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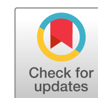


# Comparative Evaluation of Four Phenotypic Tests for Detection of Carbapenemase-Producing Gram-Negative Bacteria

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**ABSTRACT** Four screening assays aimed for rapid detection of carbapenemase production from Gram-negative bacterial isolates, i.e., the Neo-Rapid Carb kit (Rosco Diagnostica A/S), the Rapidec Carba NP test (bioMérieux SA), the β Carba test (Bio-Rad Laboratories N.V.), and a homemade electrochemical assay (BYG Carba test) were evaluated against a panel comprising 328 clinical isolates (*Enterobacteriaceae* [$n = 198$] and nonfermentative Gram-negative bacilli [$n = 130$]) with previously characterized resistance mechanisms to carbapenems. Among *Enterobacteriaceae* isolates, the BYG Carba test and the β Carba test showed excellent sensitivities (respectively, 100% and 97.3%) and specificities (respectively, 98.9% and 97.7%). The two other assays yielded poorer performances with sensitivity and specificity of 91.9% and 83.9% for the Rapidec Carba NP test and of 89.2% and 89.7% for the Neo-Rapid Carb kit, respectively. Among *Pseudomonas* spp., sensitivities and specificities ranged, respectively, from 87.3% to 92.7% and from 88.2% to 94.1%. Finally, all tests performed poorly against *Acinetobacter* spp., with sensitivities and specificities, respectively, ranging from 27.3% to 75.8% and from 75 to 100%. Among commercially available assays, the β Carba test appeared to be the most convenient for routine use and showed the best overall performances, especially against OXA-48-like producers. The excellent performance of the BYG Carba test against *Enterobacteriaceae* was confirmed (100% sensitivity and 98.9% specificity).

**KEYWORDS** *Acinetobacter*, CPE, *Pseudomonas*, antibiotic resistance, carbapenem hydrolysis, carbapenemase screening

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During the last decade, the emergence of carbapenemase-producing Gram-negative bacteria has become a major public health issue. These multidrug-resistant organisms can only be treated by limited therapeutic options and have been widely involved in nosocomial outbreaks (1, 2). Rapid detection is of paramount importance for prompt implementation of appropriate antimicrobial therapy and of isolation procedures in order to prevent cross-transmission to other patients.

A large variety of acquired carbapenem-hydrolyzing β-lactamases have spread worldwide (2, 3). Currently the most prevalent carbapenemases in *Enterobacteriaceae* in Europe belong to the Ambler class A (KPC), class B (VIM, IMP, and NDM), and class D (OXA-48-like) (2, 4). In *Acinetobacter* spp., carbapenem-hydrolyzing β-lactamases essentially belong to the Ambler class D, the most prevalent variants being OXA-23-, OXA-24-, and OXA-58-like enzymes (3, 5), while class B metallo-β-lactamases (MBL), mainly VIM and IMP to a lesser extent, are the most frequently encountered among *Pseudomonas* spp. (3, 6). Several phenotypic assays developed to detect carbapenemase production from bacterial culture within hours are now commercially available, including the Neo-Rapid Carb kit (NRCK) (Rosco Diagnostica A/S, Taastrup, Denmark),

**TABLE 1** Evaluation of the BYG Carba test, β Carba test, Rapidec Carba NP test, and Neo-Rapid Carb screen test against 198 *Enterobacteriaceae* isolates

| *Enterobacteriaceae* group | Resistance mechanism | No. of isolates | MIC range (μg/ml) for: | | | Test results (no.) for[a]: | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | BYG | | BCT | | | RCNP | | | | NRCK | | | | |
| | | | Meropenem | Imipenem | Ertapenem | P | N | P | D | N | P | D | N | NI | P | D | N | NI | |
| CPE | KPC-3 | 8 | 4–>32 | 16–>32 | 12–>32 | 8 | 0 | 7 | 1 | 0 | 8 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | |
| | NDM-1 | 18 | 3–>32 | 2–>32 | 6–>32 | 18 | 0 | 18 | 0 | 0 | 18 | 0 | 0 | 0 | 18 | 0 | 0 | 0 | |
| | VIM[b] | 13 | 1–32 | 4–>32 | 0.19–>32 | 13 | 0 | 13 | 0 | 0 | 13 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | |
| | OXA-48 | 69 | 0.19–>32 | 0.38–>32 | 0.25–>32 | 69 | 0 | 67 | 2 | 0 | 60 | 9 | 0 | 0 | 57 | 9 | 2 | 1 | |
| | NDM-1 + OXA-48 | 2 | >32 | >32 | >32 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | |
| | KPC-2 + VIM-1 | 1 | >32 | >32 | >32 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | |
| | Total CPE | 111 | 0.19–>32 | 0.38–>32 | 0.19–>32 | 111 | 0 | 108 | 3 | 0 | 102 | 9 | 0 | 0 | 99 | 9 | 2 | 1 | |
| Non-CPE | ESBL | 29 | 0.016–24 | 0.125–3 | 0.006–>32 | 0 | 29 | 0 | 0 | 29 | 0 | 6 | 23 | 0 | 0 | 2 | 26 | 1 | |
| | AmpC overexpression | 30 | 0.023–12 | 0.125–32 | 0.19–>32 | 1 | 29 | 0 | 1 | 29 | 0 | 5 | 25 | 0 | 0 | 1 | 25 | 4 | |
| | ESBL and AmpC overexpression | 13 | 0.047–4 | 0.19–16 | 0.094–>32 | 0 | 13 | 0 | 0 | 13 | 0 | 2 | 11 | 0 | 0 | 1 | 12 | 0 | |
| | Narrow spectrum β-lactamases | 15 | 0.012–4 | 0.19–2 | 0.003–24 | 0 | 15 | 0 | 1 | 14 | 0 | 1 | 14 | 0 | 0 | 0 | 15 | 0 | |
| | Total non-CPE | 87 | 0.012–24 | 0.125–32 | 0.003–>32 | 1 | 86 | 0 | 2 | 85 | 0 | 14 | 73 | 0 | 0 | 4 | 78 | 5 | |

[a]BYG, BYG Carba test, BCT, β Carba test, RCNP, Rapidec Carba NP test, NRCK, Neo-Rapid Carb screen kit; P, positive; N, negative; D, doubtful; NI, noninterpretable.
[b]VIM-1 ($n$ = 12), VIM-4 ($n$ = 1).

the Rapidec Carba NP test (RCNP) (bioMérieux SA, Marcy l'Etoile, France), and the recently marketed β Carba test (BCT) (Bio-Rad Laboratories N.V., Marnes-la-Coquette, France). In addition, we recently developed and validated the BYG Carba test (BYG), an in-house rapid electrochemical assay which allows objective detection (positivity based on a cutoff value) of carbapenemase-producing *Enterobacteriaceae* (CPE) from bacterial culture colonies in less than 30 min (7).

The Neo-Rapid Carb kit and the Rapidec Carba NP test have been evaluated by several groups (8–16), but, to our knowledge, no comparison with the novel β Carba test has been published yet. Evaluations of the colorimetric carbapenem hydrolysis assays have mostly focused on *Enterobacteriaceae*, while reports dealing with nonfermentative Gram-negative bacilli are scarce.

The aim of this study was to compare prospectively the analytical performances and qualitative characteristics (turnaround time, ease of interpretation of results, and costs) of these screening assays for the detection of the most prevalent CPE isolates. We also evaluated their performance against that of a selected panel of collection isolates of *Pseudomonas* spp. and *Acinetobacter* spp. that had been previously characterized for their resistance mechanisms to β-lactams.

## RESULTS

Tests results for *Enterobacteriaceae* and nonfermentative Gram-negative isolates are presented in Tables 1 and 2, respectively. All *Enterobacteriaceae* isolates showing discrepant results with at least one of the four assays are listed and detailed in Table S1 in the supplemental material.

Among the 198 *Enterobacteriaceae* isolates referred, 111 (56.1%) produced a carbapenemase, including OXA-48 ($n$ = 69), NDM-1 ($n$ = 18), VIM-1 ($n$ = 12), KPC-3 ($n$ = 8), VIM-4 ($n$ = 1), and combinations of OXA-48 plus NDM-1 ($n$ = 2) and of KPC-2 plus NDM-1 ($n$ = 1). Various genus and species were included, i.e., *Klebsiella pneumoniae* ($n$ = 104), *Enterobacter cloacae* ($n$ = 32), *Escherichia coli* ($n$ = 31), *Citrobacter freundii* ($n$ = 9), *Enterobacter aerogenes* ($n$ = 9), *Klebsiella oxytoca* ($n$ = 8), *Citrobacter koseri* ($n$ = 2), *Serratia marcescens* ($n$ = 2), and *Enterobacter asburiae* ($n$ = 1).

Of the 111 confirmed CPE isolates, 108 (97.3%), 102 (91.9%), 99 (89.2%), and 111 (100%) were correctly assigned by BCT, RCNP, NRCK, and BYG, respectively. Among the 87 carbapenemase-negative isolates, 85 (97.7%), 73 (83.9%), 78 (89.7%), and 86 (98.9%) were correctly identified as non-CPE by BCT, RCNP, NRCK, and BYG, respectively. BCT yielded doubtful results for three CPE isolates (OXA-48 [$n$ = 2] and KPC-3 [$n$ = 1]) and for two non-CPE isolates (one K1-OXY overexpressing *K. oxytoca* susceptible to all carbapenems and one *E. cloacae* overexpressing AmpC combined with a porin defi-

**TABLE 2** Evaluation of the BYG Carba test, β Carba test, Rapidec Carba NP test, and Neo-Rapid Carb screen kit against 130 isolates of nonfermenting Gram-negative bacteria

| Genus | Carbapenemase | No. of isolates | MIC range (μg/ml) for: | | Test results (no.) for[a]: | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | BYG | | BCT | | | RCNP | | | | NRCK | | | |
| | | | Meropenem | Imipenem | P | N | P | D | N | P | D | N | NI | P | D | N | NI |
| *Pseudomonas* spp. | GES[b] | 4 | >32 | 16–>32 | 1 | 3 | 0 | 0 | 4 | 2 | 0 | 2 | 0 | 2 | 0 | 2 | 0 |
| | KPC-2 | 1 | >32 | >32 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | VIM[c] | 34 | 32–>32 | 32–>32 | 32 | 2 | 34 | 0 | 0 | 32 | 0 | 2 | 0 | 33 | 0 | 0 | 1 |
| | IMP[d] | 11 | 24–>32 | >32 | 10 | 1 | 11 | 0 | 0 | 11 | 0 | 0 | 0 | 11 | 0 | 0 | 0 |
| | NDM-1 | 1 | >32 | >32 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | GIM | 1 | >32 | >32 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | DIM | 1 | >32 | >32 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | SPM-1 | 1 | >32 | >32 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | OXA-198 | 1 | 12 | >32 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| | Total carbapenemase producers | 55 | 12–>32 | 16–>32 | 48 | 7 | 50 | 1 | 4 | 50 | 0 | 5 | 0 | 51 | 0 | 2 | 2 |
| | Non-carbapenemase producers[e] | 34 | 0.38–>32 | 0.75–>32 | 2 | 32 | 2 | 0 | 32 | 1 | 2 | 30 | 1 | 0 | 1 | 30 | 3 |
| *Acinetobacter* spp. | GES-14 | 1 | 32 | 32 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| | VIM-4 | 1 | 32 | >32 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | IMP[f] | 3 | 16–>32 | 3–>32 | 3 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| | NDM-1 | 2 | >32 | >32 | 2 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| | GIM | 1 | 8 | 4 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | OXA carbapenemases[g] | 22 | 2–>32 | 4–>32 | 15 | 7 | 16 | 3 | 3 | 2 | 3 | 17 | 0 | 2 | 1 | 18 | 1 |
| | NDM-1 + OXA-23 | 1 | >32 | >32 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | NDM-2 + OXA-23 | 1 | >32 | >32 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | VIM-4 + OXA-58 | 1 | 32 | >32 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | Total carbapenemase producers | 33 | 2–>32 | 3–>32 | 24 | 9 | 25 | 4 | 4 | 12 | 3 | 18 | 0 | 9 | 2 | 21 | 1 |
| | Non-carbapenemase producers[h] | 8 | 0.25–32 | 0.19–8 | 0 | 8 | 1 | 0 | 7 | 0 | 2 | 6 | 0 | 0 | 0 | 8 | 0 |

[a]BYG, BYG Carba test, BCT, β Carba test, RCNP, Rapidec Carba NP test, NRCK, Neo-Rapid CARB screen kit; P, positive; N, negative; D, doubtful; NI, noninterpretable.
[b]GES-2 ($n$ = 1), GES-5 ($n$ = 1), GES-18 ($n$ = 2).
[c]VIM-1 ($n$ = 2), VIM-2 ($n$ = 24), VIM-4 ($n$ = 7), VIM-5 ($n$ = 1).
[d]Including IMP-1 ($n$ = 1), IMP-2 ($n$ = 1), IMP-7 ($n$ = 1), IMP-13 ($n$ = 5), IMP-15 ($n$ = 1), IMP-19 ($n$ = 1), and IMP-29 ($n$ = 1).
[e]Including strains producing BEL-1 ($n$ = 2), BEL-2 ($n$ = 1), GES-1 ($n$ = 2), OXA-2 ($n$ = 1), OXA-2a ($n$ = 1), OXA-4 ($n$ = 1), OXA-9 ($n$ = 1), OXA-10 ($n$ = 1), OXA-18 ($n$ = 1), OXA-28 ($n$ = 1), OXA-35 ($n$ = 1), PME-1 ($n$ = 1), SHV-2a ($n$ = 1), SHV-5 ($n$ = 1), TEM-4 ($n$ = 1), combination of OXA-10 + TEM-1 ($n$ = 1), and phenotypically assessed impermeability alone ($n$ = 16).
[f]Including IMP-1 ($n$ = 1), IMP-4 ($n$ = 1), and IMP-14 ($n$ = 1).
[g]Including OXA-23 ($n$ = 10), OXA-24 ($n$ = 1), OXA-25 ($n$ = 1), OXA-26 ($n$ = 1), OXA-27 ($n$ = 1), OXA-58 ($n$ = 4), OXA-72 ($n$ = 1), OXA-143 ($n$ = 1), OXA-255 ($n$ = 1), and combination of OXA-23 with OXA-58 ($n$ = 1).
[h]Including strains producing CARB-5 ($n$ = 1), CARB-10 ($n$ = 1), GES-12 ($n$ = 1), PER-1 ($n$ = 1), VEB-1 ($n$ = 1), phenotypically assessed impermeability ($n$ = 1), and wild type ($n$ = 2).

ciency resistant to ertapenem). For RCNP, 9 OXA-48-producing isolates and 14 non-CPE isolates belonging to various species and harboring diverse and unrelated resistance mechanisms induced a doubtful color shift. Using NRCK, 2, 9, and 1 OXA-48-producing strains gave false-negative, doubtful, and noninterpretable results, respectively, while 9 non-CPE strains generated either doubtful ($n$ = 4) or noninterpretable ($n$ = 5) results. The BYG yielded only one false-positive result for one carbapenem-susceptible DHA-producing *K. pneumoniae*.

Of the 55 carbapenemase-producing *Pseudomonas* spp., 50 (90.9%), 50 (90.9%), 51 (92.7%), and 48 (87.2%) were accurately detected by BCT, RCNP, NRCK, and BYG, respectively. BCT failed to detect any GES-type carbapenemases ($n$ = 4) and yielded a doubtful result for one OXA-198 (class D carbapenemase)-producing *P. aeruginosa*, while false-positive results were obtained for two carbapenem-resistant carbapenemase-negative strains (one OXA-35 producing *P. aeruginosa* and one *P. aeruginosa* with phenotypically assessed impermeability alone). RCNP missed carbapenemase detection for 5 *Pseudomonas* species isolates (VIM-2, VIM-4, GES-2, GES-18, and OXA-198 [$n$ = 1 each]). One noninterpretable result, two doubtful results, and one false-positive (i.e., OXA-10-producing *P. aeruginosa*) result were recorded among the carbapenemase-negative isolates. NRCK yielded two false-negative results for a GES-2 ($n$ = 1)-producing isolate and a GES-5 ($n$ = 1)-producing isolate as well as two noninterpretable results. Regarding non-carbapenemase-producing isolates, three noninterpretable results and one doubtful result, i.e., a BEL-1 extended-spectrum β-lactamase (ESBL)-producing *P. aeruginosa*, were observed. Using the BYG, false-negative results were observed for 7

**TABLE 3** Analytical performances of the BYG Carba test, β Carba test, Rapidec Carba NP test, and Neo-Rapid Carb screen kit

| Parameter[a] | Results for[b]: | | | |
|---|---|---|---|---|
| | BYG | BCT | RCNP | NRCK |
| *Enterobacteriaceae* (n = 198) | | | | |
| Sensitivity (%) | 100 | 97.3 | 91.9 | 89.2 |
| 95% CI | 100–100 | 93.4–100 | 85.4–98.4 | 81.8–96.6 |
| Specificity (%) | 98.9 | 97.7 | 83.9 | 89.7 |
| 95% CI | 96–100 | 93.7–100 | 74.1–93.8 | 81.5–97.8 |
| PPV (%) | 99.1 | 98.2 | 87.9 | 91.7 |
| 95% CI | 96.9–100 | 95–100 | 80.4–95.5 | 85–98.3 |
| NPV (%) | 100 | 96.6 | 89 | 86.7 |
| 95% CI | 100–100 | 91.8–100 | 80.4–97.7 | 77.7–95.6 |
| *Pseudomonas* spp. (n = 89) | | | | |
| Sensitivity (%) | 87.3 | 90.9 | 90.9 | 92.7 |
| 95% CI | 76–98.5 | 81.2–100 | 81.2–100 | 84–100 |
| Specificity (%) | 94.1 | 94.1 | 88.2 | 88.2 |
| 95% CI | 84–100 | 84–100 | 74.4–100 | 74.4–100 |
| *Acinetobacter* spp. (n = 41) | | | | |
| Sensitivity (%) | 72.7 | 75.8 | 36.4 | 27.3 |
| 95% CI | 53.3–92.1 | 57.1–94.4 | 15.4–57.3 | 7.9–46.7 |
| Specificity (%) | 100 | 87.5 | 75 | 100 |
| 95% CI | 100–100 | 58.3–100 | 36.7–100 | 100–100 |

[a]CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[b]BYG, BYG Carba test, BCT, β Carba test, RCNP, Rapidec Carba NP test, NRCK, Neo-Rapid CARB screen kit.

isolates (GES-type carbapenemases [*n* = 3], VIM-2 [*n* = 2], IMP-2 [*n* = 1], and OXA-198 [*n* = 1]), while false-positive results were recorded for two BEL-1-producing *Pseudomonas* species isolates.

Finally, out of 33 carbapenemase-producing *Acinetobacter* species isolates, 25 (75.8%), 12 (36.4%), 9 (27.3%), and 24 (72.7%) were correctly identified by BCT, RCNP, NRCK, and BYG, respectively. BCT yielded four doubtful color shift and four false-negative results involving class D carbapenemase producers (OXA-23, OXA-24, or OXA-58 variants [*n* = 6]), GES-14 (*n* = 1), and NDM-1 (*n* = 1), as well as one false-positive result for a GES-12-producing strain. RCNP failed to detect 20 class D carbapenemase producers and one GES-14-producing *Acinetobacter baumannii* isolate, while doubtful results were documented for 2 carbapenemase-negative carbapenem-susceptible *A. baumannii* isolates. NRCK also missed most OXA-type carbapenemase-producing strains. The assay also yielded doubtful color shifts for one OXA-143- and one NDM-1-producing *Acinetobacter* species isolate. No false-positive result and one single noninterpretable test were encountered. Last, the BYG Carba test yielded 9 false-negative results (OXA-type [*n* = 8] and GES-type [*n* = 1] carbapenemase) but no false-positive result.

The analytical performances for each test are summarized in Table 3. For *Enterobacteriaceae*, sensitivities ranged from 89.2% (NRCK) to 100% (BYG), while specificities varied between 83.9% (RCNP) and 98.9% (BYG). Slightly lower performances were obtained for *Pseudomonas* spp. with sensitivities and specificities ranging between 87.3% (BYG) and 92.7% (NRCK) and between 88.2% (RCNP and NRCK) and 94.1% (BYG and BCT), respectively. On the other hand, for *Acinetobacter* spp., analytical performances were on the whole insufficient with sensitivities below 80% for all 4 assays.

The results of the reproducibility assessment on 20 strains are detailed in Table S2 in the supplemental material. Overall, the interassay/interoperator reproducibility rates were, respectively, 90%/85% for BCT, 100%/100% for RCNP, 95%/90% for NRCK, and 95%/95% for BYG. Besides the false-negative results for each of the four assays, *Acinetobacter* species isolates were responsible for the majority of the discrepancies, confirming the poor performance of the assays in this group. Additionally, a second false-positive result using BCT with a non-carbapenemase-producing carbapenem-

resistant *K. oxytoca* isolate was documented. This strain induced a color variation ranging from dark yellow (negative) to orange (positive). Of note, one OXA-48 *K. pneumoniae* isolate gave a doubtful result three times with RCNP, and one DHA-producing *K. pneumoniae* yielded one noninterpretable result with NRCK. With regard to the BYG test, after exclusion of the *Acinetobacter* species isolates, all strains tested gave positive results with intensity signals that were clearly above the defined cutoff of 11.5 arbitrary units (AU) (range, 25.9 to 134.1 AU), with a mean within-strain coefficient of variation (CV) of 20.1%.

## DISCUSSION

BCT, RCNP, and NRCK are three rapid colorimetric assays that have been recently commercialized for the screening of carbapenemase-producing bacterial isolates. They share several advantages over in-house assays such as ready-to-use reagents and the quality control process guaranteed by the manufacturer. The BYG Carba test requires a specific testing device and is not commercially available yet but presents the advantage over the colorimetric assays of objective unequivocal results. A new BYG procedure eliminating several steps, including the preparation of a bacterial suspension and of the lysis step, was used. This simplified protocol (version 2.0) was previously validated in our laboratory comparatively to the original protocol against 207 *Enterobacteriaceae* isolates and yielded excellent performances (sensitivity, 99.3%; specificity, 99.1%) (17).

BCT and the BYG Carba test displayed excellent performances for the detection of the most prevalent carbapenemase-producing *Enterobacteriaceae* with remarkably high negative predictive values (>95%), emphasizing that a negative result by these tests may almost exclude the presence of carbapenemase and prevent further unnecessary testing on such isolates. However, results for testing of *K. oxytoca* with BCT should be taken with caution because false-positive results have been documented.

On the other hand, the calculated analytical performances of RCNP and NRCK were lower than previously reported (9, 11, 14, 16). At least two reasons might have accounted for those differences. First, the distribution of carbapenemase types among CPE isolates comprised a larger proportion of OXA-48 producers (62.2%) that are known to be less well detected by these two assays (8, 10, 12, 14). However, this high proportion of OXA-48 producers is in line with the current epidemiology of CPE observed in several European countries such as Belgium, France, Spain, or Germany (4). In comparison with this report, OXA-48 producers represented only 7.9% to 27.5% of the screened CPE isolates in other studies where better overall tests performances were reported (11, 13, 14, 16).

The second explanation might relate to the way we categorized doubtful results that were more frequently observed with RCNP and NRCK despite intense preliminary training of the operators. Repetition of doubtful tests with higher inocula had been suggested to partially solve the problem (11, 12) but was not performed here as we considered that it would lead to a delay in reporting of the results besides increasing the costs. Unlike others, we decided to classify these results as false (either false positive or false negative), since, in the routine of a clinical microbiology laboratory, only unequivocal results would be transmitted to clinicians. Visual interpretation of faint color variations such as "red-orange," "dark," or "pale" orange may vary from one observer to another and might impact analytical performances. Reporting doubtful results as false highlights the limitations of eye-read colorimetric tests and the potential benefits of optical reading device development. BYG has the clear advantage of being quantitative and objective, the results being automatically generated in real time by internal software once the positivity cutoff is crossed by the monitored signal curve.

All four assays accurately detected carbapenemase-producing *Pseudomonas* spp. but yielded suboptimal performances for *Acinetobacter* spp. Detection of carbapenemase production in *Acinetobacter* spp. is for known for being more difficult, probably due to the very low carbapenem hydrolytic activities of the class D hydrolyzing $\beta$-lactamases found in these organisms (3, 18). Further evaluation of modified proce-

**TABLE 4** Technical characteristics of BYG Carba test, β Carba test, Rapidec Carba NP test, and Neo-Rapid Carb screen kit

| Characteristic | Results for[a]: | | | |
|---|---|---|---|---|
| | BYG | BCT | RCNP | NRCK |
| Validated group of isolates (or recommended by manufacturer) | *Enterobacteriaceae* *P. aeruginosa* | *Enterobacteriaceae* | *Enterobacteriaceae* *P. aeruginosa* *A. baumannii* | *Enterobacteriaceae* *P. aeruginosa* |
| Maximal incubation time (min) | 60 | 30 | 160 | 90 |
| Total hands-on time (min) | 5 | 2 | 4 | 6 |
| No. of handling steps | 1 | 1 | 3 | 2 |
| Ease of use | Easy, little training required | Very easy, no training required | Easy, little training required | Easy, little training required |
| Catalog price per unit test in Belgium | Not commercially available | 6.6 USD[b] | 8.8 USD | 2.4 USD |
| Positive criteria | Signal attaining a fixed cutoff | Orange, red, purple | Orange-red to yellow, with a red negative control | Orange to yellow, with a red negative control |
| Overall doubtful results (%)[c] | 0 | 3 | 9.1 | 4.9 |
| Overall noninterpretable results[c] | NR[d] | NR | 0.3 | 3.4 |
| Overall reproducibility (%)[e] | 95 | 85 | 100 | 90 |

[a]BYG, BYG Carba test, BCT, β Carba test, RCNP, Rapidec Carba NP test, NRCK, Neo-Rapid CARB screen kit.
[b]USD, U.S. dollars.
[c]Proportion of doubtful and noninterpretable results among all tested isolates.
[d]NR, not reportable as no internal negative control is provided with the test.
[e]Proportion of isolates showing full agreements when tested in triplicate in a reproducibility evaluation (total $n = 20$).

dures, including increasing incubation time, inoculum preparation, and lysis might be useful for improving test performances.

Overall, BCT showed better performances for the detection of class D carbapenemases than the two other commercial assays. Indeed, BCT allowed the unambiguous detection of 97.1% of the 69 OXA-48-producing *Enterobacteriaceae* isolates, compared to only 87% and 82.6% for RCNP and NRCK, respectively.

Among the 22 class D carbapenemase-producing *Acinetobacter* species isolates, carbapenemase activity was detected in 72.7%, 9.1%, and 9.1%, respectively, using BCT, RCNP, and NRCK. Moreover, all four assays performed poorly for the detection of GES carbapenemases, again most probably because of the weak carbapenem hydrolytic activity of many variants belonging to this carbapenemase family.

The most salient technical characteristics of the four tests are compared in Table 4. BCT is a one-step assay that yields a result after a single 30-min incubation period. On the whole, the use of RCNP and of NRCK required more handling and incubation steps, resulting in slightly longer turnaround time. BCT requires a fully charged 1-μl loop. The bacterial inoculum size is at least two times larger for both NRCK and RNCP, which may require in some instances an additional subculture before the test can be performed. According to the manufacturer's instructions, BCT and NRCK have to be performed on fresh isolates, while RCNP may be performed on bacterial colonies aged up to 72 h. BCT was the less reproducible assay mainly due to discrepant results with *Acinetobacter* spp. The large number of doubtful results was an important issue for RCNP, while noninterpretable tests were more frequently documented using NRCK.

BYG requires a single handling step and yields a result after a 30-min running period when screening for CPE and after 60 min when screening for carbapenemase in Gram-negative nonfermenters. This test only requires one to three colonies, which may represent an advantage, especially in a primary culture of a clinical specimen growing a lower number of colonies or a mixture of different colonies.

There are limitations to our study. First, we acknowledge the limited diversity of carbapenemase types present in the *Enterobacteriaceae* isolates routinely collected which, however, reflects the current epidemiological patterns in Belgium, largely dominated by OXA-48 CPE. Hence, the analytical performances of these tests should be assessed in geographical areas with different epidemiological settings. A second limitation is related to the possible repeated inclusion of single epidemic clones, although in the present study the isolates were referred from a large number of different

laboratories throughout Belgium, suggesting at the least a certain level of diversity among the strains tested.

In conclusion, BCT, RCNP, and NRCK are three rapid commercial assays for the screening of carbapenemase among Gram-negative bacteria. Some difficulties of interpretations were encountered with RCNP and NRCK when dealing with OXA-48-producing *Enterobacteriaceae*. BCT appeared on the whole to perform the best, being also the easiest test to perform and to interpret and providing reliable results in short processing times among *Enterobacteriaceae* (with caution required for *K. oxytoca* isolates) and *Pseudomonas* spp. None of the assays was considered to perform sufficiently well against *Acinetobacter* spp., for which improvements are clearly required before recommending their usage. The in-house BYG appears promising since it provides quantitative, traceable (electronic data reporting), and rapid accurate results using a minimal amount of bacteria. BYG allowed accurate detection of CPE but could still be improved for the detection of carbapenemases in nonfermenters.

## MATERIALS AND METHODS

**Bacterial isolates.** A total of 198 nonduplicate, consecutive *Enterobacteriaceae* isolates referred by 56 microbiology laboratories to the National Reference Center (NRC) (CHU UCL Namur, Belgium) from December 2015 to February 2016 for confirmation of carbapenemase production were included (the selection criteria for referral were based on decreased susceptibility to at least one carbapenem using local routine methods and interpreted according to either EUCAST or CLSI recommendations). For the evaluation of nonfermentative Gram-negative bacilli (NF), 130 collection strains of *Pseudomonas* spp. ($n = 89$, including 55 carbapenemase producers) and of *Acinetobacter* spp. ($n = 41$, including 33 carbapenemase producers) were analyzed. The majority of the collection strains originated from the Belgian NRC, but some isolates were obtained from the French and from the German reference centers (see Acknowledgments). They were all characterized for their carbapenemase content by PCR and sequencing (Tables 1 and 2).

**Strain characterization.** Clinical isolates were subcultured overnight on Trypticase soy agar II with 5% sheep blood (TSA + SB) plates (Becton, Dickinson and Company, NJ, USA) to obtain fresh colonies for further investigation. Collection strains stored at −80°C were thawed and subcultured twice on the same culture media before testing.

Bacterial identification was confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Microflex LT; Bruker Daltonik GmbH, Bremen, Germany) using Bruker Biotyper 2.2 software. Patterns of resistance mechanisms to β-lactams were determined by the disk diffusion method. MICs were determined for ertapenem, imipenem, and meropenem using Etests (bioMérieux SA, Marcy l'Etoile, France) on Mueller-Hinton II agar plates (Becton Dickinson, Aalst, Belgium). Results were interpreted according to CLSI 2015 guidelines (19).

The most prevalent carbapenemases encountered in *Enterobacteriaceae* and nonfermenters were sought by two in-house ISO15189-certified multiplex PCRs targeting $bla_{OXA-48}$, $bla_{NDM}$, $bla_{KPC}$, $bla_{VIM}$ and $bla_{IMP}$, and $bla_{OXA-23}$, $bla_{OXA-24}$, and $bla_{OXA-58}$ (20). Further molecular detections targeting other less prevalent carbapenemases were only performed in case of result discrepancies between phenotypic hydrolysis-based assays (positive/doubtful results) and molecular tests (negative by the two PCRs). Finally, carbapenemase genes were sequenced using external Sanger sequencing services (Macrogen, Seoul, South Korea) for allele identification.

**Testing procedure for commercial carbapenemase detection assays.** The Rapidec Carba NP (RCNP) and Neo-Rapid Carb kit (NRCK), a variant of the former test, are based on imipenem hydrolysis detection using the pH indicator red phenol that turns from red to yellow in the presence of carbapenem-hydrolyzing enzymes (21). The β Carba test (BCT) is based on the opposite color shift from yellow to orange, red, or purple of an undisclosed chromogenic carbapenem substrate in the presence of carbapenem-hydrolyzing enzymes. The BYG Carba test (BYG) is an electrochemical assay that detects the increase of conductivity of a polyaniline-coated electrode, which is highly sensitive to modification of pH and of redox activity occurring during the imipenem enzymatic hydrolysis reaction (7).

All four screening tests (BCT, RCNP, NRCK, and BYG) were simultaneously and blindly performed on fresh isolates. Colorimetric assays were processed and interpreted according to the manufacturer's instructions (22–24). Identical procedures were applied for testing *Enterobacteriaceae* and NF. For the BYG Carba test, a simplified procedure recently validated in our laboratory was used in the present study (BYG protocol version 2.0) (17). Briefly, one to three colonies are directly spotted on two adjacent working probes of the BYG test electrode to cover the entire sensor surface. The electrode is then recovered with 50 μl of a homemade buffer with or without 3 mg/ml imipenem (6 mg/ml of Tienam, containing 3 mg/ml imipenem monohydrate and 3 mg/ml cilastatin sodium; MSD France, Courbevoie, France). A signal intensity cutoff of 11.5 arbitrary units (AU) was chosen for the discrimination between carbapenemase and non-carbapenemase producers according to the validation of the BYG 2.0 protocol (17).

Twenty strains (10 *Enterobacteriaceae* and 10 NF) covering different species and resistance mechanisms were further tested in triplicate (twice by one operator and a third time by another operator) to evaluate interassay and interoperator reproducibility rates. Interassay/interoperator reproducibility rates

were calculated by dividing the number of intraoperator/interoperator agreements by the number of strains tested.

**Test interpretation and performance analysis.** Results were blindly interpreted by a well-trained operator and recorded as positive (P), negative (N), doubtful (D), and noninterpretable (NI).

BCT was interpreted as positive if the color shifted from yellow to orange, red, or purple. For the two others colorimetric assays (RCNP and NRCK), a change from red to orange or yellow was considered positive.

Results were recorded as doubtful if the operator could not categorize the test either as positive or negative and noninterpretable whenever the negative control showed a color shift. Since there is no negative control for BCT, no noninterpretable result could be recorded for this assay. For BYG, only positive or negative results were recorded, depending on whether the measured signal curve crosses the settled cutoff or not. Doubtful and noninterpretable tests were not repeated, and these results were considered together either as false positive or as false negative in the calculation of the performance parameters.

Statistical analysis was performed using the SISA website (D. G. Uitenbroek; http://www.quantitativeskills.com/sisa/distributions/binomial.htm). A width of 95% was chosen for the confidence interval. Sensitivity and specificity for each test compared to those for molecular detection were calculated separately for *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp. The positive predictive value (PPV) and the negative predictive value (NPV) were also calculated for each test for the consecutive *Enterobacteriaceae* clinical isolates that were prospectively referred to our laboratory, considering PCR sequencing results as the gold standard.

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01853-16.

**TEXT S1,** PDF file, 0.06 MB.
**TEXT S2,** PDF file, 0.03 MB.

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S.Y. is the inventor for BYG technology patents (application no. PCT/EP2012/073011), and S.Y. and P.B. are the inventors for patent pending application no. 14773161.2.

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