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Development of rapid isothermal Recombinase Polymerase Amplification assays for the detection of carbapenemase genes

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INTRODUCTION

Acquisition of transferable carbapenemase genes has been recognized internationally as a major public health threat[1]. The so called 'big five' carbapenemase families include the KPC and OXA-48-like non-metallo-enzymes and the NDM, VIM and IMP metallo-enzymes. Rapid detection of these is crucial, not only for patient management but also for infection prevention and control of further transmission. Recombinase polymerase amplification (RPA) is an isothermal nucleic acid amplification method that is an alternative to real-time polymerase chain reaction (qPCR)[2,3]. Such assays are amenable to miniaturization on a digital microfluidic platform[4]. Here we describe the development of rapid RPA assays for the detection of five carbapenemase targets and relate this to sensitivity and specificity of detection using clinical isolates.

METHODS

RPA primers and probes were designed against homologous regions of aligned sequences of KPC, NDM, OXA-48-like, VIM and IMP carbapenemase gene targets.

Real time Recombinase Polymerase Amplification kits (TwistDx® Exo) were obtained from TwistDx, Cambridge. Limit of detection (LoD) and time to positivity (TTP) were determined for each of the assays using purified genomic DNA from clinically relevant strains and/or plasmid constructs containing representative genes of the individual carbapenemase families.

Cross-reactivity of the assays was determined by testing against purified DNA extracted from a panel of 45 isolates comprising both Gram positive and Gram negative bacteria and fungal species that are frequently encountered in a range of clinical and environmental samples, other carbapenemase/antibiotic resistance genes and background human DNA.

Isolates were prepared by emulsifying of the bacteria in 200µL of distilled water followed by incubation at 95ºC for 10 minutes and then centrifugation at 20,000 rcf. 2µL of supernatant was used per RPA reaction.

Specificity was also assessed by evaluating against a panel of 630 clinical isolates with previously defined carbapenem resistance mechanisms (Table 1.)

Species	Carbapenemase families: KPC	NDM	OXA-48 like	OXA-48 like + NDM	VIM	IMP	none
Klebsiella pneumoniae	108	88	108	3	58	0	6
Klebsiella oxytoca	6	1	3	0	7	1	0
Klebsiella sp.	0	0	0	0	11	1	0
Escherichia coli	22	43	42	2	9	2	1
Enterobacter spp.	17	14	8	0	16	8	9
Pseudomonas spp.	0	0	0	0	0	11	2
Others*	5	6	1	0	9	1	1
Total	158	152	162	5	110	24	19

*24 isolates comprising *Citrobacter* spp. (n=16), *Raoultella* spp. (n=3), *Leclercia adecarboxylata* (n=2), *Serratia marcescens* (n=2) and *Kluyvera georgina* (n=1)

Table 1. Panel of 630 isolates used for evaluation of the carbapenemase RPA assays

RESULTS

Panel	Number isolates	KPC	NDM	OXA	VIM	IMP
CRCN	19	0	0	0	0	0
KPC	158	158	0	0	0	0
NDM	152	0	152	0	0	0
OXA	162	0	0	162	0	0
NDM/OXA	5	0	5	5	0	0
VIM	110	0	0	0	110	0
IMP	24	0	0	0	0	22
Total	630					

Table 2. Isolates detected in each of the 5 carbapenemase RPA assays. With the exception of the IMP assay, which is still in development, all the assays successfully identified the expected positives with no false positives from isolates containing other carbapenemase gene targets. Carbapenem resistant, carbapenemase negative (CRCN) isolates were negative in all tests.

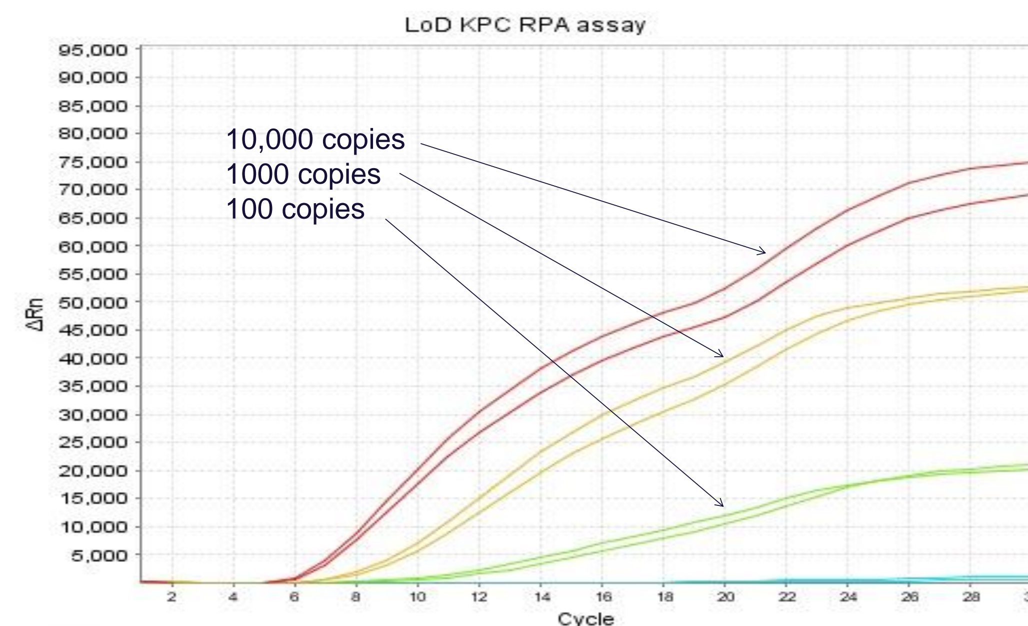

Figure 1. LoD amplification curves for KPC assay. Evaluation of serial dilution of KPC gene containing DNA demonstrated a detection limit of 100 copies in 14 minutes.

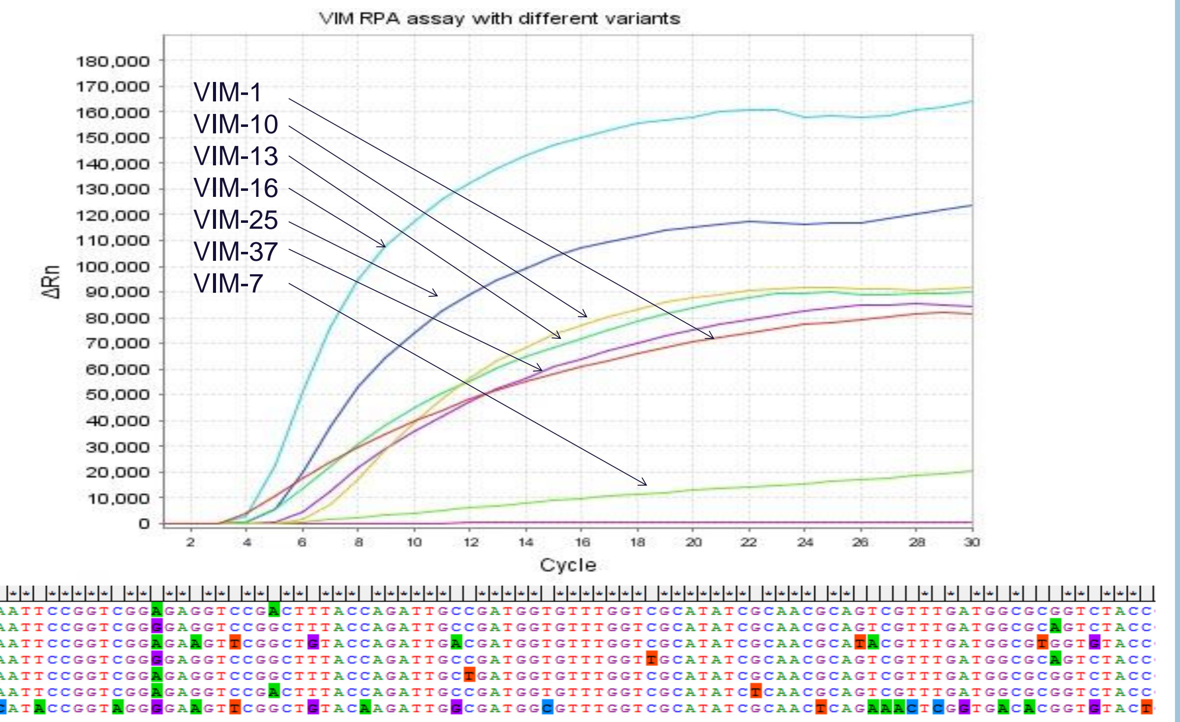

Figure 2. Detection of sequence variants on VIM RPA assay. The assay was able to detect the different sequence variants of VIM with some being better than others due to sequence homology to the primers and probes. VIM-7 is highly divergent from the other VIM sequences and therefore detected poorly by this assay. A separate assay for VIM-7 has been developed.

Target	LoD (copies)	TTP at LoD	Non-target DNA	Cross-reactivity panel	Inclusivity panel
KPC	100	14 mins	✓	100% specificity	100% sensitivity (n=158)
NDM	10	16 mins	✓	100% specificity	100% sensitivity (n=152)
OXA-48-like	10	10 mins	✓	100% specificity	100% sensitivity (n=162)
VIM	100	10 mins	✓	100% specificity	100% sensitivity* (n=110) *VIM-7 not included
IMP	100	15 mins	✓	100% specificity	91.7% sensitivity (n=24)

Table 3. Performance characteristics of the carbapenemase RPA assays. All assays demonstrated rapid detection of 100 genome copies or less in ≤16 minutes with the LoD being unaffected in the presence of 1×10^6 copies of non-target DNA. No cross-reaction was observed with a panel of non-target fungi, bacteria or other carbapenemase genes. All expected positives were detected by the individual assays with the exception of the IMP assay which is being further developed to improve the assay sensitivity.

CONCLUSIONS

- RPA assays successfully developed against KPC, NDM, OXA-48-like and VIM targets. The IMP assay requires some further development work to improve sensitivity
- Assay limit of detection between 10 and 100 genome copies
- Rapid detection (<20 mins) of target genes at LoD using RPA
- High levels of specificity and sensitivity demonstrated for all assays
- Assays to be transferred to microfluidic platform to demonstrate utility as a point-of-care device, potentially helping clinicians to manage patient antibiotic treatment options more effectively

REFERENCES

1. Canton R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen Ø, Seifert H, Woodford N and Nordmann P; the European Network on Carbapenemases (2012) Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 18(5): 413-31
2. Piepenburg O, Williams CH, Stemple DL and Armes NA. (2006) DNA detection using recombination proteins. *PLoS Biol.* 4(7): e204.
3. Ellington MJ, Findlay J, Hopkins KL, Meunier D, Alvarez-Buylla A, Horner C, McEwan A, Guiver M, McCrae LX, Woodford N, Hawkey P. (2016) Multicentre evaluation of a real-time PCR assay to detect genes encoding clinically relevant carbapenemases in cultured bacteria. *Int J Antimicrob Agents* 47(2):151-4
4. Kalsi S, Valiadi M, Tsaloglou MN, Parry-Jones L, Jacobs A, Watson R, Turner C, Amos R, Hadwen B, Buse J, Brown C, Sutton M, Morgan H. (2015) Rapid and sensitive detection of antibiotic resistance on a programmable digital microfluidic platform. *Lab Chip.* 15:3065-75

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