Introduction of molecular CPE testing during a large OXA-48 outbreak

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Introduction

Carbapenemase producing Enterobacteriaceae (CPE) pose a significant threat to public health due to rapid global dissemination, associated high mortality rates and limited treatment options. The ECDC estimate up to 89% of CPE carriers will develop infection, which has an associated mortality rates of between 50—70% [1,2,3,4]. In Ireland incidence has rapidly increased due to numerous healthcare facility outbreaks since 2009.

To contain and prevent further spread of CPE rapid, reliable screening methods need to be implemented, particularly in an outbreak scenario. Current CPE culture screening methods require a minimum time to detection of 48 hours and screening agars display variable detection sensitivity for weak carbapenemases such as IMP and OXA-48 [5,6].

Molecular diagnostics offer a same day test TAT and increased detection sensitivity of CPE, particularly for weak carbapenem hydrolysers. However such methods are not only expensive but limited to identifying gene targets predetermined by their bioinformatic design [7,8].

A real-time PCR performed on a semi-automated PCR platform could provide increased sample capacity, greater target sensitivity and the reduced TAT to allow hospital management to make safe patient placement decisions particularly in the outbreak scenario.

AIM: This study aimed to evaluate and implement a real-time PCR assay (LightMix CPE, TibMol Biol) for use on a semi-automated PCR platform (FLOWFLEX, Roche) during the largest CPE outbreak seen in Ireland to-date.

Methods

Samples were extracted on the MagnaPure 96 (Roche) and amplified on the LightCycler 480 II in 384 well format. The LightMix CPE assay (TibMol Biol) was performed in hexaplex, detecting IMP, VIM, NDM, KPC, OXA-48 plus an IAC(PhHV).

Specificity, Sensitivity & Limit of Detection study

42 CPE isolates were analysed on LightMix CPE assay for the sensitivity and specificity study. For the LoD study an 8-fold serial dilution was performed on 6 representative CPE isolates in a simulated faecal solution and processed on the LightMix CPE assay, the Gene Xpert CarbaR (Cephid) and ChromID CarbaSMART agar (BioMerieux). LoD was determined as the lowest dilutions to consistently produce a positive result.

Evaluation of culture and molecular OXA-48-like variant detection methods

An OXA-48-like variant analysis was undertaken on 7 variants (Table 3), which included a LoD study for both the LightMix CPE assay and Gene Xpert CarbaR. OXA-48-like variants were also analysed on ChromID CarbaSMART agar using a 10^6 cfu/ml conc of each variant.

Retrospective analysis of molecular CPE results

The retrospective analysis examined 93 non-repeat molecular CPE positive results over a one year period to determine what percentage molecular positive samples grew on ChromID CarbaSMART agar in 18-24 hours. Molecular CPE positives which failed to grow under standard protocols were enriched for the purpose of this study or were otherwise confirmed by the National CPE Reference Laboratory.

Results

LightMix CPE assay Specificity, Sensitivity and Limit of Detection analysis

Table 1: LightMix CPE assay specificity & Sensitivity analysis

SENSITIVITY	SPECIFICITY	POSITIVE PREDICTIVE VALUE	NEGATIVE PREDICTIVE VALUE
71%	100%	95%	100%

Low specificity due to inclusion of 2 VIM positive *Pseudomonas spps,* and low number of true negatives tested.

Table 2: Comparison of CPE detection methods Limit of Detection

CPE target	LightMix modular CPE assay LoD (CFU/ml)	GeneXpert CarbaR assay LoD (CFU/ml)	ChromID Carba SMART Agar LoD (CFU/ml)
IMP	10^3	10^2	10^4
VIM	10^2	10^3	10^6
NDM	10^1	10^2	10^3
KPC	10^2	10^2	10^2
OXA-48	10^2	10^2	10^6
OXA-181	10^3	10^3	10^4

OXA-48-like variant analysis

Table 3: Comparison of CPE detections methods against difficult to detect OXA-48-like variants

OXA-48-like variant strains	Growth on ChromID Carba Smart agar for 10^6 CFU/ml conc	LightMix CPE assay LoD (CFU/ml)		GeneXpert CarbaR LoD (CFU/ml)	
OXA-163 †	Neg	Pos	10^2	Pos	10^2
OXA-405 †	Neg	Pos	10^2	Pos	10^3
OXA-162	Pos	Pos	10^1	Pos	10^3
OXA-162 #2	Pos	Pos	10^2	Pos	10^2
OXA-204	Pos	Pos	10^2	Pos	10^3
OXA-232	Pos (single colony growth)	Pos	10^2	Pos	10^3
OXA-244	Pos (single colony growth)	Pos	10^2	Pos	10^3

Key: †= cephalosporinase activity

Retrospective analysis of CPE detected using LightMix CPE assay

Table 4: Retrospective analysis of molecular CPE result January to December 2017

Total no of CPE detected on LightMix CPE assay	No of CPE recovered on culture Day 1	No of CPE recovered via enrichment	No of non-culturable CPE confirmed by NCPERL
93	59	33	1
100%	63%	37%	

Discussion

- **The LightMix CPE assay significantly increased analytical sensitivity compared to the reference culture method used in this laboratory and was comparable to that of Gene Xpert Carba-R.**
- **LightMix CPE assay displayed increased analytical sensitivity for OXA-48-like variants than the Gene Xpert Carba-R, while both methods were a substantial improvement compared to the culture method.**
- **Molecular methods detected all OXA-48-like variants analysed as part of this study, regardless of whether they were a true carbapenemase or a cephalosporinase.**
- **A one year analysis of CPE positives detected on the LightMix CPE assay showed an additional 37% of CPE cases were detected by the introduction of molecular methods compared to culture methods alone. This increased detection rate allowed for earlier intervention in management of CPE positive cases which served to reduce the number of CPE contacts generated.**
- **The semi-automated molecular testing system easily accommodated the significant increase in CPE screens required in an outbreak scenario, from 240- 25,000 test per year.**

Conclusion

- **The reduced TAT and increased sensitivity allowed for earlier identification of CPE cases, earlier implementation of IPC measures and informed patient placement decisions.**
- **These measures helped to reduce the number of CPE contacts generated, and, likely, reduced CPE acquisition.**
- **While molecular diagnostics still remain expensive, the rapid TAT, high sensitivity and high throughput mean they are a crucial component in the management of a CPE outbreak due to early and improved detection of CPE carriers.**

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