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Development of a Real-Time PCR (RT-PCR) Assay for Carbapenemase Producing Bacteria including Enterobacteriaceae

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Introduction

Carbapenems are the ultimate drug choice for treatment of serious Gram-negative infections in many hospitals. Increasing reports of bacteria producing carbapenemases, such as New Delhi Metallo-β-lactamase (NDM) and *Klebsiella pneumoniae* Carbapenemase (KPC), especially in outbreak scenarios, are of concern. Screening policies for at risk patients are now commonplace, however most rapid methods for detecting carbapenemases remain commercial and expensive. Alternative phenotypic techniques are slower, with positive results available after 24hrs. This study aimed to develop an in-house RT-PCR assay for detecting carbapenemase producing bacteria.

ORGANISM	VIM	IMP	NDM	KPC	OXA-48	GES	TOTAL
***Acinetobacter* spp.**	7	7	26	0	0	4	**44**
***Aeromonas* sp.**	0	1	0	0	0	0	**1**
***Citrobacter* sp.**	0	0	1	0	0	0	**1**
***Enterobacter* spp.**	0	11	8	0	5	0	**24**
Escherichia coli	0	2	7	3	3	0	**15**
***Klebsiella* spp.**	11	8	7	14	22	0	**62**
M. morganii	0	0	1	0	0	0	**1**
Providencia rettgeri	0	0	1	0	0	0	**1**
***Pseudomonas* spp.**	87	17	1	0	0	1	**106**
S. maltophilia	0	11	0	0	0	0	**11**
S. marcescens	0	14	0	0	0	0	**14**
TOTAL	**105**	**71**	**52**	**17**	**30**	**5**	**280**

Table 1 (left): 280 isolates processed by the RT-PCR assay

Methods

Real-time PCR was performed using specific primers for VIM, IMP, NDM, KPC, OXA-48, GES. Three primer sets were used for IMP (named groups 1, 2 and 3) due to numerous differences in sequences within IMPs. Primer suitability was assessed by *in silico* analysis; all published enzymes were found to be covered by the assay, with an exception of VIM-7. PCR conditions were: 95°C for 5min; 30 cycles of 95°C for 20sec, 55°C for 45sec & 72°C for 30sec and high resolution melt curve analysis (between 75-95°C with 0.3°C/sec increments and data acquisition every 2sec) was used to differentiate the specific PCR amplicons. Validation was performed using a diverse set of isolates containing each of the resistance genes (Table 1).

Results

Expected melting curves for each amplified gene product are shown in Fig. 1. Distinct profile ranges were applied based on validation results to allow for accurate identification of melting curve for each enzyme: VIM: 87.9°C, IMP (groups 1 & 3): 81.4°C, IMP (group 2): 80.8°C, NDM: 83.0°C, KPC: 90.4°C, OXA48: 83.8°C & GES: 87.2°C (±0.5°C for each profile, resulting in a 1°C range and some overlap between profiles). Melt curves were recorded at the highest peak above a threshold of 1.5°C.

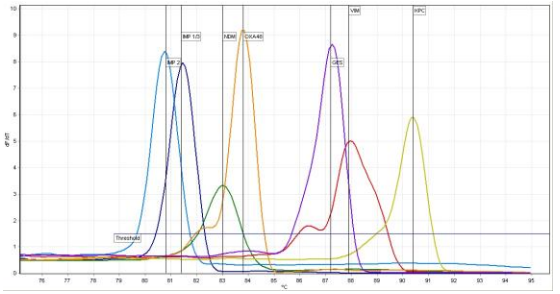

Fig. 1: Expected melting curve profiles for VIM, IMP, NDM, KPC, OXA-48 and GES carbapenemases.

All isolates containing a carbapenemase were successfully detected. One isolate – identified as VIM-11 by sequencing – had a melting curve lower than expected (87.5°C). The resulting curve sat between GES and VIM profiles (Fig 2.) due to the overlap of the profile ranges. Further analysis is required to assess detection of VIM-11 by the assay.

After processing all 280 the profile ranges were reassessed based on an average result for each and were found to be consistent with the pre-testing validation work profiles.

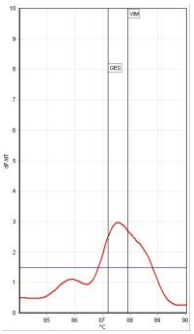

Fig. 2: A VIM-11 melt curve fell between GES and VIM melting profiles.

The performance of the assay was consistent, and similar melt curves were observed for each enzyme. VIM curves typically exhibited a shoulder peak lower than the main melting temperature (Fig. 3).

Time taken for assay set up and PCR was approximately 2 hours.

Conclusions

RT-PCR carbapenemase assay is of use for rapid detection of carbapenemase producing organisms. Rapid detection improves both antimicrobial therapy and infection control measures.

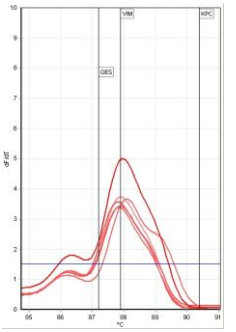

Fig. 3: Multiple VIM products exhibit similar melt curve identities but consistent main melt temperatures

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