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Rapid Detection of KPC, NDM, and OXA-48-Like Carbapenemases by Real-Time PCR from Rectal Swab Surveillance Samples

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We describe a multiplex real-time PCR assay for use on the ABI 7500 Fast TaqMan platform to detect all currently described *Klebsiella pneumoniae* carbapenemases (KPC), New Delhi metallo-β-lactamases (NDM), and the OXA-48-like family of carbapenemases from bacterial culture lysates or sample enrichment broth lysates.

The prevention of transmission of carbapenemase-producing organisms (CPO) within health care facilities requires accurate and rapid laboratory detection methods so that infection control precautions may be quickly implemented. In the midst of an outbreak, rapid case identification is required, and conventional methods can take too long and be very labor intensive, resulting in delays in infection control and significant costs to the health care system.

Molecular methods are fast and generally have higher sensitivity and specificity than phenotypic methods, and methods developed in-house are typically less expensive than commercially available kits. The published methods for the molecular detection of *Klebsiella pneumoniae* carbapenemases (KPC), New Delhi metallo-β-lactamases (NDM), and OXA-48 are typically singleplex reactions targeting only one carbapenemase or are intended for cultured isolates rather than surveillance samples (1–14). We identified the need for a highly sensitive and specific molecular assay that can detect these CPO gene targets directly from surveillance sample matrices, such as rectal swabs.

We developed a Fast multiplex real-time 5′ exonuclease-based PCR assay to detect the common carbapenemases (KPC, NDM, and OXA-48-like) from rectal swabs in enrichment broths. Although there have been several reports of real-time PCR assays in the literature (1–14), to our knowledge, this is the first Fast multiplex real-time 5′ exonuclease-based real-time PCR to detect and differentiate the 3 carbapenemases, KPC, NDM, and OXA-48-like, from rectal swabs in enrichment broths in a single reaction. A culture enrichment step was incorporated to increase the sensitivity (15).

To date, 23 KPC, 16 NDM, and 11 OXA-48-like (http://www.lahey.org/studies/other.asp#table 1) genotypes have been described. We used the Integrated DNA Technologies (IDT) OligoAnalyzer 3.1 to define a set of primers and probes that is predicted, *in silico*, to detect all described genotypes of bla_{KPC}, bla_{NDM}, and bla_{OXA-48}-like genes (Table 1). To validate this, real-time PCRs were performed on an ABI 7500 platform with the recommended Fast thermal cycling conditions in a 20-μl final volume using TaqMan Fast advanced master mix (Life Technologies, Burlington, ON), PCR-grade water (Life Technologies), and each primer and probe at a final concentration of 200 nM and 150 nM, respectively. Then 2 μl of sample lysate was added to each reaction. Real-time

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TABLE 1 Primers and probes

Sequence	Name	Probe dye	Final concn (nM)	Reference or study
GGCCGCCGTGCAATAC	KPC-F		200	1
GCCGCCCAACTCCTTCA	KPC-R		200	1
TGATAACGCCGCCGCCAATTTGT	KPC-P	MAX/ZEN	150	1
ATTAGCCGCTGCATTGATGC	NDM-F		200	7[a]
CCGGCATGTCGAGATAGGAA	NDM-R		200	7
CTG + CCA + GAC + ATT + CGGTGC[b]	NDM-P	LNA 6FAM	150	7
CAATGGYGACTATATTATTCGGGC	OXA-48F-F		200	This study[c]
CCACACATTATCATCMAGTTCAACC	OXA-48F-R		200	This study
CTAAGATTGGCTGGTGGGT	OXA-48F-P	MGB NED	150	This study

[a] With modifications to the published primers to improve annealing temperatures in TaqMan assays.
[b] LNA base pairs preceded by +.
[c] Based on the sequence described by Swayne et al. (11).

TABLE 2 Summary of positive controls used in real-time and conventional PCRs

Resistance gene	Strain	Organism	Method
KPC	ATCC 1705	*K. pneumoniae*	Real-time and conventional
NDM	ATCC 2452	*E. coli*	Real-time
	NML-1049	*E. coli*	Conventional
OXA-48	NCTC 13443	*K. pneumoniae*	Real-time
	F189043	*E. coli*	Conventional

PCR controls, ATCC strains, and clinical isolates previously characterized by conventional PCR (16) are listed in Table 2. Bacterial culture lysates were prepared using InstaGene Matrix (Bio-Rad, Mississauga, ON), according to the manufacturer's protocol. Broth lysates were prepared by 2 methods: InstaGene Matrix and a water boil. For each lysis method, approximately 30 μl of enriched culture broth was added to 1 ml of PCR-grade water. InstaGene lysates were completed according to the manufacturer's protocol. The tube for the boil method was heated to 100°C in a dry bath for 8 min to lyse the bacterial cells. A 500-bp oligonucleotide containing 1 copy of the concatenated target sequences of each assay was purchased from IDT (Toronto, ON). Pooled negative InstaGene lysates were spiked with serial dilutions of this oligonucleotide ranging from 10^1 to 10^8 copies/ml. The limit of detection was assessed in both multiplex and simplex formats. All 3 targets have a lower detection limit of 5 copies/μl extracted DNA.

A total of 155 blind-coded InstaGene lysates from archived clinical isolates previously tested by conventional PCR were tested by real-time PCR, and the results for each method were compared to determine the sensitivity and specificity. The multiplex assay correctly identified all 45 positive isolates as having at least 1 of the 3 carbapenemases tested (Table 3). The remaining 110 isolates tested were negative for KPC, NDM, and OXA-48 by both conventional PCR and real-time PCR assays. There was no evidence of cross-reactions among these three targets. The sensitivity and specificity are 100%.

A total of 268 surveillance rectal swabs collected by the participating hospital, as part of their infection control surveillance program, were used. The swabs were enriched in 5 ml of Tris-buffered saline (TSB) with a 10-μg ertapenem disc for 18 to 24 h of incubation at 37°C, in a stationary position, in an ambient air incubator and then lysed and tested with the multiplex real-time PCR. The results were compared to those for isolates recovered from the same broth (Table 4) and investigated as follows. Isolates recovered from broths were obtained by subculturing the broths to 2 MacConkey agar plates (Oxoid) with imipenem and meropenem discs (Bio-Rad, Mississauga, ON). Colonies growing within the zone of inhibition were identified to the species level, and antibiotic susceptibility testing was performed according to the 2014 CLSI guidelines. Isolates intermediate or resistant to imipenem or meropenem were submitted to the reference laboratory for conventional PCR testing. As there is no gold standard for the detection of carbapenemases directly from stool or rectal swab samples (17), we considered our current phenotypic screening followed by the conventional PCR method as the reference method for comparison.

TABLE 3 Summary of clinical isolate results, real-time PCR, and conventional PCR

Carbapenemase	Total no. of positives by: Real-time PCR[a]	Conventional PCR[a]	Species isolated (no.)
KPC	9	9	*Klebsiella oxytoca* (1)
			K. pneumoniae (7)
			Raoultella planticola (1)
NDM	30	30	*Enterobacter cloacae* (6)
			E. coli (7)
			K. oxytoca (1)
			K. pneumoniae (16)
OXA-48	8	8	*E. coli* (2)
			K. pneumoniae (6)

[a] Two 2 *K. pneumoniae* isolates tested positive for multiple targets. Isolate 45 tested as KPC positive and NDM positive. Isolate 54 tested as NDM positive and OXA-48 positive. All other isolates tested positive for only 1 target.

TABLE 4 Summary of surveillance sample results, real-time PCR, and phenotypic screen isolates

Carbapenemase	Total no. of positives by real-time PCR	Phenotypic screen isolates: Total no. of positives by conventional PCR	Species isolated (no.)
KPC	0	0	
NDM	22[a]	20	*Citrobacter freundii* (1)
			Klebsiella pneumoniae (10)
			Mixed *C. freundii*/*K. pneumoniae* (5)
			Escherichia coli (4)
OXA-48	1[b]	0	

[a] Broth 102 and broth 220 both tested as phenotypically ertapenem and meropenem susceptible; however, both tested positive for NDM.
[b] Broth 239 tested as NDM positive and OXA-48 positive, but only an NDM-positive *E. coli* was recovered from the broth. A repeat screen sample 1 week later had an OXA-48-positive *K. pneumoniae* isolated.

One hundred percent of conventionally identified KPC/NDM/OXA-48-positive screen isolates were identified by the real-time PCR assay. None of the screen isolates harbored VIM or IMP genes. Three enrichment broths had discordant results between phenotypic testing and real-time PCR. Enrichment broths 102 and 220 both tested as phenotypically ertapenem and meropenem susceptible, but both tested positive for the presence of NDM by real-time PCR. Broth 220 had a second enrichment broth tested, which had an NDM-positive *K. pneumoniae* isolate recovered. Broth 102 had no carbapenem-resistant organisms isolated, and repeat testing could not be performed. Broth 239 tested as NDM positive and OXA-48 positive by real-time PCR, but only an NDM-positive *Escherichia coli* was isolated initially. A second screen sample 1 week later from the same patient recovered an OXA-48-positive *K. pneumoniae* isolate.

This real-time PCR assay is fast and simple to perform, and the results are easier to interpret than those for the conventional PCR. Replacing the current method of conventional PCR with the real-time PCR will significantly reduce turnaround times for CPO detection, especially during an outbreak. For clinical laboratories, a real-time PCR application will reduce the turnaround time for implementing infection control measures and reduce the costs associated with CPO detection.

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