Implementation of a multiplex real-time PCR for the detection of six carbapenemase genes

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Introduction and objectives

Molecular identification of carbapenemase-producing *Enterobacteriaceae* (CPE) is a necessity as its phenotypic detection remains a challenge. For this reason, we implemented a multiplex real-time PCR assay for the detection of six different carbapenemases previously identified in Enterobacteriaceae (*bla*$_{KPC}$ type, *bla*$_{GES}$ type, *bla*$_{IMP}$ type, *bla*$_{VIM}$ type, *bla*$_{OXA-48}$ type and *bla*$_{NDM-1}$ type) in +1000 beds tertiary care Ghent University Hospital.

Methods

Bacterial isolates with decreased susceptibility to meropenem were tested: 55 were previously characterized as resistant to carbapenems and documented by uniplex PCR (Belgian National Reference Centre for multi-drug resistant gram-negative bacteria, UCL Mont-Godinne, Belgium) to carry one of the carbapenemase genes mentioned above, while the remaining 45 isolates tested negative. PCR was run on a CFX-96 cycler (Bio-Rad, Hercules, CA) using HRM PCR Master Mix (Qiagen, Hilden, Germany) including all relevant positive and negative controls. In parallel, 16S RNA gene was amplified to demonstrate successful DNA preparation.

Results

Using reference strains and clinical isolates, we noticed during implementation of the protocol by Monteiro et al. (J Antimicrob Chemother 2012; 67: 906-909) that *bla*$_{IMP}$ type genes in our isolates were not amplified (e.g. *bla*$_{IMP-1}$). Therefore, we designed *bla*$_{IMP}$ type gene primers, aiming at an amplicon T_m below 79 ° C. The table shows this and other primer pairs used in multiplex PCR, and the mean T_m analysis we measured amplifying positive isolates. An example of a HRM analysis profile of the positive controls is shown in the Figure. The T_m mean calculated from high-resolution melting curve analysis (inter- and intra-run) of the newly established multiplex PCR for the primers described by Monteiro et al. matched with what they reported.

Table: Primer specifications

Target	Primer name	Sequence (5'-3')	Amplicon size (bp)	Primer conc. (µM)[a]	T_m (° C)[b]
bla$_{KPC}$	KPC-F	TCGCTAACTCGAACAGG	785	0.2	89.6
	KPC-R	TTACTGCCCGTTGACGCCCAATCC			
bla$_{NDM-1}$	NDM1-F	TTGGCCTTGCTGTCCTTG	82	0.2	81.9
	NDM1-R	ACACCAGTGACAATATCACCG			
bla$_{GES}$	GES-F	CTATTACTGGCAGGGATCG	594	0.2	86.6
	GES-R	CCTCTCAATGGTGTGGGT			
bla$_{OXA-48}$	OXA48-F	TGTTTTTGGTGGCATCGAT	177	0.2	79.7
	OXA48-R	GTAAMRATGCTTGGTTCGC			
bla$_{IMP}$	IMP-F	TTGGGTGACGCAAATATAGA	123	0.2	78.8
	IMP-R	AAGTTTCAAGAGTGATGCGT			
bla$_{VIM}$	VIM-F	GTTTGGTCGCATATCGCAAC	382	0.2	88.2
	VIM-R	AATGCGCAGCACCAGGATAG			

[a]Final concentration in the multiplex real-time PCR

[b]Melting point calculated by CFX using Type-it HRM

The newly designed *bla*$_{IMP}$ type gene primer pair did thus not compromise the performance of the multiplex assay, and allowed to detect *bla*$_{IMP}$ type genes unambiguously, with a amplicon T_m well separated from the other carbapenemase amplicons. A concordance of 100% was found between the results of our multiplex real-time PCR assay and previously identified carbapenemase genotypes.

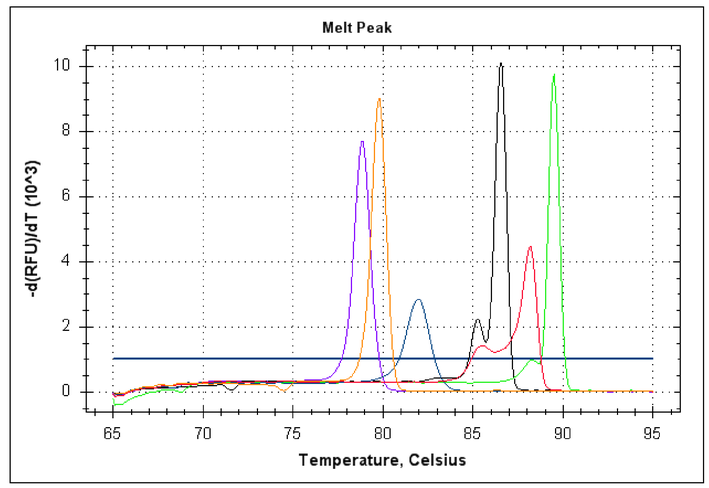

Figure: Meltcurve analysis for the 6 carbapenemase genes. *bla*IMP, T_m=78.8° C (purple); *bla*OXA-48, T_m=79.7° C (orange); *bla*NDM-1, T_m=81.9° C (blue); *bla*GES, T_m=86.6° C (black); *bla*VIM, T_m=88.2° C (red); *bla*KPC, T_m=89.6 ° C (green).

Conclusion

The multiplex real-time PCR assay modified in this study to also detect *bla*$_{IMP-1}$, is a robust assay for the screening and identification of the circulating carbapenemase genes in Europe, what is of value for adequate healthcare associated infection control.

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