Diagnostic Microbiology and Infectious Disease xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

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

Evaluation of a new real-time PCR assay (Check-Direct CPE) for rapid detection of KPC, OXA-48, VIM, and NDM carbapenemases using spiked rectal swabs☆,☆☆,★,★★

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ARTICLE INFO

Article history:
Received 25 July 2013
Received in revised form 30 August 2013
Accepted 9 September 2013
Available online xxxx

Keywords:
Screening
Carbapenem resistance
Carriers
Antibiotic

ABSTRACT

To prevent the spread of carbapenemase-producing bacteria, a fast and accurate detection of patients carrying these bacteria is extremely important. The Check-Direct CPE assay (Check-Points, Wageningen, The Netherlands) is a new multiplex real-time PCR assay, which has been developed to detect and differentiate between the most prevalent carbapenemase genes encountered in Enterobacteriaceae (bla_{KPC}, bla_{OXA-48}, bla_{VIM}, and bla_{NDM}) directly from rectal swabs. Evaluation of this assay using 83 non-duplicate isolates demonstrated 100% sensitivity and specificity and the correct identification of the carbapenemase gene(s) present in all carbapenemase-producing isolates. Moreover, the limit of detection (LoD) of the real-time PCR assay in spiked rectal swabs was determined and showed comparable LoDs with the ChromID CARBA agar. With an excellent performance on clinical isolates and spiked rectal swabs, this assay appeared to be an accurate and rapid method to detect bla_{KPC}, bla_{OXA-48}, bla_{VIM}, and bla_{NDM} genes directly from a rectal screening swab.

1. Introduction

The emergence and spread of antimicrobial resistance conferred by the production of β-lactamases amongst Gram-negative bacteria is a serious problem, in the hospital environment as well as in the community. Due to the emergence of resistance by extended-spectrum β-lactamases (ESBLs), carbapenem antibiotics are used more often to treat patients with infections caused by Gram-negative bacteria. However, the extensive use of carbapenems is probably the most significant reason for an increasing resistance to this class of β-lactams by the production of carbapenemases. The major carbapenemases reported worldwide in Enterobacteriaceae comprise carbapenemases of Ambler class A (mostly bla_{KPC}), class B (metallo-β-lactamases; mostly bla_{VIM} and bla_{NDM} and, to a lesser extent, bla_{IMP}), and class D (oxacillinases; mostly, bla_{OXA-48}) (Ambler et al., 1991; Nordmann et al., 2011).

A higher mortality rate is observed in infections caused by carbapenemase-producing Enterobacteriaceae and some nonfermenters (such as *Pseudomonas aeruginosa* and *Acinetobacter* spp.) due to inappropriate empirical therapy, which urges for rapid detection (Daikos et al., 2009; Schwaber et al., 2008; Thatrimontrichai et al., 2013). Moreover, rapid detection in a hospital setting is crucial for immediate implementation of infection control safety measures to prevent further spread of resistance in the hospital environment (Kochar et al., 2009).

However, screening for carbapenemase-producing bacteria is time-consuming since it is usually performed using selective agars such as ChromID ESBL, ChromID CARBA (bioMérieux, Marcy-l'Etoile, France), CHROMagar KPC (CHROMagar, Paris, France), or SUPERcarba medium (Nordmann et al., 2012a). Although the ChromID CARBA, the SUPERcarba medium, and the less sensitive ChromID ESBL and CHROMagar KPC media are able to detect most carbapenemase-producing isolates, the inoculated plates have to be incubated overnight (Adler et al., 2011; Carrër et al., 2010; Nordmann et al., 2012a; Vrioni et al., 2012). Subsequently, the presence of carbapenemase producers on the culture medium has to be confirmed by additional testing (e.g., modified Hodge test, gradient strips, or inhibition studies with liquid or solid media), which can take up to another 48 hours (Nordmann et al., 2012b). Although the use of molecular techniques for confirmation (such as PCR or microarray analysis) or the recently developed biochemical Carba NP assay does shorten the turnaround time to some extent, this approach is not an adequate solution for the rapid detection in patient samples, which is required (Dortet et al., 2012; Doyle et al., 2012; Monteiro et al., 2012; Nordmann et al., 2012b; Nordmann et al., 2012c).

Recently, several molecular assays have been described detecting carbapenemase genes directly from urine (bla_{KPC}) (Peter et al., 2012), rectal swabs (bla_{KPC}) (Hindiyeh et al., 2008), or stool samples (bla_{NDM} and bla_{OXA-48}) (Naas et al., 2012; Naas et al., 2011b). These tests, able to detect only 1 carbapenemase gene each (bla_{KPC}, bla_{NDM}, or bla_{OXA-48}),

☆ Funding: This work was supported by a grant from the European Fund for Regional Development, number 2011-016128. Kits containing PCR primers and molecular beacon probes were provided by Check-Points B.V. and NYtor B.V.
☆☆ ØS is supported by a grant from the Northern Norway Regional Health Authority.
★ Transparency declarations: A.A. van Zwet is an advisor for Check-Points.
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http://dx.doi.org/10.1016/j.diagmicrobio.2013.09.007

are extremely useful in an outbreak situation caused by a known carbapenemase gene. However, since a variety of genes can be responsible for carbapenemase resistance, these tests will not be appropriate for screening of carbapenemase carriers. Here, we describe the Check-Direct CPE assay (Check-Points, Wageningen, The Netherlands), a new real-time PCR test for rapid and simultaneous detection of the β-lactamase genes *bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{VIM}$, and *bla*$_{NDM}$ that is developed to apply directly on rectal swabs.

2. Materials and methods

2.1. Bacterial isolates

Specificity, sensitivity, and limit of detection (LoD) of this new Check-Direct CPE assay were assessed with 62 unique well-characterized Gram-negative bacterial isolates with proven carbapenemase activity (Table 1), along with 21 non-carbapenemase producers (Table 2). All isolates were suspended in 0.45% saline (NaCl) up to a density of approximately 0.5 McFarland (~2*10^8 CFU/ml). In addition, a subset of the carbapenemase-producing isolates (as shown in Table 3) were suspended and used for spiking experiments.

Table 1
Carbapenemase-producing clinical isolates subjected to the Check-Direct CPE.

Ambler class, carbapenemase type	Isolate	N[a]	Origin[b]	MIC range, mg/L[c]	
				IPM	MEM
Class A					
KPC type	*Enterobacter cloacae*	1	UHNN	>16	>16
(KPC-like, KPC-2)	*K. pneumoniae*	1	HPA	>16	>16
		5	MGD	>16	>16
		6	UHNN	>16	>16
		3	RS	8 to >16	>16
Class B					
NDM type	*Acinetobacter baumannii*	1	MGD	>16	4
(NDM-like,	*Acinetobacter pittii*	1	MGD	8	>16
NDM-1)	*E. cloacae*	1	MGD	>16	>16
	E. coli	2	MGD	>16	>16
		2	UHNN	8 to >16	>16
	K. pneumoniae	1	HPA	>16	>16
		2	MGD	>16	>16
		1	UHNN	>16	>16
	Morganella morganii	1	MGD	>16	>16
	Providencia rettgeri	1	MGD	8	>16
NDM- + OXA-181 type	*K. pneumoniae*	1	UHNN	>16	>16
VIM type	*Aeromonas caviae*	1	MGD	8	>16
(VIM-like, VIM-1,	*Citrobacter braakii*	1	MGD	8	>16
VIM-2, VIM-4,	*E. cloacae*	1	MGD	>16	>16
VIM-27, VIM-31)	*Klebsiella oxytoca*	1	MGD	>16	>16
		1	MGD	>16	>16
	K. pneumoniae	2	UHNN	8	>16
		1	HPA	>16	>16
	P. aeruginosa	3	MGD	>16	>16
		3	UHNN	>16	>16
VIM- + OXA-48 type	*Citrobacter freundii*	1	MGD	>16	>16
Class D					
OXA-48 type	*C. freundii*	1	MGD	>16	1
	E. aerogenes	1	MGD	>16	4
	E. cloacae	1	MGD	8	4
		1	RS	>16	4
	E. coli	1	HPA	>16	>16
		2	MGD	2	1-2
		1	RS	ND	4
	K. oxytoca	1	MGD	2	1
	K. pneumoniae	1	HPA	4	2
		2	MS	8 to >16	>16
		3	MGD	2 to >16	1 to >16
		2	RS	8 to >16	4 to >16

"-like" indicates that the gene was detected by PCR but not sequenced. IPM = imipenem; MEM = meropenem; UHNN = University Hospital of North Norway, Tromsø, Norway; HPA = Health Protection Agency, NCTC reference strains; MGD = CHU Mont-Godinne-Dinant, Louvain, Belgium; RS = Rijnstate Hospital, Velp, The Netherlands; MS = outbreak strains Maasstad Hospital, Rotterdam, The Netherlands.
[a] Number of isolates (total 62).
[b] Origin of isolates.
[c] MIC determined by Vitek2 (bioMerieux).

Table 2
Non-carbapenemase-producing clinical isolates subjected to the Check-Direct CPE assay.

Isolate	Origin	Notes
E. faecalis	ATCC 29212	
S. aureus	NCTC 10442	MRSA
S. aureus	ATCC 25923	
CoNS	Laboratory strain	
Salmonella typhimurium	Laboratory strain	
E. coli	ATCC 25922	
Campylobacter jejuni	NCTC 11351	
K. pneumoniae	Laboratory strain	
K. oxytoca	ATCC 700324	
Enterococcus casseliflavus	ATCC 700327	
Campylobacter jejuni	ATCC 29428	
K. pneumoniae	ATCC 700603	ESBL SHV
E. faecalis	ATCC 51299	VRE
E. coli	Laboratory strain	
P. aeruginosa	Laboratory strain	Meropenem resistant
E. coli	Laboratory strain	ESBL CTX-M-9, TEM WT
E. aerogenes	Laboratory strain	
C. freundii	Laboratory strain	
P. aeruginosa	Laboratory strain	Meropenem resistant, AmpC CMY-2, TEM WT
K. pneumoniae	Laboratory strain	ESBL CTX-M-1, SHV WT, TEM WT
K. pneumoniae	Laboratory strain	ESBL CTX-M-1, SHV WT, TEM WT

CoNS = coagulase-negative staphylococci.

2.2. Serial dilutions for spiking experiments

From the 0.5 McFarland suspension of each of the nineteen isolates, seven 10-fold dilutions were made in 0.45% saline (NaCl). A volume of 500 μL from a total of 100-mL liquid Amies (Sigma-transwab, MW&E, Corsham, UK) gathered from rectal swab specimens (collected for routine microbiological diagnostics) from 138 patients was aliquoted. Spiked rectal swabs were made by adding 100 μL of each dilution to the 500-μL aliquot. From every spiked sample, 200 μL was used for DNA extraction. To determine the sensitivity of the ChromID CARBA, 20 μL of each suspension was used to inoculate the plates and incubated overnight at 37 °C. The number of suspected colonies (pink- or blue-coloured according to the manual) was counted the following day. A negative control for culture and the real-time PCR was tested with an aliquot of the pool of rectal swabs without adding a carbapenemase producer. Each experiment was performed in duplicate.

2.3. DNA extraction

DNA extraction of 200 μL of each suspension was performed using the NucliSens EasyMAG (bioMérieux, Marcy-l'Etoile, France), according to the Generic 2.0.1 protocol (on-board lysis and 70-μL elution volume). Before DNA extraction, 5 μL of internal control (IC) (Check-Points) was added to each suspension as instructed by the manual. The extraction process took about 1 hour for a maximum of 24 samples.

2.4. Real-time PCR

The Check-Direct CPE assay was designed by Check-Points and detects all known variants of *bla*$_{VIM}$ (except *bla*$_{VIM-7}$), *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{OXA-48}$ including *bla*$_{OXA-162,-181,-204}$. The real-time PCR was performed on the TaqMan 7500 system (Applied Biosystems,

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Table 3
LoD determination using spiked rectal swabs.

Carbapenemase type	Isolate	Origin[a]	MIC[b], mg/L		LoD in spiked rectal swab			
			IPM	MPM	ChromID Carba		Check-Direct CPE	
					CFU/mL[c]	CFU/plate[d]	CFU/mL[c]	CFU/PCR[e]
VIM-1	*K. pneumoniae*	UHNN	8	>16	2×10^3–2×10^4	7–70	200–2000	1–10
VIM-31	*E. cloacae*	MGD	>16	>16	2×10^5–2×10^6	700–7000	2×10^3–2×10^4	10–100
VIM-1	*K. oxytoca*	MGD	>16	>16	2×10^3–2×10^4	7–70	2×10^3–2×10^4	10–100
VIM-27	*K. pneumoniae*	MGD	>16	>16	2×10^3–2×10^4	7–70	2×10^3–2×10^4	10–100
VIM-4	*P. aeruginosa*	MGD	>16	>16	2×10^6–2×10^7	7×10^3–7×10^4	2×10^4–2×10^5	100–1000
OXA-48	*K. pneumoniae*	MS	8	>16	200–2000	0.7–7	2×10^3–2×10^4	10–100
OXA-48	*K. pneumoniae*	HPA	4	2	2×10^6–2×10^7	7×10^3–7×10^4	2×10^3–2×10^4	10–100
OXA-48	*E. aerogenes*	MGD	>16	4	2×10^7–2×10^8	7×10^4–7×10^5	200–2000	1–10
OXA-48	*K. pneumoniae*	MGD	>16	>16	200–2000	0.7–7	2×10^3–2×10^4	10–100
NDM like	*E. coli*	UHNN	>16	>16	200–2000	0.7–7	2×10^3–2×10^4	10–100
NDM like	*K. pneumoniae*	HPA	>16	>16	2×10^3–2×10^4	7–70	2×10^3–2×10^4	10–100
NDM-1	*K. pneumoniae*	MGD	>16	>16	2×10^3–2×10^4	7–70	2×10^3–2×10^4	10–100
NDM-1	*K. pneumoniae*	MGD	>16	>16	200–2000	0.7–7	2×10^3–2×10^4	10–100
NDM like	*P. rettgeri*	MGD	8	>16	2×10^4–2×10^5	70–700	2×10^3–2×10^4	10–100
KPC like	*Enterobacter asburiae*	UHNN	>16	>16	200–2000	0.7–7	2×10^3–2×10^4	10–100
KPC like	*K. pneumoniae*	MGD	>16	>16	200–2000	0.7–7	2×10^3–2×10^4	10–100
KPC like	*K. pneumoniae*	MGD	>16	>16	200–2000	0.7–7	2×10^3–2×10^4	10–100
KPC-2 like	*K. pneumoniae*	MGD	>16	>16	200–2000	0.7–7	2×10^3–2×10^4	10–100
KPC like	*K. pneumoniae*	MGD	>16	>16	200–2000	0.7–7	200–2000	1–10

UHNN = University Hospital of North Norway, Tromsø, Norway; HPA = Health Protection Agency, NCTC reference strains; MGD = CHU Mont-Godinne-Dinant, Louvain, Belgium; RS = Rijnstate Hospital, Velp, The Netherlands; MS = outbreak strains Maasstad Hospital, Rotterdam, The Netherlands; IPM = imipenem; MEM = Meropenem.

[a] Origin of isolates.

[b] MIC (mg/L) determined by Vitek2 (bioMerieux).

[c] The LoD is calculated using 10-fold dilutions starting with 0.5 McFarland (~2×10^8 CFU).

[d] 20 μL of the spiked suspension is inoculated on the ChromID carba. CFU/plate is the amount of CFU calculated from the original 0.5 McFarland suspension.

[e] 10 μL of DNA isolate is used in the Check-Direct CPE. CFU/PCR is the amount of CFU calculated from the original 0.5 McFarland suspension.

Bleiswijk, The Netherlands) in a 25-μL Mastermix reaction containing 12.5 μL of TaqMan Fast Advanced Mastermix (Applied Biosystems), 2.5-μL CPE solution containing all primers and probes (Check-Points), and 10-μL DNA template. All probes are based on the molecular beacon technique and labeled with FAM, VIC, TexasRed, or Cy5 for the detection of bla_{KPC}, $bla_{NDM/VIM}$, bla_{OXA-48}, and the IC, respectively. Since the probes of both bla_{NDM} and bla_{VIM} are labeled with the fluorochrome VIC, it is not possible to differentiate between these 2 carbapenemase genes. The real-time PCR conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, and 45 cycles of 95 °C for 15 s and 60 °C for 60 s. A sample was considered carbapenemase positive when the FAM, VIC, or TexasRed signal crossed the threshold bar, which was adjusted manually in the log-linear range of the PCR, according to the manual (Ct value <45). A sample was inhibited when the Ct value of the IC was >2 higher than the Ct value of the no-template control of the corresponding run. A real-time PCR run took approximately 1 h and 40 min.

2.5. Check-MDR CT102

To confirm the results of the Check-Direct CPE assay, all DNA isolates used for the specificity and sensitivity were analyzed with the Check-MDR CT102 assay (Check-Points), using the same purified DNA as tested in the real-time PCR. The Check-MDR CT102 is able to detect most prevalent ESBL (bla_{TEM}, bla_{SHV}, bla_{CTX-M}) and carbapenemase genes (bla_{KPC}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}, bla_{NDM}) (Naas et al., 2011a).

2.6. Reproducibility

Performance of the assay (r^2 and slope over 10 log dilutions) was determined with the same serial dilutions as used to establish the LoD. The reproducibility of the assay was determined using DNA of a dilution of each carbapenemase producer with a Ct value of approximately 30. This DNA was tested 10 times on different days and different TaqMan 7500 systems.

3. Results

3.1. Analytical specificity

The sequences of the primers and probes were tested for specificity using the online BLAST search program of NCBI (http://blast.ncbi.nlm.nih.gov/). No significant matches other than those for the included bla_{KPC}, bla_{OXA-48}, bla_{VIM}, and bla_{NDM} carbapenemase genes were found as stated by the manufacturer. In addition, the performance of the Check-Direct CPE assay was tested using 62 carbapenemase-producing bacteria (comprising 16 bla_{KPC}, 13 bla_{NDM}, 1 $bla_{NDM+OXA-181}$, 17 bla_{OXA-48}, 14 bla_{VIM}, and 1 $bla_{VIM+OXA-48}$). All isolates with known carbapenemase genes were detected using DNA isolated from a 0.5 McFarland suspension and a threshold of 0.01. The isolates were all correctly detected as KPC, NDM/VIM, or OXA-48 (100% sensitivity) with a Ct value ranging from 17.4 to 18.1 (mean: 18.0), 21.3 to 26.0 (mean: 24.0), 14.7 to 18.4 (mean: 15.4), and 19.1 to 23.1 (mean: 20.6) for KPC, NDM, OXA-48, and VIM, respectively. The assay was able to detect both carbapenemase genes in the isolates containing 2 carbapenemase genes ($bla_{NDM+OXA-181}$ and $bla_{VIM+OXA-48}$). Furthermore, negative results (no amplification signal) were correctly obtained for the 21 carbapenemase-negative isolates, comprising 4 ESBL producers (1 bla_{SHV}, 1 $bla_{CTX-M-9}$, 2 $bla_{CTX-M-15}$), 2 meropenem-resistant *P. aeruginosa* (1 bla_{CMY-2}, no resistance mechanism detected in the second), 1 vancomycin-resistant *Enterococcus faecalis* (VRE, ATCC51299), 1 methicillin-resistant *Staphylococcus aureus* (MRSA, NCTC10442), and 13 other miscellaneous isolates representing the flora from the gastrointestinal tract (100% specificity). These results were confirmed using the Check-MDR CT102 (data not shown). All results were available within 3 h (1 h DNA extraction plus 1 h and 40 min real-time PCR).

3.2. LoD in rectal swabs

The LoD of the Check-Direct CPE assay, directly from rectal swabs, was determined with serial dilutions of 19 different carbapenemase-

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producing isolates spiked in a pool of rectal swabs as described in the Materials and methods section. The LoD of the assay varied from 200–2000 CFU/mL to 2×10^4 to 2×10^5 CFU/mL, corresponding to 1–10 CFU/PCR and 100–1000 CFU/PCR, as shown in Table 3. In the rectal swabs that remained carbapenemase negative, no PCR inhibition was seen. LoD for the ChromID CARBA was determined by using the same spiked samples and varied from 200–2000 CFU/mL to 2×10^7 to 2×10^8 CFU/mL (Table 3). The negative control (aliquot of the pool of rectal swabs without adding a carbapenemase producer) remained negative in both the Check-Direct CPE assay and the ChromID CARBA medium.

3.3. Dynamic range of the Check-Direct CPE assay

The detection was linear over 10 log dilutions with a mean r^2 of 0.991, 0.998, 0.987, and 0.993 and a slope of −3.91, −3.58, −3.55, and −3.90 for bla_{KPC}, bla_{OXA-48}, bla_{VIM}, and bla_{NDM}, respectively. The reproducibility of the assay, using a dilution of each carbapenemase producer with a Ct value of approximately 30, showed that the Check-Direct CPE assay is highly stable and precise. After 10 repeats, the range between minimum and maximum, mean Ct value, SD, and 95% confidence interval (CI) for each carbapenemase were as follows: bla_{VIM} (range: 2.47, mean: 30.7, SD: 0.85, CI: ±0.61), bla_{OXA-48} (range: 2.15, mean: 28.8, SD: 0.70, CI: ± 0.50), bla_{NDM} (range: 3.48, mean: 30.4, SD: 1.16, CI: ±0.83), and bla_{KPC} (range: 3.87, mean: 30.8, SD: 1.26, CI: ±0.90).

4. Discussion

The most prevalent carbapenemases worldwide are IMP, VIM, OXA-48, NDM, and KPC, with the latter 4 being mostly distributed throughout Europe (Kaase, 2012; Nordmann et al., 2011). Early identification in clinical infections and in patients colonized with carbapenemase-producing bacteria, both Enterobacteriaceae and nonfermenters (such as *P. aeruginosa* and *Acinetobacter* spp.), is important to prevent the spread of carbapenemase producers. This study describes the first evaluation of the Check-Direct CPE assay, a new real-time PCR test developed to detect the most prevalent and clinically important carbapenemase genes bla_{KPC}, bla_{OXA-48}, bla_{VIM}, and bla_{NDM} directly from rectal swabs within 3 h.

To evaluate the Check-Direct CPE assay, a total of 83 unique isolates were analyzed, comprising 62 different earlier molecularly characterized carbapenemase producers and 21 various non-carbapenemase producers. The results of the Check-Direct CPE assay showed 100% agreement with the previously defined genotypes and the results obtained with the Check-MDR CT102. For the evaluation of the Check-Direct CPE assay on rectal swabs, the LoD was determined with spiked samples, using 19 different isolates. The LoD varied within most groups of tested carbapenemase genes. The lowest LoD determined using bla_{OXA-48} was 1–10 CFU/PCR (*Enterobacter aerogenes*), while the remaining 3 isolates (all *Klebsiella pneumoniae*) gained an LoD of 10–100 CFU/PCR (Table 3). Naas et al. (2012) analyzed the LoD of an in-house developed real-time PCR for bla_{OXA-48} in spiked fecal suspensions and found 1–5 CFU/PCR using *Escherichia coli*, which is about 20 times lower than the (highest) LoD of 100 CFU/PCR bla_{OXA-48} found with the Check-Direct CPE assay. Naas et al. also analyzed the LoD in spiked fecal suspensions of another in-house developed real-time PCR, able to detect bla_{NDM}, and found an LoD of less than 1 CFU/PCR. This is about 10–100 times lower than the LoD of 10–100 CFU/PCR as determined by the Check-Direct CPE assay (Naas et al., 2011b). The in-house real-time PCRs as developed by Naas et al. were both monoplex reactions, which were detected by a TaqMan probe labeled with the most efficient reporter: FAM. Since the Check-Direct CPE assay is a complex multiplex assay, which is able to differentiate between the carbapenemase genes bla_{KPC} (FAM label), bla_{OXA-48} (Texas Red label), and $bla_{NDM/VIM}$ (both VIC label), the sensitivity can be slightly reduced. In addition, bla_{NDM} and bla_{OXA-48} are detected on the TaqMan 7500 system using the less efficient reporters VIC and TexasRed, respectively, which possibly influence the difference in LoDs. For the LoD of bla_{VIM} and bla_{KPC}, no comparison with other studies is possible, since no molecular assays determining the LoD of these carbapenemase genes in (spiked) rectal swabs or stool has been published yet. Although the analytical sensitivity of the Check-Direct CPE assay is not as good as the in-house developed monoplex real-time PCRs as described previously, the capability of the Check-Direct CPE assay to detect the 4 major carbapenemase genes simultaneously and directly differentiate between bla_{KPC}, bla_{OXA-48}, and $bla_{NDM/VIM}$ is an important feature, especially in situations where screening of carbapenemase carriers is needed and in outbreak situations.

Nowadays screening for carbapenemase-producing bacteria is most often performed with a selective medium. Therefore, in this study, we compared the sensitivity of the Check-Direct CPE assay to the ChromID CARBA medium, which has been selected because of its reported high sensitivity and specificity (Vrioni et al., 2012; Wilkinson et al., 2012). As illustrated in Table 3, LoD (CFU/mL) of the Check-Direct CPE assay shows comparable sensitivity with the ChromID CARBA concerning bla_{VIM} and bla_{NDM}. Although ChromID CARBA has a lower LoD for 2 of the bla_{OXA-48} producing *K. pneumoniae* isolates, it clearly shows the lack of correct detection when lower MICs are present. This is in concordance with the results of the study of Vrioni et al. (2012), which earlier showed a high LoD (1.1×10^7 CFU/mL) when the ChromID CARBA was tested with carbapenemase producers harboring low MICs. Finally, LoD of bla_{KPC} determined with the Check-Direct CPE assay was approximately 10 times lower than the LoD of the ChromID CARBA. A study of Wilkinson et al. (2012) earlier showed good detection of bla_{KPC} using ChromID CARBA, since this medium was able to detect 100% (12/12) of bla_{KPC} carbapenemase producers with low inoculums, whereas isolates producing bla_{VIM}, bla_{IMP}, bla_{OXA-48}, and bla_{NDM} with low inoculums were correctly detected in 83.3% (5/6), 88.9% (8/9), 66.7% (10/15), and 94.3% (83/88), respectively. Overall, in comparison with the ChromID CARBA medium, directly from rectal swabs, the Check-Direct CPE assay showed comparable results in detecting carbapenemase genes bla_{OXA-48}, bla_{VIM}, and bla_{NDM} and acceptable results for bla_{KPC}. However, when carbapenemase producers with low MICs are subjected for detection, the Check-Direct CPE assay is superior over the ChromID CARBA medium.

The turnaround time is clearly in favor of the Check-Direct CPE assay and other recently published molecular methods (all approximately 3–4 h) over any other selective agar because absence of carbapenemase producers cannot be established earlier than the next day since it will always need an overnight incubation step. Moreover, carbapenemase suspected isolates should be confirmed since ESBLs or AmpC β-lactamase producers can give false-positive results (Nordmann et al., 2012b). This confirmation step can take up to another 48 h, depending on the test used. Chromogenic media can also generate false-negative results, which might be explained by carbapenemases with very low MICs (Nordmann et al., 2012a, 2012b).

Kochar et al. (2009) demonstrated that a good infection control program, consisting of improved hand hygiene and routine rectal surveillance cultures, decreased the spread of bla_{KPC} in an intensive care unit significantly. Using the Check-Direct CPE assay, instead of routine rectal surveillance cultures, infection control programs can become more efficient.

Although the Check-Direct CPE assay has a rapid and excellent performance, it should be emphasized that this assay is only able to identify the 4 major carbapenemase genes bla_{KPC}, bla_{OXA-48}, bla_{VIM}, and bla_{NDM}. As in all molecular assays, other or newly discovered carbapenemase genes will not be detected.

This study has several limitations. First, not all known variants of the 4 carbapenemase genes were included in this study. Therefore, we were not able to evaluate the correct detection of all variants of carbapenemase genes. Second, we used spiked rectal swab samples to investigate the performance of the Check-Direct CPE assay on this

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specimen type. Since the prevalence of carbapenemase producers in The Netherlands is very low, a study of direct detection in rectal swabs from patients is almost impossible. Although spiked rectal swabs are not real clinical samples, it certainly is the most appropriate way to mimic the performance of the Check-Direct CPE assay in this specimen type. It is important to know that in our routine culture screening, we did find 4 different rectal swabs with a carbapenemase producer (3 $bla_{\text{OXA-48}}$ and 1 bla_{KPC}). These rectal swabs were set aside and tested according to the procedure as used in this study and all gained a clear positive PCR result (data not shown). Although these results of direct detection in rectal swabs from patients are very promising, further studies in patients from endemic countries are necessary.

In conclusion, this new multiplex real-time PCR assay appeared to be an accurate and stable method to detect the most prevalent and clinically important carbapenemase genes bla_{VIM}, $bla_{\text{OXA-48}}$, bla_{NDM}, and bla_{KPC}. This sensitive method could be applied directly on rectal samples and is able to differentiate between the carbapenemase genes bla_{KPC}, $bla_{\text{OXA-48}}$, and $bla_{\text{VIM/NDM}}$, obtaining results within 3 h. The assay looks promising as a carbapenemase screening method and can be an important improvement in infection control programs.

Acknowledgments

We kindly thank the National reference center for resistance in Gram-Negatives (CHU Mont-Godinne-Dinant, Belgium) for providing the strains obtained in the scope of the FP7 project TEMPOtest-QC number 241742. Furthermore, we thank Sarah Thiollet and Thijs Weijers for the technical support.

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