Journal of Global Antimicrobial Resistance 18 (2019) 100–103

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

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

Performance evaluation of the PANA RealTyper™ CRE Kit for detecting carbapenemase genes in Gram-negative bacilli

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ARTICLE INFO

Article history:
Received 11 December 2018
Received in revised form 15 January 2019
Accepted 4 February 2019
Available online 11 February 2019

Keywords:
Carbapenemase-producing organism
Carbapenemase
Evaluation
Real-time PCR
Gram-negative bacilli

ABSTRACT

Objectives: The spread of carbapenemase-producing organisms has been continuously reported over the past decade. Rapid and accurate detection of carbapenemase production is essential for adequate infection control and appropriate antimicrobial treatment in the clinical setting. In this study, the performance of the newly developed PANA RealTyper™ CRE Kit (PANAGENE, Daejeon, South Korea) for the detection of six common carbapenemase genes (bla_{KPC}, bla_{GES}, bla_{IMP}, bla_{NDM}, bla_{VIM} and $bla_{OXA-48-like}$) was evaluated.

Methods: A total of 479 non-duplicate clinical isolates of Gram-negative bacilli, including 391 carbapenemase-producers and 88 non-producers, were tested. Conventional PCR and sequencing were performed as reference methods for performance evaluation of the PANA RealTyper™ CRE Kit.

Results: The PANA RealTyper™ CRE Kit showed a reliable performance for the detection of carbapenemase-producing organisms compared with the monoplex PCR system, with sensitivity and specificity values both >95%, with only a few discrepant results for six types of carbapenemase genes.

Conclusion: This kit is rapid and accurate for simultaneously detecting various carbapenemase genes in a single reaction and could contribute to early decisions for appropriate antimicrobial treatment in the clinical setting.

1. Introduction

The emergence of antimicrobial resistance in pathogenic bacteria has become a serious threat to human public health worldwide [1]. Gram-negative bacilli (GNB) producing β-lactamases with expanded-spectrum hydrolysing activities, including extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases, are widely disseminated [2]. Notably, carbapenemase-producing organisms have been steadily increasing over the past decade [3]. Carbapenemase genes are usually located on mobile genetic elements that contribute to the spread of antimicrobial resistance between bacterial species [4]. Therefore, rapid and accurate detection of carbapenemase genes is essential for adequate infection control and appropriate antimicrobial treatment in the clinical setting.

In South Korea, since the first identification of carbapenemase-producing *Pseudomonas aeruginosa* in the early 2000s, variable classes of carbapenemases have been increasingly reported [5]. Among classes A, B and D, six genes encoding KPC, GES, IMP, NDM, VIM and OXA-48-like β-lactamases are the most prevalent in multidrug-resistant GNB, in particular *Klebsiella pneumoniae* strains carrying bla_{KPC-2} and *Escherichia coli* strains carrying bla_{NDM-1} or $bla_{OXA-48-like}$ [6].

Phenotypic confirmation tests have been developed for the detection of carbapenemases [7,8]; however, these culture-based methods are time consuming and show variable rates of detection failure when strains exhibit low-level expression of resistance genes [9]. In this regard, molecular detection for responsible genes has been considered the gold-standard method. Conventional PCR amplification in a single or multiple system is the most commonly used method [10]. Recently, real-time multiplex PCR with a peptide nucleic acid (PNA) probe-based melting curve analysis has been developed and can simultaneously amplify various target genes in a single reaction tube [11,12].

This study evaluated the performance of the PANA RealTyper™ CRE Kit (PANAGENE, Daejeon, South Korea) for the detection of six major carbapenemase genes (bla_{KPC}, bla_{GES}, bla_{IMP}, bla_{NDM}, bla_{VIM} and $bla_{OXA-48-like}$) in a single tube against clinical GNB isolates and compared it with conventional molecular methods.

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https://doi.org/10.1016/j.jgar.2019.02.002

2. Materials and methods

2.1. Clinical isolates

A total of 479 non-duplicate clinical isolates of GNB, comprising 391 carbapenemase-producers and 88 non-carbapenemase-producers, were used to evaluate the PANA RealTyper™ CRE Kit. Among the 391 carbapenemase-producing isolates, 86 (22.0%) producing KPC, 81 (20.7%) producing NDM, 82 (21.0%) producing OXA-48-like, 40 (10.2%) producing VIM, 44 (11.3%) producing IMP, 40 (10.2%) producing GES-type and 18 (4.6%) producing multiple carbapenemases were included in this study (Table 1). Bacterial species identification was performed using a Bruker MALDI MS instrument (Bruker Daltonik GmbH, Bremen, Germany).

2.2. Conventional PCR method

Conventional PCR and sequencing were performed as reference methods. Bacterial DNA was extracted by the boiling lysis method. PCR was performed using a C1000™ Thermal Cycler (Bio-Rad, Hercules, CA) under the following conditions: 94 °C for 5 min; followed by 30 cycles at 94 °C for 30 s, then 58 °C (bla_{KPC}), 55 °C (bla_{GES}) or 60 °C (bla_{IMP}, bla_{NDM}, bla_{VIM} and $bla_{OXA-48-like}$) for 30 s, and subsequently 72 °C for 30 s; and a final extension at 72 °C for 5 min. The primers used in this study are summarised in Supplementary Table S1.

2.3. PANA RealTyper™ CRE Kit assay

The PANA RealTyper™ CRE Kit is a Conformité Européenne-marked diagnostic reagent for multiplex real-time PCR. This kit is designed to detect six types of carbapenemase genes, including bla_{KPC}, bla_{GES}, bla_{IMP}, bla_{NDM}, bla_{VIM} and $bla_{OXA-48-like}$, in a single-tube PCR using PNA probe-based fluorescence melting curve analysis technology. These PNA probes are designed to hybridise to specific targets with unique melting temperature (T_m) values and fluorescent dyes (FAM, HEX, ROX and Cy5). In this study, experiments using the PANA RealTyper™ CRE Kit were performed according to the manufacturer's instructions. Briefly, the PCR reagent was prepared in 20 μL of a mixture containing 19 μL of the CRE Mix of PNA probes and primers and 1 μL of *Taq* DNA polymerase. Then, 5 μL of extracted DNA was added to the PCR reagent [13]. Each genotype-specific PNA probe, which was conjugated with a fluorescent dye and quencher, was used as a reporter in a real-time PCR. PCR was performed using a CFX96™ Real-Time System (Bio-Rad) with the following conditions: 50 °C for 2 min and 95 °C for 15 min as the two holding periods; followed by 45 cycles at 95 °C for 15 s, 58 °C for 45 s, 72 °C for 15 s, 95 °C for 5 min and 35 °C for 5 min; and a melting-curve step (from 35 °C to 80 °C with gradual 0.5 °C increments for 5 s). Detection of target genes was determined by measuring each fluorescent dye and T_m value using Bio-Rad CFX Manager software v.1.6.548.0827 (Bio-Rad).

3. Results

The results of the isolate-by-isolate relationship between the PANA RealTyper™ CRE Kit and the conventional method for detecting carbapenemases are presented in Supplementary Table S2. The overall sensitivity, specificity, positive predictive value and negative predictive value of the PANA RealTyper™ CRE Kit were 99.5% (409/411), 99.7% (2455/2463), 98.1% (409/417) and 99.9% (2455/2457), respectively (Table 2).

Among the 373 clinical isolates carrying one carbapenemase gene, all KPC-producers (100%; 86/86) were correctly detected by the PANA RealTyper™ CRE Kit; in addition, 40 GES-producers, 44 IMP-producers and 82 OXA-48-like producers agreed with the reference method. Among the NDM-producers, most (80/81;

Table 1
Clinical carbapenemase-producing isolates used in this study.

Carbapenemase (*n*)	Variant (*n*)	Species (*n*)
KPC (86)	KPC-2 (66)	*Klebsiella pneumoniae* (55), *Escherichia coli* (6), *Enterobacter* spp. (2), *Citrobacter koseri* (1), *Klebsiella oxytoca* (1), *Serratia marcescens* (1)
	KPC-3 (5)	*K. pneumoniae* (4), *E. coli* (1)
	KPC-4 (15)	*K. pneumoniae* (13), *E. coli* (1), *Enterobacter* sp. (1)
GES (40)	GES-5 (39)	*Enterobacter* spp. (12), *K. oxytoca* (11), *K. pneumoniae* (10), *Pseudomonas aeruginosa* (6)
	GES-24 (1)	*P. aeruginosa* (1)
IMP (44)	IMP-1 (4)	*K. pneumoniae* (3), *Enterobacter* sp. (1)
	IMP-4 (3)	*Enterobacter* spp. (3)
	IMP-6 (36)	*P. aeruginosa* (36)
	IMP-10 (1)	*P. aeruginosa* (1)
NDM (81)	NDM-1 (68)	*Enterobacter* spp. (26), *K. pneumoniae* (18), *E. coli* (16), *Citrobacter freundii* (6), *K. oxytoca* (1), *Citrobacter amalonaticus* (1)
	NDM-5 (10)	*E. coli* (9), *C. freundii* (1)
	NDM-7 (2)	*E. coli* (2)
	NDM-9 (1)	*E. coli* (1)
VIM (40)	VIM-1 (5)	*K. pneumoniae* (3), *C. freundii* (2)
	VIM-2 (35)	*Enterobacter* spp. (26), *K. pneumoniae* (5), *C. freundii* (1), *E. coli* (1), *K. oxytoca* (1), *S. marcescens* (1)
OXA-48-like (82)	OXA-48 (1)	*K. pneumoniae* (1)
	OXA-181 (1)	*K. pneumoniae* (1)
	OXA-232 (80)	*K. pneumoniae* (80)
Co-producers (18)	KPC-2 and NDM-1 (7)	*Raoultella planticola* (4), *Raoultella ornithinolytica* (1), *K. pneumoniae* (1), *C. freundii* (1)
	NDM-1 and OXA-232 (4)	*K. pneumoniae* (4)
	NDM-1 and OXA-181 (2)	*K. pneumoniae* (2)
	NDM-5 and OXA-181 (1)	*K. pneumoniae* (1)
	KPC-4, VIM-1 and NDM-1 (1)	*K. pneumoniae* (1)
	GES-5, VIM-2 and OXA-48 (1)	*C. freundii* (1)
	IMP-1 and VIM-2 (1)	*Enterobacter* sp. (1)
	VIM-2 and NDM-1 (1)	*E. coli* (1)

Table 2
Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the PANA RealTyper™ CRE Kit stratified according to the carbapenemase gene.

Carbapenemase gene	Sensitivity (%) (*n*/*n*)	Specificity (%) (*n*/*n*)	PPV (%) (*n*/*n*)	NPV (%) (*n*/*n*)
bla_{KPC}	98.9 (93/94)	99.7 (384/385)	98.9 (93/94)	99.7 (384/385)
bla_{GES}	100 (41/41)	99.8 (437/438)	97.6 (41/42)	100 (437/437)
bla_{IMP}	100 (45/45)	100 (434/434)	100 (45/45)	100 (434/434)
bla_{NDM}	99.0 (96/97)	99.7 (381/382)	99.0 (96/97)	99.7 (381/382)
bla_{VIM}	100 (44/44)	99.8 (434/435)	97.8 (44/45)	100 (434/434)
$bla_{OXA-48-like}$	100 (90/90)	99.0 (385/389)	95.7 (90/94)	100 (385/385)
Total	99.5 (409/411)	99.7 (2455/2463)	98.1 (409/417)	99.9 (2455/2457)

Table 3
Discrepant results between PANA RealTyper™ CRE Kit and the reference method.

Strain	Species	Reference method	PANA RealTyper™ CRE Kit
CRKP-1189	*Klebsiella pneumoniae*	KPC-4, VIM-1, NDM-1	KPC, VIM, NDM, GES
CRRPL-005	*Raoultella planticola*	KPC-2, NDM-1	NDM, OXA-48-like
CRKP-1372	*K. pneumoniae*	KPC-2, NDM-1	KPC, NDM, VIM
CREC-47	*Escherichia coli*	NDM-1	Negative
CRCB-81	*Citrobacter freundii*	VIM-2	VIM, OXA-48-like
CRENT-592	*Enterobacter* spp.	VIM-2	VIM, OXA-48-like, KPC
CRKP-2120	*K. pneumoniae*	VIM-2	VIM, OXA-48-like, NDM

98.8%) exhibited positive reactions for bla_{NDM}, but the PANA RealTyper™ CRE Kit did not yield a positive signal in the *E. coli* isolate harbouring bla_{NDM-1} (CREC-47). All 40 VIM-producers were correctly detected (100%), although three isolates (CRCB-81, CRENT-592 and CRKP-2120) also showed a positive signal for other carbapenemase genes with the PANA RealTyper™ CRE Kit (Table 3). Regarding the 88 non-carbapenemase-producers, none of the isolates presented a false-positive result by the PANA RealTyper™ CRE Kit.

Among the 18 isolates with multiple carbapenemases, most (15/18; 83.3%) were identified correctly by the PANA RealTyper™ CRE Kit, however 3 discrepant results were observed. The *K. pneumoniae* isolate (CRKP-1189) with bla_{KPC}, bla_{NDM} and bla_{VIM} identified by the reference method was shown to harbour bla_{KPC}, bla_{NDM}, bla_{VIM} and bla_{GES} by the PANA RealTyper™ CRE Kit (Table 3). In the case of one *Raoultella planticola* isolate (CRRPL-005) carrying bla_{KPC} and bla_{NDM}, only bla_{NDM} was detected by the PANA RealTyper™ CRE Kit, whereas bla_{KPC} was not detected. In addition, a false-positive signal in the $bla_{OXA-48-like}$ fraction was detected in this isolate by the PANA RealTyper™ CRE Kit. For KPC- and NDM-producing *K. pneumoniae* (CRKP-1372), both bla_{KPC} and bla_{NDM} were correctly identified by the kit; however, an additional bla_{VIM} signal was also detected, which was regarded as a false-positive result.

The seven isolates exhibiting discrepant results described above were tested by an additional confirmative method using conventional PCR with 40 cycles after newly extracting genomic DNA; however, the discrepant results did not change.

4. Discussion

The global spread of carbapenemase-producing organisms has become a critical public-health problem because carbapenems are the last resort for the treatment of infections by multidrug-resistant bacteria [1,4,14]. Therefore, accurate and rapid detection of resistance determinants to carbapenems is essential in clinical microbiology laboratories [15]. This study evaluated a newly developed real-time PANA RealTyper™ CRE Kit against a large number of non-duplicate clinical isolates of GNB.

The PANA RealTyper™ CRE Kit showed good performance in the detection of carbapenemase genes with high concordance rates (99.0%; 387/391) compared with the results of the conventional method. In addition, the PANA RealTyper™ CRE Kit was considered to have a similar performance in terms of accuracy when compared with the PANA qPCR™ CPE Detection Kit (PANAGENE Inc.), which is a previous version of this kit, and with Rapidec Carba NP (BD Diagnostic Systems, Sparks, MD), which is a phenotypic test for the detection of carbapenemase production [8,12].

Among 373 carbapenemase-producing organisms with one carbapenemase variant, there was only one false-negative result for an NDM-producer and there were three isolates with additional types of carbapenemase genes detected. For carbapenemase-producers with multiple carbapenemase variants, there were three discrepant results; however, any consistency in the carbapenemase type or the interference between fluorescent dyes was not observed. Therefore, the few discrepant results are likely considered to be random errors.

One limitation of the PANA RealTyper™ CRE Kit is that it provides only a 'positive' or 'negative' result for each carbapenemase gene without accurate genotype detected among the variants. Genotypes of carbapenemase genes are essential for epidemiological analysis in infection control [16]; however, these genotypes are not critical in treating patients with infection by a carbapenemase-producing organism in the clinical setting. This study has a limitation in that all non-carbapenemase-producers tested exhibited susceptibility to carbapenems. Although this kit is based on molecular methods, further evaluation using carbapenem-resistant Enterobacteriaceae not carrying carbapenemase genes should be performed.

In conclusion, the PANA RealTyper™ CRE Kit can identify the six most common types of carbapenemase genes with a single reaction and presents reliable performance compared with conventional methods against a large number of clinical isolates. Therefore, this kit would be a useful tool for the rapid and accurate detection of carbapenemase-producing organisms in clinical microbiology laboratories.

Funding

This work was supported by the research program funded by the Korea Centers for Disease Control and Prevention (2016-NI44001-00).

The funder of the study had no role in the study design, data collection, data interpretation, or writing of the report.

Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.02.002.

References

[1] Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, et al. The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect 2015;6:22–9.
[2] Molton JS, Tambyah PA, Ang BS, Ling ML, Fisher DA. The global spread of healthcare-associated multidrug-resistant bacteria: a perspective from Asia. Clin Infect Dis 2013;56:1310–8.
[3] Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA, et al. Carbapenemase-producing organisms: a global scourge. Clin Infect Dis 2018;66:1290–7.
[4] Hong JS, Yoon EJ, Lee H, Jeong SH, Lee K. Clonal dissemination of *Pseudomonas aeruginosa* sequence type 235 isolates carrying bla_{IMP-6} and emergence of bla_{GES-24} and bla_{IMP-10} on novel genomic islands PAGI-15 and -16 in South Korea. Antimicrob Agents Chemother 2016;60:7216–23.
[5] Lee K, Ha GY, Shin BM, Kim JJ, Kang JO, Jang SJ, et al. Metallo-β-lactamase-producing Gram-negative bacilli in Korean Nationwide Surveillance of Antimicrobial Resistance group hospitals in 2003: continued prevalence of VIM-producing *Pseudomonas* spp. and increase of IMP-producing *Acinetobacter* spp. Diagn Microbiol Infect Dis 2004;50:51–8.
[6] Yoon EJ, Kim JO, Kim D, Lee H, Yang JW, Lee KJ, et al. *Klebsiella pneumoniae* carbapenemase producers in South Korea between 2013 and 2015. Front Microbiol 2018;9:56.
[7] Maurer FP, Castelberg C, Quiblier C, Bloemberg GV, Hombach M. Evaluation of carbapenemase screening and confirmation tests with Enterobacteriaceae and development of a practical diagnostic algorithm. J Clin Microbiol 2015;53:95–104.
[8] Garg A, Garg J, Upadhyay GC, Agarwal A, Bhattacharjee A. Evaluation of the Rapidec Carba NP test kit for detection of carbapenemase-producing Gram-negative bacteria. Antimicrob Agents Chemother 2015;59:7870–2.
[9] Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G, et al. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 2018;73:909–15.
[10] Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70:119–23.
[11] Gambari R. Peptide nucleic acids: a review on recent patents and technology transfer. Expert Opin Ther Pat 2014;24:267–94.
[12] Jeong S, Kim JO, Jeong SH, Bae IK, Song W. Evaluation of peptide nucleic acid-mediated multiplex real-time PCR kits for rapid detection of carbapenemase genes in Gram-negative clinical isolates. J Microbiol Methods 2015;113:4–9.
[13] PANA RealTyper™ CRE Kit [package insert]. Daejeon, South Korea: PANAGENE Inc.; 2018.
[14] Choudhury S, Yeng JL, Krishnan PU. In vitro susceptibilities of clinical isolates of carbapenemase-producing Enterobacteriaceae to fosfomycin and tigecycline. Clin Microbiol Infect 2015;21:e75–6.
[15] Hrabak J, Chudackova E, Papagiannitsis CC. Detection of carbapenemases in Enterobacteriaceae: a challenge for diagnostic microbiological laboratories. Clin Microbiol Infect 2014;20:839–53.
[16] van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence 2017;8:460–9.