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**Poster Session IV**
**Molecular detection of bacterial resistance**

# DEVELOPMENT OF A REAL-TIME PCR (RT-PCR) ASSAY FOR CARBAPENEMASE PRODUCING BACTERIA, INCLUDING ENTEROBACTERIACEAE.

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## OBJECTIVES

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 real-time PCR assay for detecting carbapenemase producing bacteria.

## METHODS

Real-time PCR was performed using specific primers for NDM, KPC, GES, VIM, IMP and OXA-48;

| *Target* | *Forward primer* | *Reverse primer* |
|---|---|---|
| NDM | TTGGCCTTGCTGTCCTTG | ACACCAGTGACAATATCACCG |
| KPC | TCGCTAAACTCGAACAGG | TACTGCCCGTTGACGCCCAATCC |
| GES | CTATTACTGGCAGGGATCG | CCTCTCAATGGTGTGGGT |
| VIM | CCGACAGTCARCGAAATTCCG | CTACTCRRCGACTGAGCGATT |
| IMP1 | GGTCTATTTGACGGCGTCTATCAT | GCRGAYTTTGGCCAAGCTTC |
| IMP2 | GGTGTTTATGTTCATACATCG | TTCAAGAGCGACGCATCTCC |
| IMP3 | GGTTTATGTTCATACWTCG | GCGGACTTTGGCCAAGCTTC |
| OXA48 | TTGGTGGCATCGATTATCGG | GAGCACTTCTTTTGTGATGGC |

PCR conditions were: 95°C for 5 min; 30 cycles of 95°C for 20 s, 55°C for 45 s & 72°C for 30 s and high resolution melt curve analysis (between 75-95°C with 0.3°C/s increments to and data acquisition every 2 s) was used to differentiate the specific PCR amplicons. Validation was performed using a diverse set of isolates (incl. *E. coli*, *K. pneumoniae*, *E. cloacae*, S. marcescens, *P. aeruginosa, P. putida* & *A. baumannii*) containing NDM (20), KPC (15), GES (7), VIM (93), IMP (69), and OXA-48 (28). Multiple sequence types of VIM & IMP were included.

## RESULTS

Melting curves for each amplified gene product are shown in Figure 1. All melting curves were distinct with bins for each: NDM:83±0.5°C, KPC: 90.4±0.5°C, GES: 87.2±0.5°C, VIM: 87.9±0.5°C, IMP1/3: 81.4±0.5°C, IMP2: 80.8±0.5°C, & OXA48: 83.8±0.5°C. Melting curves had distinct profiles allowing accurate identification. All isolates containing a carbapenemase were successfully detected with time taken for assay set up and PCR approximately 2hrs.

## 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.

Figure 1: Melting curve profiles for NDM, KPC, GES, VIM, IMP & OXA-48 carbapenemases.