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# Comparative Evaluation of Two Chromogenic Tests for Rapid Detection of Carbapenemase in *Enterobacteriaceae* and in *Pseudomonas aeruginosa* Isolates

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**We compared the performance of the Carba NP test and the Rosco Rapid CARB screen kit for detecting carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Both tests are rapid and highly sensitive; however, the Carba NP test showed superior specificity, and several uninterpretable results were observed with the Rapid CARB screen.**

The rapid detection of carbapenemase in *Enterobacteriaceae* and in *Pseudomonas aeruginosa* is essential for early appropriate therapeutic management and infection control purposes (1). The Carba NP test was recently proposed as a cheap and easy-to-perform imipenem hydrolysis-based test with high accuracy (i.e., sensitivity and specificity) for detecting carbapenemase-producing *Enterobacteriaceae* (CPE) and *P. aeruginosa* (2, 3). However, the Carba NP test in its current format is an in-house technique requiring the purchase of several reagents and a homemade preparation of the test solutions (including the addition of imipenem). We evaluated here the ability of two imipenem hydrolysis-based rapid tests, the Carba NP test (CNP) and the commercially available Rosco Rapid CARB screen kit (RCS) (Rosco Diagnostica A/S, Taastrup, Denmark), to detect CPE and carbapenemase-producing *P. aeruginosa* (CPPA).

A total of 135 well-characterized *Enterobacteriaceae* ($n = 100$) and *P. aeruginosa* ($n = 35$) collection strains isolated from various clinical samples (66 carbapenemase producers and 69 isolates expressing other representative resistance mechanisms to β-lactams) were tested. Additionally, all nonduplicate consecutive clinical isolates referred to the national reference center (NRC) from January to June 2013 for suspected carbapenemase production were included. Using their routine testing methods and interpretative guidelines, local laboratories were requested to send all nonduplicate *Enterobacteriaceae* isolates showing decreased susceptibility to at least one carbapenem (ertapenem or meropenem) and *P. aeruginosa* isolates fulfilling all three of the following criteria: decreased susceptibility to at least one carbapenem (imipenem or meropenem), resistance to ceftazidime and/or cefepime, and resistance to at least one aminoglycoside (amikacin, tobramycin, or gentamicin).

All tested isolates were subcultured twice and tested for imipenem hydrolysis by the CNP test, as previously described (2), and by the RCS, according to the manufacturer's instructions, using the same culture grown freshly on a nonselective blood agar plate (4). Briefly, the RCS was performed as follows: two 10-μl calibrated full loops of bacterial strain were incubated in a Tris-HCl-20 mmol/liter lysis buffer (Bacterial protein extraction reagent [B-PERII]; Thermo Scientific, Rockford, IL, USA) at room temperature for 30 min. Fifty microliters of the suspension was resuspended in 100 μl saline solution in two tubes, in which one RCS test tablet and one negative-control tablet were added. The test tubes were incubated at 37°C for up to 2 h. Both tests were read after 30, 60, and 120 min of incubation. Any color change observed by the naked eye from red to yellow in a vial (for the CNP test) or in a tube (for the RCS) was considered to be a positive reaction (pink, orange, or yellow). The CNP test results were interpreted according to the guidelines by Nordmann et al. (2). The RCS results were interpreted according to the manufacturer's instructions (5). The results of the CNP and RCS tests were considered negative if the respective test vial/tube gave a negative reaction, positive if the test vial/tube gave a positive reaction and the control vial/tube gave a negative reaction (red), and uninterpretable if the control vial/tube gave a positive reaction. All isolates had been verified for the presence of carbapenemase by an in-house ISO 15189-validated multiplex PCR targeting $bla_{VIM}$, $bla_{IMP}$, $bla_{NDM}$, $bla_{KPC}$, and $bla_{OXA-48}$ (6). The *P. aeruginosa* strains were additionally tested by two other in-house multiplex PCRs targeting $bla_{GES}$ (7) and $bla_{OXA-198}$ (8).

The CNP and RCS test results for carbapenemase-producing and carbapenemase-negative *Enterobacteriaceae* and *P. aeruginosa* collection strains are detailed in Tables 1 and 2. The large majority of the characterized CPE collection strains yielded strong-positive results (orange or yellow) by the CNP (59/66) or RCS (54/66) test. Two OXA-48 CPE and one IMP-13 CPPA gave a weak-positive result (pink) by the CNP test. The CNP test missed two IMP-13 strains, one GES-18 strain, and one OXA-198 CPPA strain that showed a weak-positive result by the RCS. Two OXA-48-producing *Enterobacteriaceae* strains had uninterpretable results with the RCS. Among the 69 characterized carbapenemase-negative strains, all had negative results with the CNP test. On the other hand, 7 *Enterobacteriaceae* and 10 *P. aeruginosa* carbapenemase-negative strains yielded inconclusive ($n = 6$) or weak false-positive results ($n = 11$) by the RCS.

During the study period, a total of 356 consecutive *Enterobacteriaceae* ($n = 135$) and *P. aeruginosa* ($n = 221$) clinical isolates

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**TABLE 1** Carba NP test and Rapid CARB screen kit results for carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa* collection strains ($n = 66$)

| Group/species ($n$) | Ambler class | Carbapenemase enzyme(s) | Total no. | No. with CNP result: | | No. with RCS result of[a]: | |
|---|---|---|---|---|---|---|---|
| | | | | Positive (no. weak positive) | Negative | Positive (no. weak positive) | Uninterpretable |
| *Enterobacteriaceae* (44) | | | | | | | |
| *K. pneumoniae* | A | KPC-2/KPC-3 | 10 | 10 | | 10 | |
| | B | NDM-1 | 3 | 3 | | 3 (2)[a] | |
| | | VIM-1 | 1 | 1 | | 1 | |
| | | VIM-27 | 1 | 1 | | 1 | |
| | D | OXA-48 | 7 | 7 (1) | | 6 (2) | 1 |
| *Enterobacter cloacae* | B | VIM-1 | 2 | 2 | | 2 | |
| | | VIM-31 | 1 | 1 | | 1 | |
| | | VIM-4 | 1 | 1 | | 1 | |
| | | NDM-1 | 1 | 1 | | 1 | |
| | D | OXA-48 | 3 | 3 | | 2 | 1 |
| *E. coli* | B | NDM-1 | 2 | 2 | | 2 (1) | |
| | D | OXA-48 | 2 | 2 (1) | | 2 (1) | |
| *Klebsiella oxytoca* | B | VIM-1 | 2 | 2 | | 2 | |
| | D | OXA-48 | 1 | 1 | | 1 | |
| *Serratia marcescens* | B | VIM-1 | 1 | 1 | | 1 | |
| | | VIM-4 | 1 | 1 | | 1 | |
| *Morganella morganii* | B | NDM-1 | 2 | 2 | | 2 | |
| *Citrobacter braakii* | B | VIM-1 | 1 | 1 | | 1 | |
| *Citrobacter freundii* | D | OXA-48 | 1 | 1 | | 1 | |
| *Providencia vermicola* | B | VIM-1 | 1 | 1 | | 1 | |
| *Pseudomonas aeruginosa* (22) | A | GES-5 | 1 | 1 | | 1 | |
| | | GES-18 | 1 | | 1 | 1 (1) | |
| | | KPC-2 | 1 | 1 | | 1 | |
| | B | VIM-2 | 5 | 5 | | 5 | |
| | | VIM-4 | 2 | 2 | | 2 | |
| | | IMP-7 | 5 | 5 | | 5 | |
| | | IMP-13 | 3 | 1 (1) | 2 | 3 (2) | |
| | | SPM-1 | 1 | 1 | | 1 | |
| | | GIM-1 | 1 | 1 | | 1 | |
| | | NDM-1 | 1 | 1 | | 1 | |
| | D | OXA-198 | 1 | | 1 | 1 (1) | |

[a] No negative results were reported with RCS.

were referred by 66 laboratories throughout Belgium to the reference laboratory for suspected carbapenemase production, and the results of the CNP and RCS tests for all isolates are detailed in Table 3. Seventy-two of the 135 (53%) *Enterobacteriaceae* isolates and 55 of the 221 (25%) *P. aeruginosa* isolates were confirmed to be carbapenemase producers. OXA-48 carbapenemase was the predominant carbapenemase (82% [59/72]) found in *Enterobacteriaceae*, while VIM-type carbapenemase largely predominated (93% [51/55]) in *P. aeruginosa*. By the CNP test, all but 3 OXA-48-positive *Enterobacteriaceae* (two *Klebsiella pneumoniae* and one *Escherichia coli*) and 3 OXA-198-producing *P. aeruginosa* isolates (clustered in a single hospital and most probably corresponding to a single clone) had positive results. None of the false-negative (by either test) carbapenemase-positive isolates showed mucoid colonies (9). While no uninterpretable results were observed with the CNP test, 9% of the tested isolates (16/135 *Enterobacteriaceae* and 17/221 *P. aeruginosa*) gave an uninterpretable result, showing a positive reaction with the RCS negative-control disk.

The overall sensitivity, specificity (calculated on all tested strains), and positive and negative predictive values (calculated on consecutive isolates referred to the reference laboratory) for detecting CPE using the CNP test compared to the molecular detection results were 97%, 100%, 100%, and 95%, respectively, for *Enterobacteriaceae*, and 91%, 100%, 100%, and 96%, respectively, for *P. aeruginosa*. After excluding the uninterpretable results, the sensitivity, specificity, and positive and negative predictive values using the RCS were 98%, 83%, 81%, and 95%, respectively, for *Enterobacteriaceae*, and 96%, 54%, 39%, and 97%, respectively, for *P. aeruginosa*. If only strong color changes (orange or yellow) were considered a positive result using the RCS, the calculated specificity and positive predictive values increased to 99% and 96%, respectively, while maintaining high sensitivity and negative predictive values of 90% and 96%, respectively, for *P. aeruginosa*. In addition to the false-negative results observed for a small subset of OXA-48 CPE isolates and for GES-type CPPA isolates using the CNP test that were already reported in other studies (3, 9), we also observed false-negative results using the CNP test with two of the four IMP-13 and all four OXA-198 CPPA isolates tested. Among the total 73 OXA-48 *Enterobacteriaceae* isolates tested, which represent the main detection challenge in our setting, the numbers of strong-positive, weak-positive, negative, and uninterpretable re-

**TABLE 2** Carba NP test and Rapid CARB screen kit results for carbapenemase-negative *Enterobacteriaceae* and *Pseudomonas aeruginosa* collection strains ($n = 69$)

| Group/species (no. of isolates) | ESBL | AmpC | Other β-lactamase(s) | Nonenzymatic resistance mechanism(s) | Total no. | No. with negative CNP result: | No. with RCS result of: | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Positive[a] | Negative | Uninterpretable |
| *Enterobacteriaceae* (56) | | | | | | | | | |
| *E. coli* | CTX-M group 1 | | OXA-1 | | 1 | 1 | | 1 | |
| | CTX-M group 1 | | | | 2 | 2 | 1 | 1 | |
| | CTX-M group 2 | | | | 6 | 6 | | 6 | |
| | CTX-M group 9 | | | | 1 | 1 | | 1 | |
| | SHV-2a | ACC-1 | | | 1 | 1 | | 1 | |
| | TEM-10 | | | | 1 | 1 | | 1 | |
| | TEM-52 | | | | 1 | 1 | | 1 | |
| | | ACC-1 | | | 1 | 1 | | 1 | |
| | | CMY-42 | | | 1 | 1 | | 1 | |
| | | CMY-60 | | | 1 | 1 | | 1 | |
| | | FOX-3 | | | 1 | 1 | | 1 | |
| | | | CARB-7 | | 1 | 1 | | 1 | |
| | | | TEM-30 | | 1 | 1 | | 1 | |
| *K. pneumoniae* | CTX-M group 1 | | OXA-9 | Decreased membrane permeability | 1 | 1 | | 1 | |
| | CTX-M group 1 | | | Decreased membrane permeability | 1 | 1 | | 1 | |
| | TEM-10 | | | Decreased membrane permeability | 1 | 1 | | 1 | |
| | TEM-52 | | OXA-1 | Decreased membrane permeability | 1 | 1 | | | 1 |
| | | DHA-1 | OXA-1 | Decreased membrane permeability | 1 | 1 | 1 | | |
| | CTX-M group 1 | | OXA-1 | | 1 | 1 | | | 1 |
| | CTX-M group 1 | | | | 2 | 2 | | 2 | |
| | CTX-M group 2 | | SHV-11 | | 1 | 1 | | 1 | |
| | CTX-M group 9 | | SHV-76 | | 1 | 1 | | 1 | |
| | | CMY-2 | | | 1 | 1 | | 1 | |
| | | DHA-1 | SHV-11 | | 3 | 3 | 2 | | 1 |
| | | | LEN | | 1 | 1 | | 1 | |
| *K. oxytoca* | CTX-M group 1 | | | | 1 | 1 | | 1 | |
| | CTX-M group 2 | | | | 1 | 1 | | 1 | |
| | CTX-M group 9, SHV-12 | | | | 1 | 1 | | 1 | |
| | GES-7 | | | | 1 | 1 | | 1 | |
| *E. cloacae* | CTX-M group 1 | | | | 1 | 1 | | 1 | |
| | CTX-M group 2 | | | | 1 | 1 | | 1 | |
| | CTX-M group 9 | | | | 1 | 1 | | 1 | |
| | CTX-M group 9, SHV-12 | | | | 1 | 1 | | 1 | |
| *Enterobacter aerogenes* | CTX-M group 9 | cAmpC[b] | | | 1 | 1 | | 1 | |
| | TEM-24, SHV-2a | | | | 1 | 1 | | 1 | |
| *Enterobacter kobei* | CTX-M group 9, SHV-12 | | | | 1 | 1 | | 1 | |
| *C. freundii* | CTX-M group 1 | | OXA-1 | | 1 | 1 | | 1 | |
| | GES-7 | | | | 1 | 1 | | 1 | |
| *C. braakii* | GES-7 | | | | 1 | 1 | | 1 | |
| *Hafnia alvei* | | cAmpC | | | 2 | 2 | | 2 | |
| *S. marcescens* | CTX-M group 1 | | | | 1 | 1 | | 1 | |
| *Proteus mirabilis* | CTX-M group 2 | | | | 2 | 2 | | 2 | |
| | TEM-110 | | | | 1 | 1 | | 1 | |
| | TEM-2 | | | | 1 | 1 | | 1 | |
| | | CMY-2 | | | 1 | 1 | | 1 | |
| *Pseudomonas aeruginosa* (13) | BEL-1 | | | | 2 | 2 | 2 | | |
| | GES-1 | | | OprD deficient | 1 | 1 | | 1 | |
| | PER-1 | | OXA-2, OXA-10 | OprD deficient + MexA/B-OprM | 1 | 1 | | | 1 |
| | PER-1 | | | OprD deficient | 1 | 1 | 1 | | |
| | PER-1 | | | | 1 | 1 | 1 | | |
| | VEB-1b | | | OprD deficient + MexA/B-OprM | 1 | 1 | | | 1 |
| | VEB-1b | | OXA-10 | OprD deficient + MexA/B-OprM | 1 | 1 | | 1 | |
| | | cAmpC | OXA-10 | OprD deficient + MexA/B-OprM | 1 | 1 | 1 | | |
| | | | OXA-2 | | 1 | 1 | 1 | | |
| | | | OXA-20-like, OXA-18 | | 1 | 1 | | | 1 |
| | | cAmpC | OXA-9 | OprD deficient + MexA/B-OprM | 1 | 1 | | 1 | |
| | | | CARB | | 1 | 1 | 1 | | |

[a] All results were weakly positive.

[b] cAmpC, overexpressed chromosomal cephalosporinase.

sults were 62, 8, 3, and 0, respectively, by the CNP test, while they were 49, 14, 2, and 8, respectively, by the RCS. Based on our study experience, the RCS was technically easier to perform, as preparation of the reagents was not required as it is for the CNP test. On the other hand, some difficulty in reading the color changes in the RCS, notably due to the turbidity of the undissolved tablets, together with the nonnegligible number of false-positive and uninterpretable results, are the major drawbacks against its routine implementation.

The Carba NP test and Rapid CARB screen are rapid and highly sensitive screening tests used to exclude carbapenemase in *Enterobacteriaceae* and *P. aeruginosa*. In an epidemiological setting with a high prevalence of VIM CPPA isolates, the Rapid CARB screen can be used for confirmation of carbapenemase production in *P. aeruginosa* only if a strong-positive reaction is used as the criterion for a positive result. However, both screening tests should be used with caution in areas with higher prevalence of OXA-48 CPE and should be evaluated in other epidemiological settings in which

**TABLE 3** Carba NP test and Rapid CARB screen kit results for *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates referred to the Belgian national reference center ($n = 356$)

| Group | Test[a] | Test result | No. of isolates with indicated carbapenemase: | | | | | | | Total no. of isolates |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | OXA-48 | NDM | KPC | VIM | IMP | OXA-198 | Negative | |
| *Enterobacteriaceae* | CNP | Positive | 56 (6)[b] | 5 (3) | 4 | 4 | | | | 69 |
| | | Negative | 3 | | | | | | 63 | 66 |
| | RCS | Uninterpretable | 6 | 2 | | 1 | | | 7 | 16 |
| | | Positive | 51 (11) | 3 (1) | 4 | 3 | | | 14 (11) | 75 |
| | | Negative | 2 | | | | | | 42 | 44 |
| Total *Enterobacteriaceae* | | | 59[c] | 5[d] | 4[e] | 4[f] | | | 63[g] | 135 |
| *Pseudomonas aeruginosa* | CNP | Positive | | | | 51 (1) | 1 (1) | | | 52 |
| | | Negative | | | | | | 3 | 166 | 169 |
| | RCS | Uninterpretable | | | | 8 | | | 9 | 17 |
| | | Positive | | | | 43 (1) | 1 | | 69 (67) | 113 |
| | | Negative | | | | | | 3 | 88 | 91 |
| Total *P. aeruginosa* | | | | | | 51 | 1 | 3 | 166 | 221 |

[a] CNP, Carba NP test; RCS, Rapid CARB screen kit.
[b] The number of weak-positive results is in parentheses for all positive results.
[c] Includes 42 *K. pneumoniae*, 10 *E. coli*, 3 *K. oxytoca*, 2 *E. cloacae*, 1 *Enterobacter asburiae*, and 1 *E. kobei* isolates.
[d] Includes 2 *E. cloacae*, 1 *K. pneumoniae*, 1 *E. coli*, and 1 *C. freundii* isolates.
[e] Includes 3 *K. pneumoniae* and 1 *E. cloacae* isolates.
[f] Includes 2 *E. cloacae*, 1 *K. pneumoniae*, and 1 *C. freundii* isolates.
[g] Includes 24 *K. pneumoniae*, 10 *E. coli*, 10 *E. cloacae*, 8 *E. aerogenes*, 4 *K. oxytoca*, 2 *E. asburiae*, 2 *E. kobei*, 1 *C. freundii*, 1 *S. marcescens*, and 1 *P. mirabilis* isolates.

carbapenemases with lower hydrolytic activity of carbapenems may be found (e.g., IMP, GES, and OXA-198). The Carba NP test performed better than the Rapid CARB screen, owing to its superior specificity and the large number of uninterpretable results observed with the Rapid CARB screen.

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