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Carbapenemase Detection among Carbapenem-Resistant Glucose-Nonfermenting Gram-Negative Bacilli

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ABSTRACT Accurate detection of carbapenemase-producing glucose-nonfermenting Gram-negative bacilli (CPNFs), including *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, is necessary to prevent their dissemination within health care settings. We performed a method comparison study of 11 phenotypic carbapenemase detection assays to evaluate their accuracy for the detection of CPNFs. A total of 96 carbapenem-resistant glucose-nonfermenting isolates were included, of which 29% produced carbapenemases. All CPNFs were molecularly characterized to identify β-lactamase genes. A total of 86% of the carbapenemase-producing *P. aeruginosa* isolates produced class B carbapenemases. Several assays performed with a sensitivity of >90% for the detection of carbapenemase-producing *P. aeruginosa*, including all rapid chromogenic assays and the modified carbapenem inactivation method. Most included assays, with the exception of the Manual Blue Carba assay, the Modified Carba NP assay, the boronic acid synergy test, and the metallo-β-lactamase Etest, had specificities of >90% for detecting carbapenemase-producing *P. aeruginosa*. Class D carbapenemases were the most prevalent carbapenemases among the carbapenemase-producing *A. baumannii* strains, with 60% of the carbapenemase-producing *A. baumannii* isolates producing acquired OXA-type carbapenemases. Although several assays achieved >90% specificity in identifying carbapenemase-producing *A. baumannii*, no assays achieved a sensitivity of greater than 90%. Our findings suggest that the available phenotypic tests generally appear to have excellent sensitivity and specificity for detecting carbapenemase-producing *P. aeruginosa* isolates. However, further modifications to existing assays or novel assays may be necessary to accurately detect carbapenemase-producing *A. baumannii*.

KEYWORDS *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, carbapenem-resistant organisms, carbapenemase-producing, carbapenem-resistant

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While the critical threat of carbapenemase-producing *Enterobacteriaceae* (CPE) has become increasingly recognized over recent years (1, 2), there remains an underappreciation for the hazards posed by carbapenemase-producing glucose-nonfermenting Gram-negative bacilli (CPNFs). Increasing reports of CPNFs, dominated by *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, are worrisome as many of these carbapenemase genes have demonstrated mobility across Gram-negative species, regardless of an organism's ability to ferment glucose (3). Lack of recognition and detection of CPNFs may further perpetuate the problem of carbapenem resistance among all Gram-negative organisms.

Early and accurate detection of CPNFs is necessary to prevent their dissemination within health care settings. Multiple phenotypic methods have been described for the detection of carbapenemase production among carbapenem-resistant *Enterobacteria-*

ceae (CRE) (4). However, the performance of these assays among carbapenem-resistant glucose-nonfermenting isolates is not well described. We performed a method comparison study, including 11 phenotypic carbapenemase detection assays, to evaluate their accuracy for the detection of CPNFs.

RESULTS

Overall accuracy. The sensitivity and specificity of the various assays in detecting carbapenemase production among all carbapenem-resistant nonfermenters are shown in Table 1. The Rapidec Carba NP and Neo Rapid Carb Screen tests had overall sensitivities of 93%, the highest observed among all assays. The Neo Rapid Carb Screen, the Rapid CARB Blue Screen, and the Manual Carba NP CLSI methods achieved specificities of 99%.

There were 35 Manual Carba NP CLSI tests that yielded invalid results (*P. aeruginosa* [21/67; 31%], *A. baumannii* [11/24; 46%], and *Achromobacter* spp. [3/4; 75%]). Additionally, there were two indeterminate results with the modified carbapenem inactivation method (mCIM) (*P. aeruginosa* [1/67; 1%] and *A. baumannii* [1/24; 4%]). The 35 invalid results from the Manual Carba NP CLSI method included isolates producing VIM-4 ($n = 1$), NDM-1 ($n = 1$), OXA-23 and OXA-24 ($n = 1$), OXA-58 ($n = 1$), NDM-1 and OXA-23 ($n = 1$), and OXA-24 ($n = 2$) carbapenemases. Carbapenemase genes were not identified in the remaining 28 isolates. Regarding the mCIM, one of the indeterminate results was from an *A. baumannii* isolate producing both an OXA-23 carbapenemase and an NDM-1 carbapenemase and the second was from a *P. aeruginosa* isolate producing a VIM-2 carbapenemase. There were no isolates with negative molecular results but consistently positive results across several phenotypic assays, suggesting a low likelihood that a carbapenemase-producing gene may have been present but not detected by the included molecular techniques.

Table 2 further breaks down the carbapenem-resistant nonfermenters into *P. aeruginosa* and *A. baumannii* isolates. Several assays were found to perform with a sensitivity of 100% and specificity of >90% for the detection of carbapenemase-producing *P. aeruginosa*. On the other hand, the majority of tests performed poorly among *A. baumannii* isolates, with none achieving a sensitivity of >90%. Several assays had specificities over 90% for non-carbapenemase-producing *A. baumannii* isolates, with notable exceptions, including the Rapidec Carba NP assay, the Modified Carba NP assay, the modified Hodge test (MHT), the carbapenem inactivation method (CIM), and the mCIM.

Accuracy of assays for specific carbapenemase classes. All assays were able to accurately identify the 2 KPCs, except for the boronic acid synergy test (Table 1). This assay failed to recognize a KPC-2 carbapenemase produced by a *P. aeruginosa* isolate. The colorimetric assays had >90% sensitivity in detecting the 16 class B carbapenemases. The notable exception was the Manual Carba NP CLSI assay, which had a sensitivity of 88%, as it yielded invalid results for one NDM producer and one VIM producer. The metallo-β-lactamase (MBL) Etest identified 81% of class B carbapenemases and was particularly poor at detecting IMP carbapenemases. Both the MHT and CIM had 56% sensitivity for identifying class B enzymes. However, the mCIM was able to improve detection to 94%. It yielded an indeterminate result for an *A. baumannii* isolate producing an NDM and OXA-23. There were 10 acquired class D OXA-type carbapenemases present among the *A. baumannii* isolates. All assays performed relatively poorly in detecting OXA-type enzymes.

DISCUSSION

We evaluated 11 phenotypic assays to determine their accuracy for the detection of CPNFs. To the best of our knowledge, this is the most comprehensive evaluation of phenotypic assays for CPNF identification. The sensitivity of carbapenemase detection for *P. aeruginosa* was 93 to 100% for most assays and was comparable to the accuracy of these assays for CPE detection (4). In contrast, all tests had compromised sensitivity when used to identify carbapenemase-producing *A. baumannii*.

TABLE 1 Accuracy of 11 phenotypic assays for carbapenemase detection using 96 carbapenem-resistant non-glucose-fermenting isolates

Assay	No. of positive tests/no. of isolates producing the specific carbapenemase (% positive)													% sensitivity (95% confidence interval) for all CPNF (n = 28)	% specificity (95% confidence interval) for non-CPNF (n = 68)
	Class A	Class B					Class D								
	KPC (n = 2)	NDM (n = 4)	VIM (n = 8)	IMP (n = 3)	SPM (n = 1)	All class B (n = 16)	OXA-23 (n = 3)	OXA-58 (n = 1)	OXA-72 (n = 1)	OXA-23 + NDM (n = 1)	OXA-23 + OXA-24 (n = 1)	OXA-24 (n = 3)	All OXA types (n = 10)		
Rapidec Carba NP	2/2 (100)	4/4 (100)	8/8 (100)	3/3 (100)	1/1 (100)	16/16 (100)	3/3 (100)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)	0/3 (0)	8/10 (80)	93 (75–99)	87 (76–93)
Neo-Rapid Carb Screen	2/2 (100)	4/4 (100)	8/8 (100)	3/3 (100)	1/1 (100)	16/16 (100)	3/3 (100)	1/1 (100)	1/1 (100)	1/1 (100)	0/1 (0)	3/3 (100)	8/10 (80)	93 (75–99)	99 (91–100)
Rapid CARB Blue Screen	2/2 (100)	4/4 (100)	8/8 (100)	3/3 (100)	1/1 (100)	16/16 (100)	1/3 (33)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)	0/3 (0)	4/10 (40)	79 (59–91)	99 (91–100)
Manual Carba NP CLSI	2/2 (100)	3/4 (75)	7/8 (88)	3/3 (100)	1/1 (100)	14/16 (88)	0/3 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/3 (0)	0/10 (0)	57 (37–75)	99 (91–100)
Manual Blue Carba	2/2 (100)	4/4 (100)	8/8 (100)	3/3 (100)	1/1 (100)	16/16 (100)	2/3 (67)	1/1 (100)	0/1 (0)	1/1 (100)	0/1 (0)	0/3 (0)	4/10 (40)	79 (14–73)	76 (64–86)
Modified Carba NP	2/2 (100)	4/4 (100)	8/8 (100)	3/3 (100)	1/1 (100)	16/16 (100)	2/3 (67)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	0/3 (0)	7/10 (70)	89 (71–97)	62 (49–73)
Boronic acid synergy test	1/2 (50)														68 (58–77)
Metallo-β-lactamase Etest		4/4 (100)	7/8 (88)	1/3 (33)	1/1 (100)	13/16 (81)				0/1 (0)				76 (50–92)	87 (78–93)
Modified Hodge test	2/2 (100)	2/4 (50)	3/8 (38)	3/3 (100)	1/1 (100)	9/16 (56)	1/3 (33)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	3/3 (100)	8/10 (80)	68 (48–83)	91 (81–96)
Carbapenem inactivation method	2/2 (100)	0/4 (0)	5/8 (63)	3/3 (100)	1/1 (100)	9/16 (56)	1/3 (33)	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)	0/3 (0)	4/10 (40)	54 (34–72)	96 (87–99)
Modified carbapenem inactivation method	2/2 (100)	3/4 (75)	8/8 (100)	3/3 (100)	1/1 (100)	15/16 (94)	2/3 (67)	1/1 (100)	1/1 (100)	0/1 (0)	1/1 (100)	3/3 (100)	7/10 (70)	86 (66–95)	92 (82–97)

TABLE 2 Accuracy of 11 phenotypic assays for carbapenemase detection, including 67 carbapenem-resistant *Pseudomonas aeruginosa* isolates and 24 carbapenem-resistant *Acinetobacter baumannii* isolates

Assay	% sensitivity (95% confidence interval)		% specificity (95% confidence interval)	
	Carbapenemase-producing *Pseudomonas aeruginosa*[a] ($n = 14$)	Carbapenemase-producing *Acinetobacter baumanii*[b] ($n = 14$)	Non-carbapenemase-producing *Pseudomonas aeruginosa* ($n = 53$)	Non-carbapenemase-producing *Acinetobacter baumannii* ($n = 10$)
Rapidec Carba NP	100 (73–100)	86 (56–97)	91 (76–96)	70 (35–92)
Neo-Rapid Carb Screen	100 (73–100)	86 (56–97)	98 (89–100)	100 (66–100)
Rapid CARB Blue Screen	100 (73–100)	57 (30–81)	98 (89–100)	100 (60–100)
Manual Carba NP CLSI	93 (64–100)	21 (6–51)	100 (92–100)	100 (60–100)
Manual Blue Carba	100 (73–100)	57 (30–81)	77 (63–87)	90 (54–99)
Modified Carba NP	100 (73–100)	79 (49–94)	60 (46–73)	80 (44–96)
Boronic acid synergy test	50 (3–97)		57 (44–69)	96 (76–100)
Metallo-β-lactamase Etest	75 (43–93)	80 (30–99)	84 (71–92)	94 (71–100)
Modified Hodge test	64 (36–86)	71 (42–90)	98 (89–100)	70 (35–92)
Carbapenem inactivation method	79 (49–94)	29 (10–58)	100 (92–100)	80 (44–96)
Modified carbapenem inactivation method	100 (73–100)	71 (42–90)	98 (89–100)	70 (35–92)

[a]The carbapenemases harbored by the *P. aeruginosa* isolates were as follows: KPC ($n = 2$); VIM ($n = 8$); IMP ($n = 3$); SPM ($n = 1$).
[b]The carbapenemases harbored by the *A. baumannii* isolates were as follows: NDM ($n = 4$); NDM and OXA-23 ($n = 1$); OXA-23 ($n = 3$); OXA-24 ($n = 3$); OXA-58 ($n = 1$); OXA-72 ($n = 1$); OXA-23 and OXA-24 ($n = 1$).

Data from the U.S. National Healthcare Safety Network indicate that the proportions of *P. aeruginosa* and *A. baumannii* health care-associated isolates resistant to carbapenems in 2014 were notable at approximately 25% and 53%, respectively (5). Unlike the data from carbapenem-resistant *Enterobacteriaceae* (CRE) (6), the proportions of carbapenem-resistant glucose-nonfermenting isolates due to carbapenemases are unknown, according to U.S. estimates, but likely differ between *P. aeruginosa* and *A. baumannii*. Although carbapenem resistance among *P. aeruginosa* strains is predominantly mediated by non-carbapenemase mechanisms, including the loss of OprD porin expression (most common) and/or upregulation of MexAB-OprM efflux pumps (7, 8), increasing numbers of carbapenemase-producing *P. aeruginosa* isolates are being reported both in the United States and globally (9–14). Similarly to what others have reported (8), the majority of the 14 carbapenemase-producing *P. aeruginosa* isolates included in our cohort belonged to Ambler class B MBLs, primarily VIM and IMP carbapenemases (8). Based on our findings, most phenotypic tests commonly applied to CRE isolates for detection of carbapenemase producers can be applied to carbapenem-resistant *P. aeruginosa*.

In contrast to carbapenem-resistant *P. aeruginosa*, carbapenemase production is the primary resistance mechanism among carbapenem-resistant *A. baumannii* isolates (15). The most common acquired carbapenemases produced by *A. baumannii* are from Ambler class D—predominantly of the OXA-23 type (15, 16). Differences between *P. aeruginosa* and *A. baumannii* in the heterogeneity of carbapenemases likely accounted for some of the variability in accuracy estimates of phenotypic assays in our cohort. These differences may be due to the intrinsic low cell membrane permeability of *A. baumannii* isolates, requiring a lysis solution different from that used for *Enterobacteriaceae* and *P. aeruginosa*, in turn making it more difficult for carbapenemases to be detected by rapid colorimetric assays that rely on lysis and release of carbapenemases. Additionally, the differences observed between *P. aeruginosa* and *A. baumannii* with respect to phenotypic carbapenemase detection may be due to weaker carbapenemase activity of the class D OXA enzymes that have only been reported in *A. baumannii* to date. Although they are weaker carbapenemases, *A. baumannii* isolates that harbor class D OXA-type enzymes often demonstrate high carbapenem resistance due to the presence of other resistance mechanisms such as porin mutations or efflux pumps (17).

Attempts to detect carbapenemases among *A. baumannii* isolates have been previously attempted by modifying existing approaches for the identification of carbapenemase producers. Most modifications include the use of different extraction methods

to overcome the intrinsic low permeability of isolates for more efficient lysis and increasing the inoculum to increase the amount of enzyme present to overcome the weaker hydrolytic activity of OXA-type carbapenemases. In our study, the modified Carba NP had improved sensitivity compared with the manual Carba NP CLSI method for detection of carbapenemase-producing *A. baumannii*, with the sensitivity increasing from 21% to 79%, in part due to changes in the extraction reagent (18, 19). Of note, CLSI is reevaluating their endorsement of the Carba NP assay for detecting carbapenemase producers among carbapenem-resistant *A. baumannii* isolates. For more details on the relative pros and cons of the various phenotypic assays tested in this study, we refer the reader to previous work from these investigators (4).

A notable limitation to this work is that there were small numbers of isolates producing any particular carbapenemase enzyme. In addition, the paucity of other CPNF isolates (e.g., *Achromobacter* spp.) precludes any discussion of detection accuracy estimates for these organisms. Detection of CPNFs by phenotypic assays needs to be repeated in a larger, more diverse cohort of isolates to provide more-accurate sensitivity and specificity estimates. Of note, we did not evaluate the CarbAcineto NP test, which was designed specifically to detect carbapenemase production among *Acinetobacter* spp. and which has been reported to have a sensitivity of 94% and specificity of 100% (16).

Overall, our findings suggest that existing phenotypic assays generally appear to have excellent sensitivity and specificity for detecting carbapenemase-producing *P. aeruginosa* isolates. This is particularly important as we are continuing to witness increasing numbers of carbapenemase-producing *P. aeruginosa* isolates. However, further modifications to existing assays or novel assays may be necessary to accurately detect carbapenemase-producing *A. baumannii*.

MATERIALS AND METHODS

This study included 96 carbapenem-resistant glucose-nonfermenting isolates obtained from (i) The Centers for Disease Control and Prevention and the Food and Drug Administration Antimicrobial Resistance Isolate Bank (CDC-FDA) (20) ($n = 26$), (ii) International Health Management Associates, Inc. (IHMA) ($n = 7$), and (iii) The Johns Hopkins Hospital (JHH) Medical Microbiology Laboratory ($n = 63$). JHH isolates were prospectively collected carbapenem-resistant (specifically, meropenem-resistant) glucose-nonfermentering isolates from unique patients encountered in the clinical laboratory over a 1-month period.

The CDC-FDA and IHMA isolates were previously molecularly characterized to identify β-lactamase genes using whole-genome sequencing and/or PCR. For the JHH clinical isolates, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics Inc., Billerica, MA) was used for genus and species identification. A BD Phoenix automated system (BD Diagnostics, Sparks, MD) was used for antimicrobial susceptibility testing (AST), and carbapenem AST results were confirmed using the Etest method (bioMérieux, Marcy-l'Étoile, France). Carbapenem resistance was defined according to the Clinical and Laboratory Standards Institute (CLSI) breakpoint criteria (18). Acquired β-lactamase genes in the JHH clinical isolates were identified using the Check-MDR CT103XL kit microarray-based assay (Check-Points, Wageningen, Netherlands).

The genera and species of the 96 carbapenem-resistant glucose-nonfermenting isolates were as follows: *P. aeruginosa* ($n = 67$, 70%), *A. baumannii* ($n = 24$, 25%), *Achromobacter* spp. ($n = 4$, 4%), and *Ralstonia picketti* ($n = 1$, 1%). Twenty-eight (29%) isolates produced carbapenemases. All *Achromobacter* and *R. picketti* isolates were non-carbapenemase producing. The carbapenemase genes identified in the *P. aeruginosa* isolates included the following: bla_{KPC} ($n = 2$), bla_{VIM} ($n = 8$), bla_{IMP} ($n = 3$), and bla_{SPM} ($n = 1$). The carbapenemase genes identified in the *A. baumannii* isolates included the following: bla_{NDM} ($n = 4$), bla_{NDM} and bla_{OXA-23} ($n = 1$), bla_{OXA-23} ($n = 3$), bla_{OXA-24} ($n = 3$), bla_{OXA-58} ($n = 1$), bla_{OXA-72} ($n = 1$), and bla_{OXA-23} and bla_{OXA-24} ($n = 1$).

Isolates were subcultured from frozen stock to tryptic soy agar (TSA) with 5% sheep blood agar with a second subculture performed prior to phenotypic testing. All tests were set up on the same calendar day from a common blood agar plate containing pure cultures of the isolates. A total of 1,056 tests (96 isolates tested by 11 assays) were evaluated. *K. pneumoniae* ATCC 1705 and ATCC 1706 were included daily as positive and negative controls, except for the metallo-β-lactamase (MBL) Etest, where *Stenotrophomonas maltophilia* ATCC 13636 was included as the positive control.

The 11 phenotypic carbapenemase detection methods included represented a combination of commercially available tests as follows: (i) Rapidec Carba NP (bioMérieux, Marcy-l'Étoile, France; package insert version 98024-04/03/2016); (ii) Neo-Rapid Carb Screen kit (Rosco Diagnostica, Taastrup, Denmark; package insert version DBV0040L-05/01/2017); and (iii) Rapid Carb Blue Screen (Rosco Diagnostica, Taastrup, Denmark; package insert version DBV0-04/09/2014). The manual rapid phenotypic tests were as follows: (iv) Manual Carba NP CLSI method (18); (v) Manual Blue Carba (21); and (vi) Modified Carba NP (19). The growth-based methods were as follows: (vii) boronic acid synergy test using 10-μg

ertapenem disks (22); (viii) metallo-β-lactamase (MBL) Etest containing imipenem (IP) and imipenem/EDTA (IPI) (bioMérieux; package insert 16248 2010/11); (ix) modified Hodge test (MHT) (18); (x) the carbapenem inactivation method (CIM; set up with a 10 μl loopful of CRNF) (23); and (xi) the modified carbapenem inactivation method (mCIM; set up with a 10-μl loopful of CRNF as described in the CLSI January 2017 AST Subcommittee meeting minutes [http://clsi.org/standards/micro/microbiology-files/]) (4, 18). All methods were performed as previously described or according to the instructions in the package insert for commercially available assays. For consistency, imipenem-based solutions for use with the manual rapid colorimetric assays were made on the same day of testing to prevent imipenem hydrolysis from prolonged storage. The Rapidec Carba NP assay has been cleared by the U.S. Food and Drug Administration for use with *P. aeruginosa*. The Neo-Rapid Carb Screen kit, Rapid Carb Blue Screen test, and MBL Etest are research-use-only tests. The Manual Carba NP CLSI method, the Manual Blue Carba assay, the Modified Carba NP assay, the boronic acid synergy test, the modified Hodge test, CIM, and mCIM are laboratory-developed tests.

The investigators performing the phenotypic testing were blind to the genus, species, and genotype of the isolates. The sensitivity and specificity of each test were identified, with the molecular genotype serving as the reference method. Carbapenem-resistant glucose-nonfermenting isolates that were not carbapenemase producers were used to assess the specificity of the assays. For isolates with invalid (manual Carba NP) results or indeterminate (mCIM) results, testing was repeated. If results were still invalid or indeterminate, isolates were considered negative for carbapenemase production.

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