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Comparison of the Carba NP, Modified Carba NP, and Updated Rosco Neo-Rapid Carb Kit Tests for Carbapenemase Detection

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The accurate detection of carbapenemase-producing organisms is a major challenge for clinical laboratories. The Carba NP test is highly accurate but inconvenient, as it requires frequent preparation of fresh imipenem solution. The current study was designed to compare the Carba NP test to two alternative tests for accuracy and convenience. These were a modified Carba NP test that utilized intravenous (i.v.) imipenem-cilastatin, which is less expensive than reference standard imipenem powder, and an updated version of the Rosco Neo-Rapid Carb kit, which does not require the preparation of imipenem solution and has a shelf life of 2 years. The comparison included 87 isolates that produced class A carbapenemases (including KPC-2, -3, -4, -5, -6, and -8, NMC-A, and SME type), 40 isolates that produced metallo-β-lactamases (including NDM-1, GIM-1, SPM-1, IMP-1, -2, -7, -8, -18, and -27, and VIM-1, -2, and -7), 11 isolates that produced OXA-48, and one isolate that produced OXA-181. Negative controls consisted of 50 isolates that produced extended-spectrum β-lactamases (ESBLs), AmpCs (including hyperproducers), K1, other limited-spectrum β-lactamases, and porin and efflux mutants. Each test exhibited 100% specificity and high sensitivity (Carba NP, 100%; Rosco, 99% using modified interpretation guidelines; and modified Carba NP, 96%). A modified approach to interpretation of the Rosco test was necessary to achieve the sensitivity of 99%. If the accuracy of the modified interpretation is confirmed, the Rosco test is an accurate and more convenient alternative to the Carba NP test.

The accurate detection of carbapenemase-producing organisms (CPOs) is a major challenge for clinical laboratories. In some laboratories, detection of carbapenem-resistant *Enterobacteriaceae* (CRE) is the primary focus, and the need to detect carbapenemase production is considered optional and for epidemiologic purposes only (1). CRE detection is based on detection of resistance or nonsusceptibility of *Enterobacteriaceae* to carbapenems and certain cephalosporins and does not distinguish between carbapenemase producers and non-carbapenemase producers. Although carbapenem-resistant non-carbapenemase producers are important, they should not trigger the same level of concern as CPOs such as NDM- and other carbapenemase-producing isolates (2–6). Furthermore, the focus on CRE detection ignores carbapenemase producers that either are not *Enterobacteriaceae* or are carbapenemase-producing *Enterobacteriaceae* that are carbapenem susceptible, such as the VIM-producing *Klebsiella pneumoniae* isolates with imipenem MICs as low as 0.12 μg/ml that were involved in a large outbreak with high mortality in Greece (7).

Clinical laboratories aiming to detect carbapenemase producers need a test that is accurate and convenient. The Carba NP test is highly accurate (8, 9) but labor-intensive and inconvenient due to the instability of imipenem in solution, which necessitates extemporaneous preparation (1, 10). Another disadvantage of this test is the high cost of reference standard imipenem powder ($317.00 for 100 mg [catalog no. 1337809; Sigma-Aldrich, St. Louis, MO]). With the aim of identifying a less expensive or more convenient test with similar accuracy, a study was designed to investigate two alternative tests. These were a modified Carba NP test that utilized less expensive therapeutic intravenous (i.v.) imipenem-cilastatin (approximately $4.00 per 100 mg of imipenem) and the updated version 98024 of the Neo-Rapid Carb kit (Rosco Diagnostica A/S, Taastrup, Denmark).

MATERIALS AND METHODS

Isolates. Study isolates consisted of 189 *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* isolates that were previously characterized by molecular, phenotypic, and biochemical tests for types of β-lactamase production (11). Table 1 provides a breakdown of number of isolates of each species, β-lactamase types, and the laboratories that provided the isolates. In brief, 87 isolates produced class A carbapenemases that included KPC-2, KPC-3, KPC-4, KPC-5, KPC-6, KPC-8, NMC-A and SME enzymes, 40 isolates produced class B carbapenemases (metallo-β-lactamases) that included NDM-1, GIM-1, SPM-1, IMP-1, IMP-2, IMP-7, IMP-8, IMP-18, IMP-27, VIM-1, VIM-2, and VIM-7, and 12 isolates produced the class D carbapenemases OXA-48 ($n = 11$) and OXA-181($n = 1$). Non-carbapenemase-producing controls included 50 isolates that produced extended-spectrum β-lactamases (ESBLs), AmpCs (including hyperproducers), K1, limited-spectrum β-lactamases (non-ESBL, non-AmpC β-lactamases such as TEM-1), and porin and efflux mutants. The isolates studied included three quality control (QC) strains that are available to clinical laboratories: KPC-producing *Klebsiella pneumoniae* ATCC BAA 1705, NDM-producing *Escherichia coli* ATCC BAA 2452, and negative-control *K. pneumoniae* ATCC BAA 1706.

Carba NP test. We used the originally published procedure (8) and not the modified procedure recommended by CLSI (1). In brief, the procedure involved lysis of a heavy bacterial suspension in 100 μl of B-Per II

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TABLE 1 Summary of isolates and β-lactamase types

Carbapenemase	Species	β-Lactamase[a]	No. of isolates	Referring laboratory(ies)[b]
Class A				
KPC	*C. freundii*	KPC-3 like	1	A
	E. cloacae	KPC	12	A
		KPC-2 like	5	A
	Enterobacter sp.	KPC-2	1	A
	E. coli	KPC	2	A
		KPC-3 like	4	A
		KPC-3	1	A
	K. oxytoca	KPC	3	A
		KPC-4	1	A
	K. pneumoniae	KPC	12	A
		KPC-2	1	A
		KPC-2 like	21	A
		KPC-3	6	A
		KPC-3 like	6	A
		KPC-4	1	A
		KPC-6	1	A
		KPC-8	2	A
	P. aeruginosa	KPC	2	A
		KPC-5	2	A
NMC-A	*E. cloacae*	NMC-A	1	A
SME	*S. marcescens*	SME-type	2	A
Class B				A
GIM	*P. aeruginosa*	GIM-1	1	A
IMP	*E. cloacae*	IMP-2	1	A
	K. pneumoniae	IMP-8	1	A
	P. mirabilis	IMP-27	1	A
	S. marcescens	IMP-1	1	A
	P. aeruginosa	IMP-7	3	A
		IMP-18	1	A
NDM	*C. freundii*	NDM-1	2	B
	E. cloacae	NDM	2	B
	E. coli	NDM-1	3	B
	K. pneumoniae	NDM	1	B
		NDM-1	7	B
	P. rettgeri	NDM-1	1	B
	S. marcescens	NDM-1	1	B
SPM	*P. aeruginosa*	SPM-1	1	A
VIM	*A. baumannii*	VIM-2	1	A
	K. pneumoniae	VIM	1	A
		VIM-1	1	A
	P. aeruginosa	VIM	2	A
		VIM-2	7	A
		VIM-7	1	A
Class D				
OXA	*K. pneumoniae*	OXA-48	11	B, C
		OXA-181	1	B
None[c]	*C. freundii*		1	A
	C. koseri		1	A
	E. aerogenes		4	A
	E. cloacae		5	A
	E. coli		17	A
	K. pneumoniae		11	A
	K. oxytoca		4	A
	M. morganii		1	A
	P. mirabilis		1	A
	S. enterica		1	A
	P. aeruginosa		4	A

[a] "like" designates similarity to KPC-2 or KPC-3 as determined by melting point but sequencing not done. KPC, NDM, or VIM without a number designates PCR positivity for KPC, NDM, or VIM, respectively, but sequencing not done.
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[c] Non-carbapenemase-producing controls, included producers of ESBLs, AmpCs (including hyperproducers), K1, limited-spectrum β-lactamases (i.e., non-ESBL, non-AmpC β-lactamases such as TEM-1), and porin and efflux mutants.

extraction buffer (Thermo Scientific, Rockford, IL) followed by vortexing, incubation for 30 min at room temperature, and centrifugation for 5 min at 13,000 rpm, after which 30 μl of the supernatant was inoculated into 100 μl of a solution containing reference standard imipenem (Sigma-Aldrich, St. Louis, MO), phenol red, NaOH, and $ZnSO_4$ as per the published procedure (8). This reaction mixture was incubated in a test tube for up to 2 h at 35 to 37°C, with a positive result interpreted as a color change from red to yellow or orange.

Modified Carba NP test. The procedure described above was modified by utilizing i.v. imipenem-cilastatin (Hospira Inc., Lake Forrest, IL) obtained from the hospital pharmacy as the substrate. This was provided as 500 mg each of imipenem and cilastatin and 20 mg of $NaHCO_3$. Weight adjustment was performed to ensure that identical amounts of imipenem were used in both forms of the Carba NP test.

Rosco Neo-Rapid Carb test. This test is similar in principle to the Carba NP test but utilizes tablets containing imipenem plus an indicator and negative-control tablets. It does not require laboratory preparation of imipenem-containing solution from reference standard imipenem powder. The study used the 98024 version of the test, which is more sensitive than the previous 98021 version. The test procedure, provided in the manufacturer's update of 19 November 2014 (available from Key Scientific, Stamford, TX), consisted of adding several loopfuls of the test strain to a mixture of 100 μl 0.9% NaCl and 100 μl of B-Per II extraction buffer to produce a suspension of at least a McFarland standard 4. The suspension was vortexed and maintained at room temperature for 30 min, after which 50 μl was transferred to a tube containing 100 μl of 0.9% NaCl. A tablet containing imipenem plus indicator was added, and the tube closed and vortexed briefly (1 to 2 s) to disintegrate the tablet and then incubated at 35 to 37°C for 30 min, 1 h, and 2 h. This process was duplicated in parallel with a negative-control tablet substituted for the imipenem-containing test tablet. A positive result was interpreted as a color change from pink or red to yellow or orange/yellow, providing that the negative control remained pink or red. Positive results after 30 min or 1 h were interpreted at that time and not later to avoid falsely negative tests due to subsequent fading of the positive reaction. If results were difficult to interpret, resolution was attempted using the following modifications to the manufacturer's methodology: (i) holding the tube in vertical orientation above eye level and inspecting the bottom of the tablet for yellow color (interpreted as positive) and (ii) comparison of test and negative-control tubes by viewing them side by side, tilting gently to horizontal, and examining in bright light above a white background (Fig. 1). If the result remained unclear, the test was repeated with a higher inoculum and interpreted using the modifications described above.

RESULTS

All three test methods exhibited a high level of accuracy, with 96% or higher sensitivity and 100% specificity (Table 2). The Carba NP

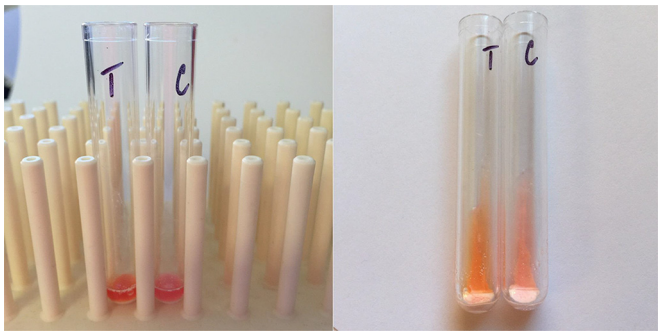

FIG 1 Improved interpretation by comparing difficult-to-interpret test (T) and negative-control (C) tubes in vertical position (left) with tubes in horizontal position above white background (right). Tube T is clearly positive (orange/yellow) in horizontal position but equivocal in vertical position.

TABLE 2 Sensitivity and specificity for isolates producing carbapenemases of classes A, B, and D and non-carbapenemase producers

Carbapenemases	No. of isolates	Carba NP		Modified Carba NP		Rosco	
		% sensitivity	% specificity	% sensitivity	% specificity	% sensitivity	% specificity
Class A (KPC, NMC-A, SME)	87	100	100	97	100	99	100
Class B (metallo-β-lactamase NDM, GIM, SPM, IMP, VIM)	40	100	100	95	100	98	100
Class D (OXA-48, OXA-181)	12	100	100	92	100	100	100
All producers	139	100	100	96	100	99	100

test was positive for all 139 carbapenemase-producing isolates and negative for all 50 non-carbapenemase producers (100% sensitivity and specificity).

On initial testing with the Rosco test, eight isolates yielded results that could not be resolved, to yield an overall sensitivity of 93%, with individual sensitivities for each carbapenemase class as follows: class A carbapenemases, 85/87 (98%); class B carbapenemases, 33/40 (83%); and OXA carbapenemases (class D), 11/12 (92%). After reviewing the reference results for the eight problematic isolates, it was decided to retest them using the modified test interpretation approach. This yielded a final set of positive results for all but two carbapenemase producers, and the Rosco test then exhibited 99% sensitivity and 100% specificity. Two falsely negative results occurred with a KPC-4-producing *K. pneumoniae* isolate and a *Proteus mirabilis* isolate that produced IMP-27. The eight isolates which required modified interpretation produced NDM-1 ($n = 6$), KPC-like ($n = 1$), and OXA-48 ($n = 1$) carbapenemases.

The modified Carba NP test was the least sensitive of the three tests but still exhibited high accuracy, with 96% sensitivity and 100% specificity. Falsely negative results occurred with two KPC-4-producing *K. pneumoniae* isolates, an SME-like-carbapenemase-producing *Serratia marcescens* isolate, and one isolate each of OXA-48-producing *K. pneumoniae*, NDM-producing *Providencia rettgeri*, and IMP-27-producing *P. mirabilis*. Each of these six isolates had either an imipenem or meropenem MIC in the range 2 to >128 μg/ml.

DISCUSSION

The high level of accuracy of the three tests was notable because the study included many carbapenemase producers that may not be detected by clinical laboratories that use insensitive carbapenemase detection tests or focus only on detection of carbapenem-resistant *Enterobacteriaceae*, which excludes carbapenemase-producing *Pseudomonas* spp., *Acinetobacter* spp., and carbapenemase-producing *Enterobacteriaceae* that are carbapenem susceptible. These isolates were OXA-48-producing *K. pneumoniae*, KPC-4-producing *K. pneumoniae*, KPC-5-producing *Pseudomonas aeruginosa*, NDM-1-producing *Providencia rettgeri*, IMP-7-producing *P. aeruginosa*, IMP-18-producing *P. aeruginosa*, IMP-27-producing *Proteus mirabilis*, VIM-2-producing *P. aeruginosa* and *Acinetobacter baumannii*, VIM-7-producing *P. aeruginosa*, and SPM-1-producing *P. aeruginosa*. For microbiology laboratories to achieve maximum effectiveness in combating the challenge of increasing Gram-negative resistance, it is essential that they aim to detect the correct diagnostic target, carbapenemase-producing organisms (CPOs), and not focus solely on CRE. For this purpose, it is essential to use a carbapenemase test that has high accuracy.

The high level of accuracy of the Carba NP test in this study was consistent with previous reports (8, 9, 12–15). The achievement of almost comparable sensitivity with the Rosco test was due to enhancing the clarity of test interpretation with a modified approach to reading what were otherwise uninterpretable tests.

Other evaluations of the Rosco Neo-Rapid Carb kit have reported the occurrence of uninterpretable results. These reports cannot be directly compared to the current study because they tested different strains and either used the less sensitive 98021 version of the test or did not indicate which test version was used. In a study involving 150 isolates, of which 49 produced various types of carbapenemases, Simner et al. attributed the achievement of overall sensitivity of 98.0% and specificity of 100% to two modifications: use of a 50% lower-than-recommended inoculum and performing the test in a tube with a rounded rather than a conical bottom to effect complete rather than partial dissolution of the tablets (16). While the current study also used round-bottom tubes, the value of a reduced inoculum density to detect carbapenemase activity warrants caution, as it would reduce the number of carbapenemase molecules in the test and hence the amount of carbapenemase activity and the sensitivity of the test. This might offset the value of the lower inoculum producing increased clarity of the test solution. By omitting uninterpretable results from their analysis of 135 *Enterobacteriaceae* and 35 *P. aeruginosa* isolates, 66 of which produced various types of carbapenemases, Huang et al. reported 98.0% sensitivity and 83% specificity for *Enterobacteriaceae* and 96% sensitivity and 54% specificity for *P. aeruginosa* (17). Yusuf et al. reported a study with the less sensitive 98021 test version that included 92 *Enterobacteriaceae* and 19 *P. aeruginosa* isolates, 51 of which were carbapenemase producers. They obtained 100% specificity but lower sensitivity (73.3% for *Enterobacteriaceae* and 66.7% for *P. aeruginosa*) than reported for the 98024 version (18).

The modified Carba NP test was the least sensitive test in the study but might be made more sensitive by modifications such as adjustments to the inoculum density and/or imipenem concentration or the substitution of intramuscular (i.m.) imipenem-cilastatin for i.v. imipenem-cilastatin. The utilization of i.m. imipenem/cilastatin would eliminate any buffering effect due to $NaHCO_3$ retarding the rate of pH change; however, this formulation was unavailable during the time of the study. Even if increased sensitivity could be achieved, it would not overcome the inconvenience of having to prepare imipenem solution extemporaneously.

Several observations relevant to carbapenemase detection arose in this study. The lack of false-positive results was notable because the study included high-level AmpC-producing isolates, which are notorious for producing false-positive results with many phenotypic carbapenemase detection tests. This finding raises the possibility that

higher-than-recommended inocula might be employed to investigate difficult-to-interpret results. Also relevant to inoculum density is that the amount of β-lactamase activity is a more critical determinant of test sensitivity than the number of cells tested. Therefore, standardization of inoculum density does not guarantee optimal test performance. To ensure that a threshold level of detectable carbapenemase activity is achieved, it is best to test the highest inoculum density that will not generate a false-positive result.

In conclusion, both the Rosco and modified Carba NP tests offered greater suitability for the needs of busy clinical laboratories in terms of lower cost or greater convenience than the Carba NP test. If the accuracy of the modified interpretation procedures of the Rosco test is confirmed, its greater convenience and reagent stability indicate that it is a test with the attributes required for phenotypic carbapenemase detection in routine clinical laboratories.

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