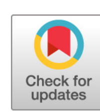
MECHANISMS OF RESISTANCE



NG-Test Carba 5 for Rapid Detection of Carbapenemase-Producing *Enterobacterales* from Positive Blood Cultures

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ABSTRACT The immunochromatographic assay, NG-test Carba 5 (NG Biotech), has been evaluated for detection of carbapenemase-producing *Enterobacterales* (CPE) from spiked blood cultures ($n = 205$). It detected and discriminated in less than 30 minutes KPC, IMP, VIM, NDM, and OXA-48-like producers with a sensitivity and specificity of 97.7% and 96.1%, respectively. Thus, it might help the rapid optimization of treatment of bloodstream infections due to CPE.

KEYWORDS IMP, KPC, NDM, OXA-48-like, VIM, immunochromatography, rapid diagnostic, septicemia

Carbapenem-resistance in *Enterobacterales* is increasing worldwide, mostly due to the dissemination of carbapenemase-producing isolates (1, 2). Most often, carbapenemase-producing *Enterobacterales* (CPE) are resistant to almost all β-lactams, and treatments options are very limited (1, 2). However, a few novel antimicrobial combinations, such as ceftazidime-avibactam (3–5) and meropenem-vaborbactam (6), have been commercialized recently for the treatment of KPC and OXA-48-like carbapenemase producers (for ceftazidime-avibactam). Unfortunately, ceftazidime-avibactam is not active on metallo-β-lactamase (MBL) producers, and other alternatives have to be explored, such as combinations of remaining active antimicrobials (e.g., colistin, aminoglycosides, and/or fosfomycin) (7), or aztreonam in combination with ceftazidime-avibactam or amoxicillin-clavulanate (8–10). In this context, it is crucial to rapidly detect CPE isolates and identify the carbapenemase family to prevent further spread in hospitals and implement the most adequate therapy in case of infection.

Recently, a rapid diagnostic test (<15 min), the NG-Test Carba 5 (NG Biotech, Guipry, France), based on the immunochromatographic detection of the five most widespread carbapenemase families (i.e., KPC, NDM, VIM, IMP, and OXA-48-like enzymes) has been developed (11). This newly commercialized assay was demonstrated to perform well on bacterial colonies (11). Here, we evaluated the ability of the NG-Test Carba 5 to detect CPE isolates from positive blood cultures. Implementation of this technique might help to improve antimicrobial stewardship of patients with sepsis.

A total of 205 well-characterized isolates were included in this study. These strains included carbapenemase producers of KPC-type ($n = 22$) (Table S1), VIM-type ($n = 20$) (Table S2), IMP-type ($n = 10$) (Table S3), NDM-type ($n = 22$) (Table S4), OXA-48-type ($n = 43$) (Table S5), multiple-carbapenemase ($n = 15$) (Table S6), other-carbapenemase ($n = 15$) (Table S7), and noncarbapenemase producers ($n = 58$) (Table S8).

The detection of carbapenemase producers was performed on positive blood cultures, which were spiked with the isolates, as previously described (12). Briefly, a bacterial suspension of 0.5 McFarland (~10^8 CFU/ml) was diluted in sterile water to

Citation Takissian J, Bonnin RA, Naas T, Dortet L. 2019. NG-Test Carba 5 for rapid detection of carbapenemase-producing *Enterobacterales* from positive blood cultures. Antimicrob Agents Chemother 63:e00011-19. https://doi.org/10.1128/AAC.00011-19.

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Received 3 January 2019
Returned for modification 30 January 2019
Accepted 17 February 2019
Accepted manuscript posted online 25 February 2019
Published 25 April 2019

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obtain 10^4 CFU/ml, and 1 ml of this suspension was finally inoculated in 10 ml of sterile total human blood ($\sim 1 \times 10^3$ CFU/ml), which was subsequently transferred into the blood culture bottles (Bact/Alert aerobic and anaerobic bottles without charcoal; bioMérieux, La Balme-les-Grottes, France).

Blood culture bottles were incubated until positivity of the blood culture was detected by the Bact/Alert 3D system (bioMérieux). Detection times ranged from 6 to 15 h, as previously described (11). The NG-Test Carba 5 was performed on positive blood cultures (Fig. S1). Briefly, 500 μl of the positive blood culture was centrifuged at 12,000 $\times$ g for 2 min. The bacterial pellet was resuspended in Triton 1$\times$ (1/10 volume/volume dilution of Triton 100X; Sigma), and centrifuged at 12,000 $\times$ g for 2 min. The supernatant was discarded, and the pellet was resuspended in 5 drops of the NG-Test Carba 5 lysis buffer. One hundred μl of the suspension were loaded onto the NG-Test Carba 5 cassette using the pipette included in the kit. Test results were read by eye after 15 min of migration at room temperature. All of the samples were blindly tested in a blind manner.

The NG-Test Carba 5 allowed the detection of 100% of KPC (Table S1), IMP (Table S3), OXA-48-like (Table S5), and multiple-carbapenemase producers (Table S6). It detected 90% and 95.5% of the VIM (Table S2) and NDM producers (Table S4), respectively. Although 3 undetected isolates (2 VIM and 1 NDM) did not have very high MIC values, which might corroborate low expression of the carbapenemase, other VIM and NDM producers with lower MICs to carbapenems were detected. All rare carbapenemases (IMI, NMC-A, GES, SME, FRI-1, GIM-1, and OXA-372) that are not targeted by the test (Table S7) and isolates that did not produce any carbapenemase (Table S8) were not detected. Of note, since the NG-Test Carba 5 was not able to discriminate OXA-48-like variants with carbapenemase activity (OXA-48, OXA-162, OXA-181, OXA-204, OXA-232, and OXA-244) from variants deprived of carbapenemase activity (OXA-163-like and OXA-405) (11), the 2 OXA-163- and the OXA-405-producing isolates might be considered to be falsely positive for OXA-48-like carbapenemase producers (Table S5). Overall, the sensitivity and specificity of the NG-Test Carba 5 for the detection and identification of the "big five carbapenemase"-producing *Enterobacterales* isolates from positive blood cultures were 97.7% (95% confidence interval [CI] = 92.8% to 99.4%) and 96.1% (95% CI = 88.1% to 99.0%), respectively (Table 1). These results are in agreement with the recent evaluation of the Resist-3 O.K.N. immunochromatographic lateral flow test (ICT) (Coris BioConcept, Gembloux, Belgium), another immunochromatographic assay designed to detect OXA-48-like, KPC, and NDM carbapenemase, which was tested on positive blood cultures (13). In this study, addition of 10 μl of 0.5 M ZnSO4 to the aliquot of positive blood cultures and an additional incubation for 15 min before the first pelleting step were added in the protocol to restore a positive signal for some NDM producers that were not detected. This protocol was applied for three undetected metallo-β-lactamase producers (2 VIM and 1 NDM), but the signal remained negative.

In countries with high CPE prevalence, the use of the NG-Test Carba 5 on positive blood cultures might be useful for the rapid management of patients, in particular for the early administration of ceftazidime-avibactam in case of KPC or OXA-48-like positivity (3, 4, 14). In the case of a positive signal with an MBL (NDM, VIM, or IMP), other therapeutic alternatives might be considered (7–10).

This study has some limitations. First, it remains a proof-of-principle study performed on spiked blood cultures that do not necessarily reflect a real clinical scenario. Further prospective studies are needed to assess the performance of the NG-Test Carba 5 in routine diagnostics. In addition, only blood culture bottles from the Bact/Alert 3D system (bioMérieux) were used, and results have to be confirmed with other systems. Finally, this protocol still requires a few centrifugation and pipetting steps; there might be a room for improvement in order to work directly on the positive blood culture sample.

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TABLE 1 Summary of NG-Test Carba 5 results from positive spiked blood cultures

β-Lactamase	No. of positive tests/total no. of isolates	Sensitivity (% [95% CI])	Specificity (% [95% CI])
KPC enzymes	22/22	100 (81.5–100)	100 (97.3–100)
KPC-2	19/19		
KPC-3	3/3		
NDM enzymes	21/22	95.5 (75.1–99.8)	100 (97.4–100)
NDM-1	12/13		
NDM-4	2/2		
NDM-5	2/2		
NDM-6	2/2		
NDM-7	2/2		
NDM-9	1/1		
VIM enzyme	18/20	90.0 (66.9–98.2)	100 (97.5–100)
VIM-1	11/13		
VIM-2	2/2		
VIM-4	3/3		
VIM-5	1/1		
VIM-19	1/1		
IMP enzymes	10/10	100 (65.5–100)	100 (97.6–100)
IMP-1	4/4		
IMP-8	5/5		
IMP-11	1/1		
OXA-48-like enzymes	43/43	100 (89.8–100)	100 (97.1–100)
OXA-48	20/20		
OXA-162	2/2		
OXA-163[a]	2/2		
OXA-181	8/8		
OXA-204	5/5		
OXA-232	3/3		
OXA-244	2/2		
OXA-405[a]	1/1		
Multiple carbapenemases	15/15	100 (74.7–100)	100 (97.5–100)
NDM-1 + OXA-48	3/3		
NDM-1 + OXA-181	5/5		
NDM-1 + OXA-232	2/2		
NDM-5 + OXA-48	1/1		
NDM-5 + OXA-232	1/1		
NDM-7 + OXA-181	1/1		
VIM-4 + OXA-48	1/1		
KPC-4 + NDM-7	1/1		
Detection of the "big five" carbapenemases[b]		97.7 (92.8–99.4)	96.1 (88.1–99.0)
Other carbapenemases	0/15		
IMI-1	0/1		
IMI-2	0/2		
NMC-A	0/1		
SME-1	0/1		
SME-2	0/1		
GES-5	0/1		
GIM-1	0/1		
FRI-1	0/1		
OXA-23	0/5		
OXA-372	0/1		
Noncarbapenemase producers	58/58		
Acquired cephalosporinase	0/4		
ESBLs	0/14		
ESBL or cephalosporinase + impermeability	0/40		

[a]OXA-163 and OXA-405 are truly detected as OXA-48-like enzymes, but they are deprived of any carbapenemase activity.
[b]OXA-163 and OXA-405 producers were considered false-positive results for sensitivity and specificity calculations.

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SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00011-19.

SUPPLEMENTAL FILE 1, PDF file, 4.7 MB.

ACKNOWLEDGMENTS

This work was partially supported by the University Paris-Sud, France, and Assistance Publique des Hôpitaux de Paris, Paris, France.

L.D., R.A.B., and T.N. are members of the Laboratory of Excellence in Research on Medication and Innovative Therapeutics (LERMIT), which is supported by a grant from the French National Research Agency (ANR-10-LABX-33).

We have no conflicts of interest to declare.

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