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Performance of the CLSI Carba NP and the Rosco Carb Screen Assays Using North American Carbapenemase-Producing *Enterobacteriaceae* and *Pseudomonas aeruginosa* Isolates

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This study compared the performance of the Carba NP assay, published by the Clinical and Laboratory Standards Institute, and the Rosco Rapid Carb Screen kit. Carba NP had superior sensitivity, but both assays required an increased inoculum to detect carbapenemase production in isolates with *bla*$_{NDM}$, *bla*$_{IMP}$, and *bla*$_{OXA-48}$.

Rapid detection of carbapenemase production in multidrug-resistant organisms may help to mitigate institutional outbreaks by expediting initiation of infection control procedures (1). Nordmann et al. first described the Carba NP assay and reported sensitivity and specificity of 100% (2). Carbapenemase activity is detected using a pH indicator (phenol red) that changes color with the hydrolysis of the β-lactam ring of imipenem. Dortet et al. (3) published a modified procedure in 2014. The bacterial inoculum was reduced, testing was streamlined using whole lysed bacterial cells instead of supernatant, and the concentration of imipenem was raised from 3 to 6 mg/ml (3). Reported sensitivity and specificity were 100% (3). In 2015, based on the work of Vasoo et al. (4), the Clinical and Laboratory Standards Institute (CLSI) published a similar Carba NP procedure using a bacterial inoculum that was further reduced to 1 μl (5).

Carba NP is labor-intensive. Reagents need to be prepared in-house, and some have shelf lives as short as 72 h. In contrast, the Rapid Carb Screen kit (98021; Rosco Diagnostica A/S, Taastrup, Denmark) provides tablets containing a pH indicator, with and without imipenem. No reagent preparation is required, and the shelf life is >12 months. In this study, we evaluated the performance of the Carba NP assay (CNP) as published by CLSI and the Rapid Carb Screen (RCS).

In total, 49 organisms were tested. These included 39 isolates that were previously characterized as carrying *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, and *bla*$_{OXA-48}$. Real-time PCR was performed to detect *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ as described elsewhere (6, 7). *bla*$_{IMP}$, and *bla*$_{OXA-48}$ were detected using conventional PCR with primers as follows: for *bla*$_{IMP}$, 5′-GTTTATGT TCATACWTCG-3′ (forward) and 5′-GGTTTAAYAAAACAA CCAC-3′ (reverse); for *bla*$_{OXA-48}$, 5′-ATGCGTGTATTAGCCT TATCGGCTG-3′ (forward) and 5′-CTAGGGAATAATTTTTT CCTGTTTG-3′ (reverse). In addition, we included 10 isolates that tested negative for all targets. Imipenem, meropenem, and ertapenem MICs were produced using GN4F Sensititre Gram-negative MIC plates (Thermo Fisher Scientific, Oakwood Village, OH) and interpreted using CLSI interpretive breakpoints (5). Organisms were identified to the species level using the Vitek 2 system (bioMérieux, Durham, NC). Prior to testing, isolates were subcultured and incubated twice in ambient air at 37°C on Trypticase soy agar with 5% sheep's blood (Remel, Lenexa, KS). All testing runs included positive (ATCC BAA-1705) and negative (ATCC BAA-1706) controls. CNP also had a reagent-only control.

CNP was performed strictly as stated by CLSI (3). Briefly, solution A with 0.5% phenol red solution (made with phenol red indicator powder; Fisher Science Education, Nazareth, PA) and 10 mM zinc sulfate heptahydrate solution (made with zinc sulfate heptahydrate powder; Fisher Scientific, Fair Lawn, NJ) was prepared. On the day of testing, solution B was prepared, consisting of solution A with 6 mg/ml of imipenem monohydrate (USP, Rockville, MD). A single 1-μl loop of bacteria was inoculated into a 1.5-ml microcentrifuge tube (Sarstedt Inc., Newton, NC) with 100 μl of KPEX bacterial protein extraction reagent (Key Scientific Products, Stamford, TX) and vortexed for 5 s. KPEX is the equivalent of B-PER II, referenced in early Carba NP studies (1, 2, 7). Solution A (100 μl) was added, and the tube was vortexed again. The procedure was then repeated with 100 μl of solution B. Reaction mixtures were incubated in ambient air at 35 to 37°C and examined at 30 min, 1 h, and 2 h. CNP was performed on all isolates with inocula of 1 μl, 3 μl, and 5 μl.

Rapid Carb Screen kits were obtained through Key Scientific

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Products, Stamford, TX. RCS was performed according to the manufacturer's instructions with a modification in the lysis step. The manufacturer states that an organism suspension of at least 4 McFarland standard needs to be prepared in a 100-μl fluid volume. Because both visual and spectrophotometric determination of organism density in such a small volume was not feasible, we pursued an alternative approach using 1-μl loops. One, two, three, four, and five loops of *Klebsiella pneumoniae* BAA 1705, the recommended positive control, were inoculated into tubes with 100 μl of KPEX. As the positive-control isolate tested positive with all 5 inocula, the lowest inoculum (i.e., one 1-μl loop) was chosen as a starting point. To ensure that the bacterial concentration was sufficient compared to the 4 McFarland recommended by the manufacturer, we simulated the 1-μl inoculum by adding 30 μl of *Escherichia coli* (ATCC 35218) to 3 ml of sterile saline. The suspension was vortexed. According to the DensiCHEK instrument (bioMérieux, Durham, NC), the density was >4 McFarland, indicating that the density of the lowest inoculum of 1 μl was greater that the recommended 4 McFarland. Bacterial suspensions in KPEX were vortexed for 60 s and incubated at room temperature for 30 min. A 50-μl portion of this suspension was then added to 100 μl of sterile saline. A negative-control tablet was added. The tube was vortexed for 1 to 2 s to disintegrate the tablet. The process was then repeated with a tablet containing imipenem plus indicator. Reaction mixtures were incubated in ambient air at 35 to 37°C and examined at 30 min, 1 h, and 2 h. RCS was performed on all isolates using inocula of 1 μl, 3 μl, and 5 μl.

Two blinded laboratory staff read all reaction results independently. For CNP, the readers assigned one of 6 colors (red, red-orange, orange, light orange, dark yellow, and yellow) to each reaction mixture using the color figure provided by CLSI (5). A color change of red to light orange, dark yellow, or yellow was considered a positive reaction. Orange was interpreted as indeterminate. Reaction mixtures that remained red or red-orange were negative. Tests where the negative control was any color but red or red-orange were deemed invalid (5). For RCS, a color change of red to yellow was interpreted as positive. Reaction mixtures with an orange color were considered indeterminate. Reactions where tubes remained red were interpreted as negative. A reaction where the negative-control tube was any color besides red was deemed invalid. We observed that positive (yellow) RCS reaction mixtures could revert to a red or red-orange color if the reaction was allowed to continue. RCS reactions therefore had to be read at all of the recommended time points (30 min, 1 h, and 2 h) to ensure that a positive reaction was not missed. We did not observe this phenomenon with CNP. For study purposes, the strongest positive RCS result was used for analysis.

Results are summarized in Table 1. All positive and negative isolate controls were appropriately positive and negative, respectively, for both assays. For CNP, all reagent-only controls were negative. Finally, all negative-control tubes (with no imipenem) were appropriately negative. All reactions involving non-carbapenemase producers ($n = 10$) tested negative by both assays.

With a bacterial inoculum of 1 μl, CNP tested false negative in 7/39 (18%) carbapenemase-producing isolates. These included 2 of 14 (14%) isolates with bla_{NDM}, 1 of 1 with bla_{IMP}, and 4 of 4 with bla_{OXA-48}. At this inoculum, there were no indeterminate results. All 20 isolates with bla_{KPC} and bla_{VIM} tested positive. With 3 μl, 5 of these 7 isolates tested indeterminate, one tested positive, and an isolate with OXA-48 (Jefferson-73) tested negative again. With 5 μl, all 39 carbapenemase-producing isolates tested positive with the exception of an NDM-producing isolate (CHLA-1; indeterminate) and the Jefferson-73 isolate (negative).

With a bacterial inoculum of 1 μl, 26/39 (67%) carbapenemase-producing isolates tested indeterminate or negative with RCS. These included 4/13 (31%) isolates with KPC (all indeterminate), 13/14 (93%) isolates with NDM (11 indeterminate and 2 negative), 4/7 (57%) isolates with VIM (2 indeterminate and 2 negative), 1/1 isolate with IMP (negative), and 4/4 isolates with OXA-48 (all negative). With 3 μl, 25 of these 26 isolates (96%) tested either indeterminate or positive. Jefferson-73 remained negative. With 5 μl, 6/39 (15%) isolates still tested indeterminate (2 NDM-, 1 VIM-, 1 IMP-, and 2 OXA-48-producing isolates), but there were no false-negative results.

There were two discrepant test reads. With CNP and 3 μl of organism, the Jefferson-74 (IMP) reaction was read as red by one reader and red-orange by the other. This did not impact the interpretation. However, with RCS and 5 μl of organism, the Jefferson-73 (OXA-48) reaction was read as red (negative) by one reader and orange (indeterminate) by the other.

At various study sites, in addition to isolates with bla_{KPC}, clinical isolates harboring bla_{NDM} and bla_{VIM} have been recovered from patients who have traveled to the Indian subcontinent and Europe, respectively. With rapidly expanding international medicine programs and diverse ethnic patient population bases, these cases have underscored the importance of supporting infection prevention measures that are designed to avert catastrophic institutional outbreaks involving multidrug-resistant organisms. Rapid detection of carbapenemase activity in suspicious isolates is an essential part of this endeavor.

Among bacterial isolates without molecular evidence of carbapenemase carriage, all CNP and RCS testing results were appropriately negative, including those performed with inocula of 3 μl and 5 μl. This is consistent with published specificity data in the literature (2–4, 8).

Using the current CLSI CNP procedure on 120 previously characterized isolates harboring bla_{KPC}, bla_{NDM}, bla_{VIM}, and bla_{SME}, Vasoo et al. (4) reported sensitivity and specificity of 100%. They noted, however, that only 9 isolates were non-KPC. They also reported an isolate of *P. aeruginosa* with bla_{VIM} that tested orange, which would be interpreted by the published CLSI procedure as indeterminate. In our study, we studied a larger number of isolates producing non-KPC carbapenemases. Using the recommended 1-μl inoculum, CNP was tested with isolates harboring bla_{OXA-48} ($n = 4$), bla_{NDM} ($n = 2$), and bla_{IMP} ($n = 1$). Six of the 7 isolates had imipenem, meropenem, and ertapenem MICs that were interpreted as intermediate or resistant by M100-S25 interpretive criteria. In contrast, others have reported a particular propensity for isolates with low carbapenem MICs to test false negative by Carba NP (8). Also, Tijet et al. reported that increasing the bacterial inoculum yielded positive CNP results in such isolates, particularly in those harboring bla_{OXA-48} (8). We also observed this phenomenon in our study in 3 isolates with bla_{OXA-48}. If detection of carbapenemases other than KPC is epidemiologically important in a given setting, raising the bacterial inoculum to at least 5 μl (which is closer to the inoculum used in the study by Dortet et al. [3]) may reduce false-negative results with CNP. We attributed the poor performance of CNP with OXA-48 isolates to weak catalytic activity (9). The false-negative

TABLE 1 Carba NP and Rapid CARB Screen test results for carbapenemase-producing *Enterobacteriaceae* and *P. aeruginosa*[a]

Isolate (*n*)	MIC (μg/ml) by BMD			Result of: Rapid Carb Screen			Carba NP		
	IPM	MPM	EPM	1 μl	3 μl	5 μl	1 μl	3 μl	5 μl
KPC producing (13)									
ARUP-1 (*K. pneumoniae*)	8	>8	>8	+	+	+	+	+	+
ARUP-2 (*K. pneumoniae*)	>8	>8	>8	Ind	+	+	+	+	+
ARUP-3 (*K. pneumoniae*)	8	>8	>8	+	+	+	+	+	+
ARUP-4 (*K. pneumoniae*)	>8	>8	>8	+	+	+	+	+	+
ARUP-5 (*E. coli*)	4	8	>8	+	+	+	+	+	+
ARUP-6 (*K. pneumoniae*)	8	>8	>8	+	+	+	+	+	+
ARUP-7 (*K. pneumoniae*)	8	>8	>8	+	+	+	+	+	+
ARUP-8 (*K. pneumoniae*)	4	8	>8	Ind	+	+	+	+	+
ARUP-9 (*K. pneumoniae*)	4	4	8	Ind	+	+	+	+	+
ARUP-10 (*K. pneumoniae*)	4	8	8	+	+	+	+	+	+
ARUP-11 (*K. pneumoniae*)	>8	>8	>8	Ind	+	+	+	+	+
ARUP-12 (*K. pneumoniae*)	4	4	8	+	+	+	+	+	+
ATCC BAA 1705 (*K. pneumoniae*)	8	>8	>8	+	+	+	+	+	+
NDM producing (14)									
ATCC BAA-2471 (*E. coli*)	>8	>8	>8	Ind	+	+	+	+	+
ATCC BAA-2146 (*K. pneumoniae*)	>8	>8	>8	Ind	Ind	+	+	+	+
ATCC BAA-2469 (*E. coli*)	8	>8	>8	Ind	Ind	+	+	+	+
ATCC BAA-2472 (*K. pneumoniae*)	>8	>8	>8	Ind	+	+	+	+	+
ATCC BAA-2473 (*K. pneumoniae*)	>8	>8	>8	Ind	+	+	+	+	+
ATCC BAA-2452 (*E. coli*)	4	8	>8	−	Ind	+	+	+	+
CHOP-1 (*K. pneumoniae*)	>8	>8	>8	Ind	+	+	+	+	+
CHLA-1 (130541189) (*K. pneumoniae*)	4	8	>8	Ind	Ind	+	−	Ind	Ind
CHLA-2 (121260484) (*E. coli*)	>8	>8	>8	−	Ind	Ind	+	+	+
CHLA-3 (130340513) (*K. pneumoniae*)	4	8	>8	Ind	Ind	Ind	-	+	+
ARUP-B1279 (*K. pneumoniae*)	8	>8	>8	+	+	+	+	+	+
MAYO- EC (*E. coli*)	8	>8	>8	Ind	Ind	+	+	+	+
MAYO-2 (*K. pneumoniae*)	>8	>8	>8	Ind	+	+	+	+	+
Jefferson-45 (*K. pneumoniae*)	>8	>8	>8	Ind	Ind	+	+	+	+
VIM producing (7)									
CHOP-2 (*P. aeruginosa*)	>8	8	-	+	+	+	+	+	+
K. pneumoniae (CDC 1002235)	>8	>8	>8	-	+	+	+	+	+
P. aeruginosa (CDC 1200559)	>8	>8	>8	−	Ind	Ind	+	+	+
E. cloacae (CDC 1301491)	4	1	0.5	Ind	+	+	+	+	+
K. pneumoniae (CDC 1301489)	4	4	2	+	+	+	+	+	+
Jefferson-77 (*P. aeruginosa*)	>8	>8	-	Ind	+	+	+	+	+
Jefferson-78 (*P. aeruginosa*)	>8	>8	-	+	+	+	+	+	+
IMP producing (1)									
Jefferson-74 (*K. pneumoniae*)	<0.5	<0.5	<0.25	−	Ind	Ind	−	Ind	+
OXA-48 producing (4)									
Jefferson-72 (*K. pneumoniae*)	2	2	4	−	Ind	+	−	Ind	+
Jefferson-75 (*E. coli*)	2	4	8	−	Ind	Ind	−	Ind	+
Jefferson-76 (*K. pneumoniae*)	2	2	4	−	Ind	+	−	Ind	+
Jefferson-73 (*K. pneumoniae*)	4	>8	>8	−	−	Ind	−	−	−

[a] BMD, broth microdilution using Trek panel GN4F; IPM, imipenem; MPM, meropenem; EPM, ertapenem; Ind, indeterminate.

results for the isolates with bla_{NDM} and bla_{IMP} may also be related to insufficient zinc content in the subculture medium or CNP reagents (3, 8).

RCS had lower sensitivity and produced more indeterminate results than CNP. With 5 μl of organism, 6 carbapenemase producers still tested indeterminate by RCS (2 NDM producers, 1 VIM producer, 1 IMP producer, and 2 OXA-48 producers), but there were no false negatives. Huang et al. tested 66 carbapenemase-producing *Enterobacteriaceae* and *P. aeruginosa* isolates and reported positive results for 58/66 isolates (10). False-negative results included isolates harboring bla_{OXA-48} ($n = 1$), $bla_{OXA-198}$ ($n = 1$), bla_{NDM} ($n = 3$), bla_{IMP} ($n = 2$), and bla_{GES-18} ($n = 1$). Of note, the manufacturer recommends interpreting orange reactions as indeterminate, but the authors analyzed both yellow and orange reactions as strongly positive. Therefore, the reported proportion of carbapenemase-producing isolates with true-positive

results may have been somewhat inflated. Also, the negative-control tube of test pairs was positive in 6/66 isolates (9%), rendering a significant portion of the tests invalid.

A confirmatory test could be useful for isolates with indeterminate results. DNA PCR and microarrays are commercially available, but they can be labor-intensive and expensive and may have slow turnaround compared to CNP and RCS (11). Adding to the challenge is that there are currently no FDA-cleared molecular assays that are designed to detect carbapenemase determinants from bacterial colonies on the U.S. market. The modified Hodge test has slow turnaround, and false-negative results have been reported with isolates producing metallo-β-lactamases (4, 12). The use of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for detection of carbapenemase activity is also an emerging platform with some promise (13).

Only 1 of the 294 test reactions (0.3%) yielded an interpretive discrepancy between the two blinded readers. This corroborates the observations of others (4). The color figure provided by CLSI appears to be an effective tool for reaction color assignment when CNP is read (5). The manufacturer's instructions for interpreting RCS were simpler, with only three options (red, orange, and yellow).

With regard to end-user experience, CNP was predictably more labor-intensive due to the reagent preparation requirements and the 72-hour shelf life of solution B. With experience, we still required at least 30 min to prepare reagents. Five to 10 min of hands-on time was required to test a single clinical isolate with controls. Also, CLSI recommends weighing out a minimum of 10 mg of imipenem to minimize error. This requires preparation of at least 16 tubes of solution B every time CNP testing is performed, which may be wasteful in low-incidence settings. Finally, the CLSI procedure does not specify which bacterial protein extraction reagent to use. The KPEX bacterial protein extraction reagent worked well in our evaluation (2, 3). On the other hand, the RCS procedure was simpler and faster, requiring about 5 min of hands-on time per isolate. However, the tablets were difficult to emulsify, and if tubes were disturbed during incubation or reading, the white, chalky tablet material could cloud the tube, lightening the reaction color and rendering it difficult to read.

In summary, the Carba NP procedure published by CLSI had sensitivity superior to that of the Rosco Rapid Carb Screen assay at similar organism inocula, but both required at least 5 μl of organism to improve sensitivity in isolates with bla_{NDM}, bla_{IMP}, and bla_{OXA-48}. CNP was more labor-intensive during test setup, but RCS required more attention during incubation and reading.

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