Diagnostic Microbiology and Infectious Disease 98 (2020) 115127

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Bacteriology

Fast and reliable detection of carbapenemase genes in various Gram negatives using a new commercially available fluorescence-based real-time polymerase chain reaction platform

Mustafa Sadek [a], Anthony Demord [b], Laurent Poirel [a,b,c], Patrice Nordmann [a,b,c,d,*]

[a] Medical and Molecular Microbiology, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland
[b] Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg
[c] INSERM European Unit (IAME), University of Fribourg, Fribourg
[d] Institute for Microbiology, University of Lausanne and University Hospital Centre, Lausanne, Switzerland

ARTICLE INFO

Article history:
Received 10 March 2020
Received in revised form 24 June 2020
Accepted 30 June 2020
Available online 8 July 2020

Keywords:
Carbapenemase
Gram-negatives
Rapid diagnostics
Real-time PCR

ABSTRACT

The Revogene® Carba C assay is a real-time polymerase chain reaction–based assay that runs on the microfluidic Revogene platform. It was recently designed for the detection of genes encoding the 5 major carbapenemases (NDM, VIM, IMP, KPC, and OXA-48) from various Gram-negatives. A total of 145 clinical Gram-negative strains (96 carbapenemase producers and 49 non–carbapenemase producers) were tested. The overall sensitivity and specificity were 100%. All strains co-producing double carbapenemases have been correctly detected. All non–carbapenemase producers and nontargeted carbapenemase producers gave a negative result. The sample preparation was easy to handle, taking around 5 to 10 min per isolate, with a run time of approximately 70 min. This assay is a rapid, easy-to-perform, reliable tool to detect the most common carbapenemases, with excellent sensitivity and specificity regardless of the host bacteria. Given its user friendliness, simplicity, and short time to result, the Revogene® Carba C assay is suitable for microbiology laboratories.

Currently, the alarming emergence and spread of carbapenem resistance among Gram-negative pathogens constitute a major global health threat associated with significant mortality (Pitout and Laupland, 2008; Schwaber and Carmeli, 2008). Resistance to carbapenem is mainly mediated by overproduction of an AmpC- or extended-spectrum β-lactamase–type enzyme combined with porin loss/modification, or by acquisition of plasmid or chromosomal resistance genes encoding serine carbapenemases or metallo-β-lactamase enzymes (Nordmann et al., 2011; Thomson, 2010; Yang et al., 2009). A variety of carbapenemases have been reported in Enterobacterales (Nordmann et al., 2012a) and *Pseudomonas* spp. (Gupta, 2008; Mesaros et al., 2007). The major carbapenemases are grouped into 3 classes according to their amino acid identity: *Klebsiella pneumoniae* carbapenemase (KPC) enzymes (Ambler class A); New Delhi metallo-β-lactamase (NDM), the Verona Integron-encoded metallo-β-lactamase (VIM), and the IMiPenemase (IMP) enzymes (all Ambler class B); and OXA-48-types and its derivatives (Ambler class D) (Nordmann et al., 2011). The early and accurate detection of carbapenemase-producing organisms (CPOs) in patients being infected or colonized is of utmost importance not only to minimize the CPOs spread but also to assist the clinical decision making for treating infected patients and for infection control purposes. Phenotypic tests recently developed for the rapid detection of carbapenemases are based on the biochemical detection of the carbapenem hydrolysis (Mancini et al., 2017; Nordmann et al., 2012b).

Recently, a novel real-time polymerase chain reaction (PCR)–based assay, Revogene® Carba C assay (formerly GenePOC™ Carba Assay), has been designed. It is a multiplex nucleic acid–based in vitro diagnostic test which allows detection of the 5 major carbapenemases (NDM, VIM, IMP, KPC, and OXA-48) in Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* isolates.

It was reported to be 100% sensitive and specific for carbapenemase-producing Enterobacterales (Girlich et al., 2020). It detects the main carbapenemases encountered in Enterobacterales (KPC, GES, NDM, VIM, IMP, OXA-48-like) and in *P. aeruginosa* (VIM, IMP, NDM, and KPC) but only the less prevalent ones in *A. baumannii* (VIM, IMP, NDM) (Baeza et al., 2019; Girlich et al., 2020). As part of the mission of our national reference center in Switzerland, the present study aimed to evaluate the Revogene® Carba C assay using carbapenemase and non–carbapenemase producers of various Gram-negative species.

A well-characterized panel of 145 clinical Gram-negative strains (96 carbapenemase producers and 49 non–carbapenemase producers) from the Medical and Molecular Microbiology Unit, Faculty of Science and Medicine, University of Fribourg, Switzerland, was tested with the

* Corresponding author. Tel.: +41-26-300-9581.
E-mail address: patrice.nordmann@unifr.ch (P. Nordmann).

https://doi.org/10.1016/j.diagmicrobio.2020.115127

Table 1
Results of the Revogene® Carba C assay on Enterobacterales.

Species (*n*)	Resistance determinant (*n*)	Revogene® Carba C assay
KPC ($n = 15$)		
Klebsiella pneumoniae (13)	KPC-2 (6)	Positive
	KPC-3 (6)	Positive
	KPC-11(1)	Positive
Enterobacter cloacae (1)	KPC-2 (1)	Positive
Escherichia coli (1)	KPC-3 (1)	Positive
NDM ($n = 13$)		
Klebsiella pneumoniae (2)	NDM-1 (1)	Positive
	NDM-7 (1)	Positive
Escherichia coli (9)	NDM-1 (1)	Positive
	NDM-4 (2)	Positive
	NDM-5 (5)	Positive
	NDM-6 (1)	Positive
Citrobacter freundii (1)	NDM-1 (1)	Positive
Enterobacter cloacae (1)	NDM-1 (1)	Positive
VIM ($n = 4$)		
Klebsiella pneumoniae (2)	VIM-1 (1)	Positive
	VIM-19 (1)	Positive
Escherichia coli (1)	VIM-19 (1)	Positive
Proteus mirabilis (1)	VIM-1 (1)	Positive
IMP ($n = 2$)		
Serratia marcescens (1)	IMP-11 (1)	Positive
Klebsiella pneumoniae (1)	IMP-8 (1)	Positive
OXA-48-like with carbapenemase activity ($n = 15$)		
Klebsiella pneumoniae (6)	OXA-48 (1)	Positive
	OXA-162 (1)	Positive
	OXA-181 (1)	Positive
	OXA-204 (1)	Positive
	OXA-232 (2)	Positive
Escherichia coli (8)	OXA-48 (2)	Positive
	OXA-181 (1)	Positive
	OXA-204 (1)	Positive
	OXA-244 (3)	Positive
Citrobacter freundii (1)	OXA-162 (1)	Positive
Double carbapenamases ($n = 9$)		
Klebsiella pneumoniae (6)	NDM-1 + OXA-181 (5)	Positive
	NDM-1 + OXA-48 (1)	Positive
Escherichia coli (1)	OXA-48+ VIM-1 (1)	Positive
Morganella morganii (2)	NDM-1+ OXA-232 (1)	Positive
	NDM-1+ OXA-181 (1)	Positive
OXA-48-like without carbapenemase activity ($n = 8$)		
Klebsiella pneumoniae (2)	OXA-436 (1)	Negative
	OXA-163 (1)	Negative
Enterobacter cloacae (3)	OXA-163 (2)	Negative
	OXA-427 (1)	Negative
Enterobacter asburiae (1)	OXA-436 (1)	Negative
Citrobacter freundii (1)	OXA-436 (1)	Negative
Serratia marcescens (1)	OXA-405 (1)	Negative
Carbapenem-resistant non–carbapene mase producers ($n = 20$)		
Klebsiella pneumoniae (9)	CTX-M gp1 (4), DHA (2), CMY (1), SHV-11+ TEM-1 + DHA (1), CTX-M-15 + TEM-1 + SHV-11 (1)	Negative
Enterobacter cloacae (3)	Overexpressed cephalosporinase (1), wild type (2)	Negative
Enterobacter aerogenes (2)	Wild type (2)	Negative
Morganella morganii (2)	Wild type (2)	Negative
Proteus mirabilis (3)	TEM-211 (1), TEM (1), wild type (1)	Negative
Serratia marcescens (1)	Wild type (1)	Negative
Carbapenem-susceptible strains ($n = 20$)		
Escherichia coli (10)	Wild type (3), OXA-1 (2), OXA-10 (1), TEM (2), CTX-M gp1 (1), CTX-M gp9 (1),	Negative
Klebsiella pneumoniae (5)	CTX-M gp1 (3), SHV-5 (1), CTX-M gp1 + TEM+ SHV (1)	Negative
Klebsiella oxytoca (2)	Koxy (2)	Negative
Enterobacter aerogenes (1)	TEM-116(1)	Negative
Proteus mirabilis (1)	Wild type (1)	Negative
Serratia marcescens (1)	Overexpressed cephalosporinase (1)	Negative

GenePOC™ Carba Assay using the Revogene instrument. This collection included 66/96 Enterobacterales (*Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter cloacae*, *Enterobacter asburiae*, *Citrobacter freundii*, *Proteus mirabilis*), 19/96 *Acinetobacter* spp. (*Acinetobacter junii, A. baumannii*), and 11/96 *Pseudomonas* spp. (*P. stutzeri*, *P. putida*, and *P. aeruginosa*). The carbapenemase types were as follows: KPC ($n = 15$), VIM ($n = 10$), IMP ($n = 10$), NDM ($n = 14$), and OXA-48-like ($n = 15$; OXA-162, OXA-181, OXA-204, OXA-232, OXA-244 and OXA-48). There were also producers of OXA-48-like enzymes lacking carbapenemase activity ($n = 8$; OXA-436, OXA-163, OXA-427, and OXA-405) and nontargeted carbapenemase producers ($n = 15$; OXA-23, OXA-40, OXA-58, OXA-58+ IS*Aba1*, OXA-

58 + IS*18*). Noteworthy, 8 isolates coproducing the carbapenemases NDM + OXA-48-like and a single isolate coproducing VIM + OXA-48-like enzymes were included. In addition, 49 non–carbapenemase producers were tested that included 26 carbapenem-resistant (20 Enterobacterales and 6 *P. aeruginosa*) strains but not CPE and 23 carbapenem-susceptible strains (20 Enterobacterales, 2 *P. aeruginosa*, and 1 *A. baumannii*). The 26 carbapenem-resistant strains were resistant or intermediate at least to 1 carbapenem. The antimicrobial resistance profiles of the strains were determined by disk diffusion according to EUCAST (http://www.eucast.org/clinical_breakpoints/) guidelines. The minimal inhibitory concentrations (MICs) of ertapenem, meropenem, and imipenem were determined using Etest strips (bioMérieux, La Balme-les-Grottes, France) on Mueller–Hinton agar plates at 37 °C, and the results were interpreted according to the latest EUCAST breakpoints (data not shown). Phenotypic carbapenemase production was confirmed by using the Carba NP test (Nordmann et al., 2012b). All strains had previously been characterized for their ß-lactamase content and carbapenemase encoding genes by PCR approaches followed by subsequent DNA sequencing.

Revogene® Carba C assay. The Revogene® Carba C assay was performed according to the manufacturer's recommendations on fresh overnight bacterial colonies grown on URISelect™ 4 agar plates (Bio-Rad, Cressier, Switzerland) or Mueller–Hinton agar plates for 16 h at 37 °C. Bacterial colonies were suspended in NaCl 0.9% to obtain 0.5 McFarland suspension. Then, using a micropipette, 15 μL of each bacterial suspension was added to the sample buffer tube. After subsequent vortexing for 15 s, 120–200 μL of sample buffer was transferred into the sample loading chamber of the microfluidic cartridge. Following the manufacturer's instructions, a maximum of 8 cartridges was used for every run in the Revogene® instrument, using mock cartridges when less than 8 samples were processed. All strains have been tested in duplicate. Around 5 to10 min per isolate was needed for sample preparation, with a run time of approximately 70 min on the instrument for extraction, real-time PCR, and detection.

Out of 145 clinical Gram-negative isolates evaluated with Revogene® Carba C assay, 96 were carbapenemase producers. The results were interpreted by the Revogene® software and provided a positive or negative test result, without Ct values, for a given gene. The results were reported in Tables 1 and 2. The Revogene® Carba C assay allowed the identification of all KPC, OXA-48-types, NDM, VIM, and IMP producers. Noteworthy, this assay was able to detect the 2 carbapenemases in those isolates co-producing NDM-1 and OXA-48-type enzymes and in those isolates co-producing VIM and OXA-48-like enzymes, resulting in a sensitivity and specificity of 100%. For Enterobacterales, the Revogene® Carba C assay detected all KPC, NDM, VIM, IMP, and OXA-48 producers including OXA-48-like variants exhibiting carbapenemase activity (OXA-162, -181, -204, -232), and those conferring unusual and known to be difficult to detect when relying on phenotypic assays, such as OXA-244 known to be difficult to detect when relying only on phenotypic approaches (Hoyos-Mallecot et al., 2017; Potron et al., 2015). A single NDM-5–producing *E. coli* strain, which initially tested indeterminate, was positive for NDM upon repetition. Another OXA-232–producing *K. pneumoniae* strain, which initially tested positive for OXA-48-like, turned OXA-48 and NDM positive. The same strain tested positive for MBL phenotype and was NDM-1 positive by PCR and sequencing.

The Revogene® Carba C assay failed to detect those OXA-48-like variants (OXA-163, OXA-405, OXA-427, and OXA-436) that lack carbapenemase activity. The Revogene® Carba C assay has this particular advantage of differentiating those latter OXA-48 variants. This result is consistent with previous study indicating that OXA-163, OXA-405, and OXA-436 may not be detected by the Revogene® Carba C assay (Girlich et al., 2020). None of the non–carbapenemase producers yielded positive results. All double carbapenemase producers ($n = 9$) were correctly detected. The overall sensitivity for carbapenemase detection of the Revogene® Carba C assay was 100%. In addition, it showed a specificity of 100%. For detection of carbapenemase-producing *Pseudomonas* and *Acinetobacter* species, all the tested IMP (IMP-1, -4, -5, -10, -13, -15, -19, -29), VIM (VIM-2, -4, -5), and NDM-1 producers were perfectly detected (Table 2), resulting in a sensitivity and specificity of 100%. The most widespread OXA-carbapenemases usually encountered in *A. baumannii* (OXA-23-like, OXA-40-like, OXA-58-like) are not targeted by this assay. All carbapenem-resistant non–carbapenemase producers and carbapenem-susceptible isolates gave negative results.

One limitation point of Revogene® Carba C assay is its ability to detect only known enzymes compared to the Rapidec Carba NP or the β-Carba test, biochemical tests previously proven to be sensitive for rare carbapenemase detection (Mancini et al., 2017; Nordmann et al., 2012b). Nevertheless, we believe that this assay may be useful as a second-line test after usage of the broad-spectrum carbapenemase screening rapid tests based on biochemistry. In conclusion, the Revogene® Carba C assay is a rapid, easy-to-perform, reliable tool to detect the most common carbapenemase families identified in Gram-negative bacteria, with excellent sensitivity and specificity regardless of the host bacteria. Given its user friendliness, simplicity, and short time to result, the Revogene® Carba C assay is suitable for microbiology laboratories.

Declaration of competing interest

This authors have no conflict of interest to declare. PN was member of the advisory board of GenPoC until beginning of 2019.

Table 2
The results of the Revogene® Carba assay on *Pseudomonas* and *Acinetobacter* sp.

Species (n)	Resistance determinant	Revogene® Carba C assay
IMP ($n = 8$)		
Pseudomonas aeruginosa (5)	IMP-10 **(1)**	Positive
	IMP-13 **(1)**	Positive
	IMP-15 **(1)**	Positive
	IMP-19 **(1)**	Positive
	IMP-29 **(1)**	Positive
Acinetobacter baumannii (2)	IMP-4 **(1)**	Positive
	IMP-5 **(1)**	Positive
Acinetobacter junii (1)	IMP-1 **(1)**	Positive
VIM ($n = 6$)		
Pseudomonas aeruginosa (3)	VIM-2 **(1)**	Positive
	VIM-4 **(2)**	Positive
Pseudomonas stutzeri (1)	VIM-2 **(1)**	Positive
Pseudomonas putida (2)	VIM-2 **(1)**	Positive
	VIM-5 **(1)**	Positive
NDM ($n = 1$)		
Acinetobacter baumannii (1)	NDM-1 **(1)**	Positive
Carbapenem-resistant non–carbapenemase producers (6)		
Pseudomonas aeruginosa (6)	GES-5 **(1)**	Negative
	Overexpressed cephalosporinase and/or decreased permeability **(5)**	Negative
Nontargeted carbapenemase producers		
Acinetobacter baumannii (15)	OXA-23 (4), OXA-40 (4), OXA-58 (5), OXA-58 (+IS*Aba2*) (1), OXA-58 (+IS18) (1)	Negative
Carbapenem-susceptible strains (3)		
Pseudomonas aeruginosa (2)	Wild type **(2)**	Negative
Acinetobacter baumannii (1)	Wild type **(1)**	Negative

Acknowledgments

This work was funded by the Swiss National Science Foundation (project numbers FNS-407240_177381 and FNS-407240_177382).

Authors' statement

LP and PN designed the study. MS and AD performed the experiments. MS drafted the manuscript. LP and PN wrote the final version of the manuscript.

References

Baeza LL, Pfennigwerth N, Hamprecht A. Rapid and easy detection of carbapenemases in Enterobacterales in the routine laboratory using the new GenePOC Carba/Revogene Carba C assay. J Clin Microbiol 2019;57, e00597–19.

Girlich D, Laguide M, Dortet L, Naas T. Evaluation of the Revogene® Carba C Assay for detection and differentiation of carbapenemase-producing Gram negative bacteria. J Clin Microbiol 2020;58, e01927-19.

Gupta V. Metallo-β-lactamases in *Pseudomonas aeruginosa* and *Acinetobacter* species. Expert Opin Investig Drugs 2008;17:131–43.

Hoyos-Mallecot Y, Naas T, Bonnin RA, Patino R, Glaser P, Fortineau N, et al. OXA-244–producing *Escherichia coli* isolates, a challenge for clinical microbiology laboratories. Antimicrob Agents Chemother 2017;61, e00818–17.

Mancini S, Kieffer N, Poirel L, Nordmann P. Evaluation of the RAPIDEC® CARBA NP and β-CARBA® tests for rapid detection of carbapenemase-producing *Enterobacteriaceae*. Diagn Microbiol Infect Dis 2017;88:293–7.

Mesaros N, Nordmann P, Plésiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, et al. *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect 2007;13:560–78.

Nordmann P, Dortet L, Poirel L. Carbapenem resistance in *Enterobacteriaceae*: here is the storm! Trends Microbiol 2012a;18:263–72.

Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 2011;17:1791–8.

Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 2012b;18:1503–7.

Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. Lancet Infect 2008;8:159–66.

Potron A, Poirel L, Dortet L, Nordmann P. Characterisation of OXA-244, a chromosomally-encoded OXA-48-like β-lactamase from *Escherichia coli*. Int J Antimicrob Agents 2015;47:102–3.

Schwaber MJ, Carmeli Y. Carbapenem-resistant *Enterobacteriaceae*: a potential threat. JAMA 2008;300:2911–3.

Thomson KS. Extended-spectrum-β-lactamase, AmpC, and carbapenemase issues. J Clin Microbiol 2010;48:1019–25.

Yang D, Guo Y, Zhang Z. Combined porin loss and extended spectrum β-lactamase production is associated with an increasing imipenem minimal inhibitory concentration in clinical *Klebsiella pneumoniae* strains. Curr Microbiol 2009;58:366–70.