity of each assay was determined. For sensitivity, discrepant results from each assay compared to reference genotype were arbitrated with MIC and/ or PCR testing to assess loss of plasmid-mediated resistance. Xpert Carba-R was evaluated for resistance genes in their FDA claim (i.e., the genes encoding KPC; NDM; VIM; IMP; and OXA-48).

The sensitivity for the assays was: MIC (N = 96), 96.8%, (discrepant analysis to 98.9% [2 cured plasmids]); Xpert Carba-R (N = 85), 97.6% (discrepant analysis to 100% % [2 cured plasmids]); EPI-CRE (N = 96), 91.7% (discrepant analysis to 91.7%); MALDI-TOF MS (N = 96) ertapenem hydrolysis using Compass software for interpretation (2 h incubation), 92.7% (discrepant analysis to 94.7% % [2 cured plasmids]); MALDI-TOF MS (N = 96) imipenem hydrolysis (1 h incubation), 97.9% (discrepant analysis to 98.9% % [1 cured plasmid]). The specificity for each assay was: MIC (N = 29), 100%; EPI-CRE (N = 29), 96.6%; MALDI-TOF MS ertapenem hydrolysis (N = 29), 100%; MALDI-TOF MS imipenem hydrolysis (N = 29), 96.6%. All isolates tested to ensure specificity demonstrated susceptible MIC results for carbapenems and did not qualify for testing with Xpert Carba-R.

No single assay detected all of the known genetic markers of carbapenem hydrolysis.

1. Introduction

Carbapenem-resistant organisms (CROs) are of great significance to the medical community and are associated with higher mortality rates than carbapenem-susceptible organisms [1]. The Centers for Disease Control and Prevention (CDC) campaign to: Detect and Protect Against Antibiotic Resistance Initiative (known as the AR Initiative), specifically cites detection and tracking of carbapenem-resistant *Enterobacteriaceae* as highest priority [2].

As such, accurate, rapid, diagnostic modalities to detect carbapenemase- producing *Enterobacteriaceae* (CPE) are needed to meet

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Table 1
Summary of Carbapenem-resistant Enterobacteriaceae tested.

Organism	Resistance Marker	N
Citrobacter freundii	NDM	1
Enterobacter aerogenes	IMP 1	1
Enterobacter cloacae	IMI	3
	KPC	3
	KPC + CTX-M-1	1
	VIM	1
Escherichia coli	KPC	2
	KPC 3	2
	NDM + CTX-M-1	1
	NDM	7
	NDM 5	2
	NDM 6	2
Klebsiella pneumoniae	IMP 1	1
	IMP 4	1
	KPC	12
	KPC 2	4
	KPC 3	13
	KPC 4	1
	NDM + OXA 232	1
	NDM	9
	NDM 1	2
	OXA 48	6
	OXA 181	3
	VIM	1
	VIM 27	1
Klebsiella oxytoca	KPC	1
Kluyvera ascorbata	KPC	1
Morganella morganii	KPC	1
Proteus mirabilis	KPC	2
	NDM	1
Raoultella ornithinolytica	KPC	1
Salmonella serovar *Seftenberg*	NDM	1
Serratia marcescens	SME	7
Total		96

these goals. Detecting these organisms is confounded due to the many ways organisms may become resistant to this class of antimicrobics. These include mutations in porins that limit access of drugs to their site of action, alteration of penicillin-binding protein sites, upregulation of efflux pumps, and production of specific carbapenemases. The carbapenemases include: Class A serine carbapenemases, including KPC, SME, and GES enzymes; Class B metallo-β-lactamases (MBL) including IMI, IMP, VIM, and NDM enzymes; and Class D OXA enzymes including OXA-48, OXA-181 and OXA-like enzymes [3]. Many of the genes for these enzymes have been identified on mobile genetic elements including plasmids and integrons; thus, transmission of carbapenemase-mediated resistance has been detected among the *Enterobacteriaceae* as well as other Gram-negative rods, in particular *Pseudomonas* and *Acinetobacter* species [3]. This horizontal spread contributes to a reservoir of organisms both in clinical and environmental locations. Resistance can be mediated by a single mechanism, or combinations of the above, and when present with other extended-spectrum beta-lactamases (e.g., ESBLs and AmpC), confers widespread resistance to multiple antimicrobics [4].

In this study, we evaluated different methods of detecting CRE that have been previously identified by genotypic methods. We evaluated routine minimum inhibitory concentration testing (MIC) testing with the GNX2F MIC panel, TREK Sensititre (Thermo Fisher Scientific, Waltham, MA), Xpert® Carba-R polymerase chain reaction (PCR) (Cepheid, Sunnyvale, CA), the EPI-CRE assay (Pilots Point, LLC,Sarasota, Florida), and matrix- assisted -laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS), (Bruker Daltonics, Billerica, MA) to detect hydrolysis of ertapenem and imipenem.

2. Materials and methods

2.1. Bacterial strains

For the sensitivity studies, 96 *Enterobacteriaceae* isolates possessing genes encoding carbapenemases from collections of Children's Healthcare of Atlanta (CHOA) and the U.S. CDC were tested (Table 1). Isolates were obtained from a number of clinical laboratories throughout the country and tested for the presence of carbapenemase genes by polymerase chain reactions (CHOA) or whole genome sequencing (CDC). Bacteria were cultivated on Remel blood agar plates (Thermo Fisher Scientific, Waltham, MA) with either a10 μg

ertapenem or 10 μg meropenem Bauer-Kirby disc (Becton Dickinson, Sparks, MD) in the primary streak area and incubated in ambient air overnight at 35 °C. Fresh overnight cultures were used for the tests.

For the specificity study, 29 *Enterobacteriaceae* that were susceptible to all carbapenems were tested. These included *Citrobacter freundii* (N = 1), *Escherichia coli* (N = 19), *Enterobacter aerogenes* (N = 1), *Enterobacter cloacae* (N = 2), *Klebsiella pneumoniae* (N = 4), *Kluyvera ascorbata* (N = 1), and *Proteus mirabilis* (N = 1). No further tests for resistance genes were performed.

2.2. MIC testing

Panel GNX2F of the TREK Sensititre system (Thermo Fisher Scientific, Waltham, MA) was used to perform doripenem, ertapenem, imipenem, and meropenem MIC determinations. The assays were performed per manufacturer specifications. Plates were read in the TREK Vizion manual-reader with enhanced digital visualization (Thermo Fisher Scientific, Waltham, MA). All quality control was performed per Clinical and Laboratory Standards Institute (CLSI, M-100 S26).

2.3. Carba-R PCR assay

The Xpert Carba-R assay (Cepheid, Sunnyvale, CA) was used to detect organisms harboring CRE genes including those encoding KPC, NDM, VIM, IMP and OXA-48 (including OXA-181 and OXA-232). The procedural set-up followed the manufacturer's recommendations and results were available in 48 min. Positive controls from Maine Molecular Quality Controls, Inc. (Scarborough, Maine) were used to verify the assay and included segments if the genes encoding KPC, NDM, VIM, IMP-1, and OXA 48 cloned in an *Escherichia coli* vector.

2.4. EPI-CRE colorimetric resistance test

Dry reagents were supplied from Pilots Point, LLC (Sarasota, Florida). Reagents were hydrated with nuclease-free grade water. Organisms were inoculated into aliquots of the reagent and incubated at 35 °C. A positive result was denoted with a color change from purple to yellow and/or formation of a violet precipitate at the bottom of the tube. *E. coli* ATCC 25922 was used as the β-lactamase negative control strain and *K. pneumoniae* strain, CHOA2, with a $bla_{\text{IMP-4}}$ gene was used as the positive control strain.

2.5. MALDI-TOF MS hydrolysis assay

Ertapenem and imipenem were supplied by Merck Research Laboratories (Rahway, NJ). Ertapenem was dissolved in 10 mM ammonium bicarbonate, pH 8.0 buffer to a final concentration of (0.1 mg/mL). Imipenem was dissolved in the same buffer to a final concentration of (0.5 mg/mL). As previously described, organisms were cultivated on Remel blood agar plates (Thermo Fisher Scientific, Waltham, MA) with either a10 μg ertapenem or 10 μg meropenem Bauer-Kirby disc (Becton Dickinson, Sparks, MD) in the primary streak area and incubated in ambient air overnight at 35 °C. Using a 1 μL inoculation loop, organisms were scraped from the plate and resuspended in 50 μL of the antibiotic solution. Following incubation at 35 °C for 2, 3, and 4 h (ertapenem assay) or 1 and 2 h (imipenem assay), the tubes were centrifuged for two minutes at 13,000 × *g* at room temperature. The supernatant was then tested for the parent molecule or hydrolysis products of the parent molecule (indicating carbapenemase activity).

One microliter of supernatant was spotted onto a polished steel target plate, and dried spots were then overlaid with matrix, composed of 10 mg/mL α-cyano-4-hydroxy-cinnamic acid [α-HCCA] in 50% acetonitrile-2.5% triflouroacetic acid (Bruker Daltonics, Billerica, MA). The spotted target was air dried, and then placed in a Microflex LT bench-top mass spectrometer (Bruker Daltonics, Billerica, MA) containing a 60-Hz nitrogen laser. Laser parameter settings were optimized for the low mass range- ion source 1 [IS1] 20 kV; [IS2], 17.5 kV; lens, 6.5 kV; detector gain, 2650 kV; gating, none, as detailed by Sparbier et al. [5]. Spectra were recorded in the positive linear mode in the mass range of 100 Da to 1000 Da at maximum laser frequency. A standard composed of bradykinin (1–5) (Sigma- Aldrich, St. Louis, MO) and bradykinin (1–7) (Sigma-Aldrich, St. Louis, MO) was used to calibrate the mass spectrometer. Calibration was accomplished via detection of the HCCA peaks $[M+H]^+$ at 190.05 Da and $[2M+H]^+$ at 372.02 Da, the bradykinin (1–5) peak $[M+H]^+$ at 573.31 Da, and the bradykinin (1–7) peak $[M+H]^+$ at 757.40 Da. A total of 240 shots were acquired in one position for one spectrum [5].

Acquired MALDI-TOF MS spectra were analyzed with the software Compass Flex Control 3.4 (Bruker Daltonics, Billerica, MA). Settings and analysis were performed as in Sparbier et al. [5]. In brief, the baseline was subtracted for each spectrum (algorithm, TopHat) and peaks were smoothed (algorithm, SavitzkyGolay; width, 0.2*m/z*; cycles 1). Peaks were selected manually with the following parameter settings: peak detection algorithm, centroid; signal-to-noise threshold, 2; relative intensity threshold, 0%; minimum intensity threshold, 100; peak width, 0.2*m/z*; height, 80% baseline subtraction, Top Hat. Hydrolysis products were automatically analyzed to determine sensitivity or resistance to ertapenem based on presence and intensity of the following peaks: sensitive strains, 498.7 Da, 514.7 Da, 520.7 Da, 536.8 Da, and 542.7 Da; resistant strains, 494.7 Da, 516.6 Da, 538.7 Da, 450.7 Da, 472.0 Da, and 488.0 Da [5]. Data were presented from the instrument as hydrolyzed (resistant) or non-hydrolyzed (susceptible). For the imipenem assay, peak intensity at 300.0 Da was read manually. Strains were classified as resistant if there was a decrease in intensity of the imipenem peak (300.0 Da) of 80% or more of the intensity of the non-hydrolyzed form (negative control). *E. coli* ATCC 25922 was used as the β-lactamase negative control species for both ertapenem and imipenem hydrolysis, and *K. pneumoniae* strain, CHOA2, containing a $bla_{\text{IMP-4}}$ gene or *K. pneumoniae* strain, CHOA24, containing a bla_{KPC} gene were used as positive control in the ertapenem assay. *K. pneumoniae* strain, CHOA24, harboring a bla_{KPC} gene was used as the positive control species in the imipenem

Table 2
CRE: comparison of Carba-R, minimum inhibitory concentrations, and EPI-CRE assays, and susceptibility profiles.

Resistance Mechanism[b]		Organism	Detection Method							
			CARBA-R		MIC OR MIC RANGE (μg/mL)[a]				EPI-CRE	
Designation	N		N	% POS	Doripenem	Ertapenem	Imipenem	Meropenem	N	% POS
IMI	3	*Enterobacter cloacae*	3	NA	2– > 8	2.0–16.0	> 8– > 32	2–16	3	100
IMP 1	2	*Enterobacter aerogenes*	1	100	2	2	2	2	1	100
		Klebsiella pneumoniae	1	100	> 2	> 4	> 8	> 8	1	100
IMP 4	1	*Klebsiella pneumoniae*	1	NA	1	≤1	≤1	≤1	1	100
KPC	23	*Klebsiella pneumoniae*	12	100	0.5– < 2	2– > 4	≤1.0– > 8	≤1.0– < 8	12	100
		Escherichia coli	2	100	2– > 2	4	4–8	4–8	2	100
		Enterobacter cloacae	3	100	2–8	4– > 8	4–8	4– > 8	3	100
		Proteus mirabilis	2	100	2–4	1–2	16	0.5–2	2	100
		Klebsiella oxytoca	1	100	0.5	0.5	4	1	1	100
		Kluyvera ascorbata	1	100	4	8	4	8	1	100
		Morganella morganii	1	100	4	8	8	4	1	100
		Raoultella ornithinolytica	1	100	2	1	4	1	1	100
KPC 2	4	*Klebsiella pneumoniae*	4	100	> 2	> 4– > 16	4–16	4– > 16	4	100
KPC 3	15	*Escherichia coli*	2	100	4	2–8	4	4	2	100
		Klebsiella pneumoniae	13	84.6 (2)[c]	≤0.12– > 8	≤0.25– > 16	≤1.0– > 64	≤1.0– > 16	13	100
KPC 4	1	*Klebsiella pneumoniae*	1	100	≤0.12	≤0.25	≤1.0	≤1.0	1	100
KPC + CTX-M-1	1	*Enterobacter cloacae*	1	100	4	> 8	8	8	1	100
NDM + CTX-M-1	1	*Escherichia coli*	1	100	> 8	> 8	8	> 8	1	100
NDM + OXA 232	1	*Klebsiella pneumoniae*	1	100	> 8	> 8	64	> 8	1	100
NDM	19	*Citrobacter freundii*	1	100	> 8	> 8	16	> 8	1	100
		Escherichia coli	7	100	> 2– > 8	> 4– > 16	8–32	8– > 16	7	100
		Klebsiella pneumoniae	9	100	> 2– > 8	> 4– > 8	4– > 64	8– > 8	9	100
		Proteus mirabilis	1	100	> 8	4	32	4	1	0
		Salmonella sv. Seftenberg	1	100	8	> 8	4	8	1	100
NDM 1	2	*Klebsiella pneumoniae*	2	100	> 8	> 8	≤0.5–32	> 8	2	100
NDM 5	2	*Escherichia coli*	2	100	> 8	> 8	16	> 8	2	100
NDM 6	2	*Escherichia coli*	2	100	> 2– > 8	> 4– > 8	> 8–16	> 8	2	100
OXA 48	6	*Klebsiella pneumoniae*	6	83.3	> 2–8	> 4– > 8	4– > 8	8– > 8	6	100
OXA 181	3	*Klebsiella pneumoniae*	3	100	2–4	8– > 8	2–4	2–4	3	100
SME	7	*Serratia marcescens*	7	NA	> 2	> 4– > 16	> 8– > 32	8– > 16	7	0
VIM	2	*Enterobacter cloacae*	1	100	4	2	4	2	1	100
		Klebsiella pneumoniae	1	100	> 8	> 4	8	8	1	100
VIM 27	1	*Klebsiella pneumoniae*	1	100	> 2	> 4	> 8	> 8	1	100
Initial % +				96.5						91.7
Post DA[d] % +				100						91.7

[a] Organisms resistant (CLSI standards) to imipenem, meropenem, doripenem, ertapenem.
[b] Determined by routine PCR.
[c] Not 100% sensitivity because organisms cured plasmid.
[d] DA, discrepant analysis.

assay.

2.6. Discrepant testing

For any isolates known to harbor resistance genes that tested negative in a given assay, concurrent repeat testing of that assay along with MICs (and Xpert Carba-R for appropriate organisms) were performed from the same inoculum. For any isolates known not to harbor resistance genes that tested as positive in a given assay, concurrent repeat testing with MICs was performed from the same inoculum. For MALDI-TOF MS sensitivity studies, isolates that failed to hydrolyze ertapenem at 2 h were tested at 3 and 4 h, and isolates that failed to hydrolyze imipenem at 1 h were tested at 2 h.

3. Results

3.1. MIC testing

The initial MIC assay detected carbapenem resistance in 93 of 96 isolates with known CRE-associated mutations, for an initial sensitivity of 96.8% (Table 2). Two KPC-3-harboring *K. pneumoniae* isolates and one KPC-4-harboring *K. pneumoniae* isolate were susceptible to all carbapenem antibiotics by MIC testing. Discrepant analysis showed the KPC-4 isolate retained its plasmid by PCR

Table 3
Non-CRE: comparison of EPI-CRE and MALDI-TOF MS hydrolysis of Ertapenem and Imipenem.

Organism	Routine MIC[a]		EPI-CRE		Ertapenem % hydrolysis by time 2 h		Imipenem % hydrolysis by time 1 h	
	N	% NEG	N	% NEG	N	% NEG	N	% NEG
Citrobacter freundii	1	100	1	100	1	100	1	100
Enterobacter aerogenes	1	100	1	100	1	100	1	100
Enterobacter cloacae	2	100	2	50	2	100	2	100
Escherichia coli	19	100	19	100	19	100	19	94.7
Klebsiella pneumoniae	4	100	4	100	4	100	4	100
Kluyvera ascorbata	1	100	1	100	1	100	1	100
Proteus mirabilis	1	100	1	100	1	100	1	100
TOTAL	29	100	29	96.6	29	100	29	96.6

[a] Minimum inhibitory concentration (μg/ml).

testing and two of the KPC-3 harboring isolates cured their plasmids despite placing an ertapenem disc on the purity plate to maintain selective pressure. As a result, the final sensitivity for routine MIC testing was determined to be 98.9%. One IMP-4-producing *K. pneumoniae* isolate was classified as intermediate to doripenem only (MIC = 2 μg/mL) and susceptible to all other carbapenem antibiotics. None of the organisms tested in the specificity study were resistant by MIC testing to the carbapenems.

3.2. Xpert Carba-R PCR assay

The initial Xpert Carba-R PCR assay detected carbapenemase resistance in 83 of 85 isolates with known CRE-associated mutations. This gives an initial sensitivity of 97.6% (Table 2). Two *K. pneumoniae* isolates harboring $bla_{\text{KPC-3}}$ genes produced negative results, but were found to cure their plasmid on discrepant analysis. The final sensitivity was 100% as denoted in Table 2. None of the organisms in the specificity study met the inclusion criteria for testing in the Xpert Carba-R assay, as all tested susceptible to the carbapenems.

3.3. EPI-CRE colorimetric resistance test

The initial EPI-CRE assay detected carbapenemase activity in 88 of 96 isolates with known CRE-associated mutations to give a sensitivity of 91.7%, as denoted in Table 2. Of particular note, the two KPC-3 harboring *K. pneumoniae* tested positive in this assay, but cured their plasmids in the MIC and Xpert Carba-R assays. One *P. mirabilis* strain harboring a bla_{NDM} gene tested negative in the EPI-CRE assay, in addition to all seven *S. marcescens* strains harboring bla_{SME} genes. All other isolates tested positive, as seen in Table 2. Sensitivity did not change with discrepant analysis. In the specificity study, one *E. cloacae complex* isolate with no known resistance genes tested positive in the EPI-CRE assay, yielding a specificity of 96.6% (Table 3). All other isolates with no known CRE markers tested negative (Table 3).

3.4. MALDI-TOF MS hydrolysis assay

On the initial runs, the MALDI-TOF MS hydrolysis assay detected carbapenemase activity in 89 of 96 CRE when using ertapenem as the antibiotic target, and 94 of 96 isolates using imipenem as the target. The initial sensitivity was 92.7% for ertapenem and 97.9% for imipenem, respectively (Table 4).

Interpretation of ertapenem data was performed by the instrument using the proprietary Compass software (Bruker Daltonics). Software is being developed for the imipenem assay.

One *K. pneumoniae* strain harboring a bla_{NDM} and two *K. pneumoniae* strains containing bla_{OXA48} hydrolyzed ertapenem at 3 but not at 2 h, and hydrolyzed imipenem at 1 h (Table 2). One *P. mirabilis* strain harboring a bla_{KPC} required 2 h for imipenem hydrolysis. Four isolates were subjected to discrepant analysis. One *K. pneumoniae* strain harboring a $bla_{\text{KPC-3}}$ gene did not hydrolyze ertapenem or imipenem, but had lost its plasmid. Another *K. pneumoniae* strain harboring a $bla_{\text{KPC-3}}$ gene, a *E. coli* harboring a bla_{KPC}, and a *P. mirabilis* with a bla_{NDM} hydrolyzed imipenem at one hour, but failed to hydrolyze ertapenem (at 2,3,and 4 h). This *K.pneumoniae* harboring a $bla_{\text{KPC-3}}$ gene cured its plasmid, while the *E. coli* harboring a bla_{KPC} and *P.mirabilis* with a bla_{NDM} retained their plasmids. The adjusted sensitivity for ertapenem hydrolysis was 94.7% and for imipenem was 98.9%.

One *E. coli* strain with no known resistance showed hydrolysis of imipenem but did not hydrolyze ertapenem. All other strains that were known not to contain carbapenemase genes did not hydrolyze ertapenem or imipenem in the MALDI-TOF MS assay. Following discrepant analysis, the specificity for ertapenem and imipenem hydrolysis was 100% and 96.6%, respectively (Table 3).

4. Discussion

CRE are both public health and therapeutic challenges. Detecting, preventing, and controlling these organisms requires a strategic and sustained effort. As the organisms are constantly altering their resistance mechanisms, it is critical to identify these organisms as

Table 4
CRE: MALDI-TOF MS hydrolysis of Ertapenem and Imipenem.

Resistance	Organism (N)	Ertapenem % hydrolysis by time			Imipenem % hydrolysis by time	
Mechanism[a] (N)		2 h % POS	3 h % POS	4 h % POS	1 h % POS	2 h % POS
IMI (3)	*Enterobacter cloacae* (3)	100			100	
IMP 1 (2)	*Enterobacter aerogenes* (1)	100			100	
	Klebsiella pneumoniae (1)	100			100	
IMP 4 (1)	*Klebsiella pneumoniae* (1)	100			100	
KPC (23)	*Klebsiella pneumoniae* (12)	100			100	
	Escherichia coli (2)	50	0	0	100	
	Enterobacter cloacae (3)	100			100	
	Proteus mirabilis (2)	100			50	100
	Klebsiella oxytoca (1)	100			100	
	Kluyvera ascorbata (1)	100			100	
	Morganella morganii (1)	100			100	
	Raoultella ornithinolytica (1)	100			100	
KPC 2 (4)	*Klebsiella pneumoniae* (4)	100			100	
KPC 3 (15)	*Escherichia coli* (2)	100			100	
	Klebsiella pneumoniae (13)	84.6(2)[b]	0(2)[b]	0(2)[b]	92.3(1)[b]	0(1)[b]
KPC 4 (1)	*Klebsiella pneumoniae* (1)	100			100	
KPC + CTX-M-1 (1)	*Enterobacter cloacae* (1)	100			100	
NDM + CTX-M-1 (1)	*Escherichia coli* (1)	100			100	
NDM + OXA 232 (1)	*Klebsiella pneumoniae* (1)	100			100	
NDM (19)	*Citrobacter freundii* (1)	100			100	
	Escherichia coli (7)	100			100	
	Klebsiella pneumoniae (9)	88.9	100	100	100	
	Proteus mirabilis (1)	0	0	0	100	
	Salmonella sv. Seftenberg (1)	100			100	
NDM 1 (2)	*Klebsiella pneumoniae* (2)	100			100	
NDM 5 (2)	*Escherichia coli* (2)	100			100	
NDM 6 (2)	*Escherichia coli* (2)	100			100	
OXA 48 (6)	*Klebsiella pneumoniae* (6)	66.7	100	50	100	
OXA 181 (3)	*Klebsiella pneumoniae* (3)	100			100	
SME (7)	*Serratia marcescens* (7)	100			100	
VIM (2)	*Enterobacter cloacae* (1)	100			100	
	Klebsiella pneumoniae (1)	100			100	
VIM 27 (1)	*Klebsiella pneumoniae* (1)	100			100	
Initial % +		92.7			97.9	
Post DA[c] % +		94.7			98.9	

[a] determined by PCR or whole genome sequencing.
[b] cured plasmid hence not 100%.
[c] DA, discrepant analysis.

rapidly and efficiently as possible. In this report, we examined routine MICs, PCR (Cepheid, Xpert Carba-R), hydrolysis detection of ertapenem (using an automated interpretation tool) and imipenem by MALDI-TOF MS, and a colorimetric assay (EPI-CRE) for their ability to detect CRE.

The Xpert Carba-R assay detected all of the isolates with resistant mechanisms within their FDA claim (KPC, NDM, VIM, IMP and OXA-48, including OXA-181 and OXA-232). These mechanisms constitute the most commonly seen carbapenemases worldwide [4,6,7]. Initially 2 KPC-3 harboring *K. pneumoniae* tested negative in the Xpert Carba-R assay. Discrepant analysis indicated the organisms had lost their resistance plasmids. All of the OXA-48-containing isolates were detected in our study. However, lower detection rates of isolates harboring bla_{OXA} genes has been cited in the literature primarily due to either use of a previous version of the assay that did not detect $bla_{OXA\text{-}181}$, or testing of non-Enterobacteriaceae that contained OXA enzymes other than OXA-48 [8–11]. Cortegiani et al. cites a sensitivity of 50% (N = 23) due to poor Xpert Carba-R detection of OXA-23 producing *A. baumannii* and GES-producing *P. aeruginosa,* but the assay was not designed to detect either of these resistance determinants [8]. Tato et al. noted a sensitivity of 95.0% (N = 38) for isolates possessing $bla_{OXA\text{-}48}$ genes [9]; additional literature shows sensitivities of 39.2% (N = 47) and 83% (n = 100) for OXA-48-like-producers, again using a previous version of the assay [10,11].

The EPI-CRE assay detected 91.7% of the CRE. The SME-producing *S. marcescens* strains (N = 7) that gave negative results in the EPI-CRE assay showed resistance to at least 1 carbapenem (doripenem, ertapenem, imipenem, or meropenem) in discrepant MIC testing (Table 2). The *E. cloacae complex* isolate that did not contain carbapenemase genes but produced a positive EPI-CRE result was carbapenem-susceptible by discrepant MIC testing. While this appears to be an anomaly, there may be other mechanisms extent to cause the shift in pH to be interpreted as positive.

Few studies have been done using EPI-CRE assay to detect carbapenem resistance. Slesar and Schreckenberger tested 13 isolates

harboring KPC enzymes, 26 organisms with an MBL, 3 containing OXA-48, and 20 ESBL producing *Enterobacteriaceae* in addition to non-CPEs [12]. They reported 100% specificity and 100% sensitivity for all isolates [12]. Our EPI-CRE results were similar, as most KPC, MBL, and OXA-48-producers in our study were detected, with the exception of one NDM (an MBL) producing *P. mirabilis.* This may be a result of the low level hydrolysis and the lack of pH shift. The *E.cloacae* strain that was non-CRE testing positive in the assay may have been due to instability of reagents with hydrolysis of the carbapenem.

MALDI-TOF MS detection of hydrolysis of ertapenem and imipenem showed favorable results with sensitivities of 94.7% and 98.9%, respectively in post discrepant analysis. A single *E.coli* isolate (non-CRE) testing positive in the imipenem assay is most probably due to self-degradation of the carbapenem. To our knowledge, this is the first publication in the United States detailing the use of the automated Compass software to evaluate the ertapenem hydrolysis assay. The software performed well. Imipenem hydrolysis intensity, evaluated at greater than or equal to 80% of the positive control was very easy to interpret visually by examining the 300 Da peak. Differing from molecular assays, limited by their targets, MALDI-TOF MS carbapenem hydrolysis was able to detect a wide range of carbapenemases as has been previously demonstrated by Yi et al. [13].

From the technical aspects in the routine laboratory, the Carba-R assay required preparation of a standard inoculum, transferring it to a sample reagent tube and inoculation of the Cepheid cartridge. Once in the instrument, results were generated in less than an hour. The EPI-CRE assay required hydration of the reagent, distribution to individual tubes, inoculation of organisms, and overnight incubation. No instrumentation was necessary and interpretation was by visual examination. Color changes were robust and were not subject to nuances in the color variation. The MALDI-TOF MS hydrolysis assays required daily preparation of antimicrobics, distribution to tubes, inoculation, incubation, centrifugation, plating on target and addition of matrix. Interpretation of results for ertapenem hydrolysis was performed by the instrument via the Compass software, while hydrolysis of imipenem was calculated by comparing to the control. MALDI TOF MS results were available on average at 3–4 h (for routine runs) at a minimal cost for reagents.

Our study had several limitations. Most importantly, we tested a limited, but diverse number of isolates for both sensitivity and specificity studies. Each assay had limitations. The Xpert Carba-R PCR performed well for the respective targets in the assay (KPC, NDM, VIM, IMP and OXA-48), but as expected, did not detect all types of carbapenemases, most notably GES, IMI, non-IMP-1, and SME- producing isolates [4]. In addition, this assay requires the 6-channel instrument, with updated software. As with any molecular assay, it will be critical to track genetic drift among the markers tested via periodic assessment of sensitivity by comparison to sequence analysis. In EPI-CRE, only 1 lot of reagent was available for testing from the manufacturer (Pilots Pointe LLC, Sarasota, Florida). It would be important to determine if slight modifications in the components could be made to detect the SME-producing strains. For the MALDI-TOF MS assays, Compass software is only available for ertapenem hydrolysis, and imipenem hydrolysis must be analyzed manually. Compass software for imipenem hydrolysis is currently in development for automatic interpretation of resistance and susceptibility. It is key to note that the current Compass software for ertapenem hydrolysis evaluates resistance or susceptibility based on the difference between the positive control value and negative control value. Bruker Daltonics recommends using clinical control strains according to recommendations of CLSI or EUCAST.

5. Conclusions

On initial testing, simulating the clinical laboratory routine, carbapenemase resistance was confirmed for greater than 95% of the CRE isolates in this study using MICs, Xpert Carba-R PCR, and MALDI-TOF MS imipenem hydrolysis. Discrepant analysis raised these values to 98.9%, 98.8%, and 98.9%, respectively. Both EPI-CRE and MALDI-TOF MS hydrolysis of ertapenem were greater than 90% on initial testing. With discrepant analysis, these values were 94.7% and 91.7%, respectively. No assay tested in this study accurately detected all 96 CREs.

Conflicts of interest

None. This research did not receive any specific grant from funding agencies in the public, commercial, or not- for- profit sectors.

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