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Short Communication

Multicentre evaluation of a real-time PCR assay to detect genes encoding clinically relevant carbapenemases in cultured bacteria

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ABSTRACT

The performance and portability of a multiplex real-time PCR assay to detect KPC, NDM, OXA-48-like and VIM carbapenemase gene families from bacterial isolates was assessed using Rotor-Gene Q and ABI 7500 instruments. Gram-negative bacterial isolates ($n = 502$) were comprised of 100 isolates each with KPC, NDM, VIM or OXA-48-like carbapenemases (including 17 with OXA-181) and 2 isolates with NDM + OXA-48-like enzymes (including 1 with OXA-181) as well as 100 assay-negative isolates comprised of 24 IMP-producers, 24 carbapenem-resistant isolates with no known carbapenemase gene and 52 extended-spectrum β-lactamase-producing carbapenem-susceptible isolates. A multicentre evaluation was carried out in five laboratories using a subset of 100 isolates comprised of 22 isolates each with KPC, NDM, OXA-48-like or VIM alleles and 12 isolates that were negative for the assay targets. Initial validation of the assay on both the Rotor-Gene Q and ABI 7500 instruments demonstrated 100% sensitivity amongst the 402 isolates that were positive for KPC, NDM, OXA-48-like (including OXA-181) and VIM carbapenemase genes, whilst the 100 assay-negative samples were correctly identified indicating 100% specificity. During the multicentre evaluation the same 100% level of sensitivity and specificity was observed in each of the five centres that participated. Neither invalid nor false-positive results were observed. In conclusion, the assay offers a portable and reliable option for the detection of bacteria carrying clinically significant carbapenemases encoded by KPC, NDM, VIM and OXA-48-like carbapenemase genes using either of the two most common real-time PCR instrument platforms.

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1. Introduction

Resistance to carbapenem antimicrobials represents one of the most pressing threats to public health [1], with the greatest emphasis placed on those bacteria that have transferable gene(s) encoding carbapenemases. The host range of carbapenemase genes and their associated mobile genetic elements is broad across Gram-negative bacteria but they are particularly prevalent amongst the Enterobacteriaceae, particularly *Klebsiella pneumoniae* and *Escherichia coli* [2,3]. Nosocomial outbreaks involving bacteria carrying each of the five most globally prevalent carbapenemase families (KPC, OXA-48-like, NDM, VIM and IMP) have been reported worldwide [4–8], but geographical differences exist in their relative importance. In Europe and the USA, a mixture of KPC, OXA-48-like, NDM and VIM enzymes predominates [9], whilst in the Far East and Australia IMP enzymes are more prevalent [4,10].

Rapid confirmation of carbapenemase production informs patient management and the prompt deployment of infection prevention and control measures in order to minimise spread. However, differentiation of genuine carbapenemase-producers from isolates that are resistant to carbapenems through other mechanisms [e.g. porin loss combined with either extended-spectrum β-lactamase (ESBL) or AmpC activity] requires supplemental tests owing to the wide range of carbapenem minimum inhibitory

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Table 1
Sequences and label characteristics of oligonucleotide primers and probes.

Name	Sequence (5′ → 3′)	5′ label	3′ label
Primers			
KPC_fwd	GCAGCGGCAGCAGTTTGTTGATT	–	–
KPC_rev	GTAGACGGCCAACACAATAGGTGC	–	–
NDM_fwd	CCAGCAAATGGAAACTGGCGAC	–	–
NDM_rev	ATCCAGTTGAGGATCTGGGCG	–	–
OXA_48_fwd	GATTATGGTAATGAGGACATTTCGGGC	–	–
OXA_48_rev	CATATCCATATTCATCGCAAAAAACCACAC	–	–
VIM_fwd	TTGCTTTTGATTGATACAGCGTGGGG	–	–
VIM_rev	GTACGTTGCCACCCCAGCC	–	–
Probes[a]			
VIM_probe	TCTCGCGGAGATTGAAAAGCAAATTGGACTTCC	CY5	BHQ-3
OXA_48_probe_ABI7500*	CCATTGGCTTCGGTCAGCATGGCTTGTTT	JOE	BHQ-1
OXA-48_probe_RGene*	CCATTGGCTTCGGTCAGCATGGCTTGTTT	Cy5.5	BHQ-3
KPC_probe	CAGTCGGAGACAAAACCGGAACCTGC	ROX	BHQ-2
NDM_probe_ABI7500*	ACCGAATGTCTGGCAGCACACTTC	TAM	BHQ-2
NDM_probe_RGene*	ACCGAATGTCTGGCAGCACACTTC	JOE	BHQ-1

[a] Probes marked.
* denote labels specific for use with Rotor-Gene® or ABI7500 instruments.

concentrations (MICs) observed in carbapenemase-producers. Molecular diagnostics allow rapid screening for carbapenemase genes, and many in-house and commercial assays are based on real-time PCR. Here we compared the performance of a validated in-house diagnostic assay across four different centres within England using two different real-time PCR platforms and a panel of bacterial isolates with previously defined carbapenem resistance mechanisms.

2. Materials and methods

2.1. Real-time PCR assay and data analysis

For the novel detection assay, carbapenemase genes recorded at http://www.lahey.org/Studies/ (accessed 14 May 2014) were downloaded and aligned using ClustalW. Oligonucleotide sequences were selected for KPC, OXA-48-like, NDM and VIM genes (Table 1). Primers and probes were obtained from MWG-Eurofins (Ebersberg, Germany) and Sigma–Aldrich (Poole, UK). Template DNA was prepared using one to five colonies of test organism suspended in 100 μL of molecular-grade water. The suspension was incubated for 30 min at 98 °C and 5 μL of the supernatant was used in the real-time PCR assay. A *Taq* polymerase PCR enzyme and dNTP mixture (Platinum® Quantitative PCR SuperMix-UDG; Invitrogen, Paisley, UK) was mixed with primers and probes and template DNA. Rotor-Gene® Q (5-plexHRM) instruments (QIAGEN, Crawley, UK) were programmed as follows: 60 °C for 1 min; 95 °C for 10 min; then 50 cycles of 95 °C for 15 s and 58 °C for 1 min; followed by a single step of 60 °C for 5 min and 4 °C hold. The same parameters were used for ABI 7500 (Life Technologies, Paisley, UK) instruments, except that the extension step was performed at 60 °C. Results from real-time PCR assays were all interpreted using a cut-off value ($C_t \leq 36$) to identify carbapenemase-positive isolates. Rotor-Gene Q v.2.0.3 software and ABI 7500 v.2.0.4 were used to analyse data. This assay is subject to International patent application no. PCT/GB2015/053276.

2.2. Reference laboratory – assay validation

The isolates ($n = 502$) comprised 490 Enterobacteriaceae and 12 *Pseudomonas* spp. (Table 2) that had been submitted to Public Health England's (PHE's) Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit for investigation of 'unusual' resistance (mostly to carbapenems) [11]. Of 450 previously characterised carbapenem-resistant isolates, 426 were carbapenemase-positive with 100 each of KPC, NDM, VIM and OXA-48-like producers, 2 isolates were positive for NDM as well as an OXA-48-like enzyme and 24 encoded an IMP enzyme. An additional 24 isolates had carbapenem resistance contingent upon ESBL and/or AmpC activity plus porin loss (Table 2) [11]. The remainder of the 502 isolates ($n = 52$) were carbapenem-susceptible with

Table 2
Species distribution and carbapenemase status of isolates used for validation ($n = 502$) or multicentre evaluation ($n = 100$) of the carbapenemase gene detection real-time PCR assay.[a]

Species	Carbapenemase gene family							Total
	KPC	NDM	VIM	OXA-48-like	NDM + OXA-48-like	IMP	None	
Klebsiella pneumoniae	62/10	52/11	55/9	59/9	2/0	0/0	25/2	255/41
Klebsiella oxytoca	6/2	0/0	12/5	3/1	0/0	1/1	0/0	22/9
Klebsiella sp.	0/0	0/0	0/0	0/0	0/0	1/1	0/0	1/1
Escherichia coli	9/2	28/5	8/4	29/8	0/0	2/1	25/2	101/22
Enterobacter spp.	18/4	14/3	16/1	8/3	0/0	8/2	23/2	87/15
Pseudomonas spp.	0/0	0/0	0/0	0/0	0/0	11/0	1/0	12/0
Others	5/4	6/3	9/3	1/1	0/0	1/1	2/0	24[b]/12[c]
Total	100/22	100/22	100/22	100/22	2/0	24/6	76[d]/6[e]	502/100

[a] Dividers separate isolates from the validation and evaluation panels.
[b] Comprised of *Citrobacter* spp. ($n = 16$), *Raoultella* spp. ($n = 3$), *Leclercia adecarboxylata* ($n = 2$), *Serratia marcescens* ($n = 2$) and *Kluyvera georgiana* ($n = 1$).
[c] Comprised of *Citrobacter* spp. ($n = 6$), *Raoultella* spp. ($n = 2$), *L. adecarboxylata* ($n = 2$), *S. marcescens* ($n = 1$) and *K. georgiana* ($n = 1$).
[d] Extended-spectrum β-lactamase (ESBL)-producing organisms ($n = 52$) that were carbapenem-susceptible, and carbapenem-resistant isolates ($n = 24$) that did not produce a carbapenemase.
[e] Isolates that were carbapenem-resistant but did not produce a carbapenemase.

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