Using a human in-vitro gut model to evaluate carbapenemase-producing Enterobacteriaceae screening methods

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Introduction

Global spread of carbapenemase producing Enterobacteriaceae (CPE) is a critical threat to public health(1). Rapid, sensitive and specific methods of screening patients for CPE colonisation are paramount, especially with increasing interest in medical tourism. Using a clinically reflective in-vitro human gut model we compared the relative accuracy of routine screening methods commonly used in clinical practice . A well validated, triple-stage chemostat was used to replicate the environment of the human large intestine(2).

Methods

Three gut models were seeded with pooled faeces from four healthy volunteers. Following an initial equilibration phase, the models were spiked with increasing inocula of carbapenemase-producing (CP) *Klebsiella pneumoniae* (range 1.8-8.9 log10 cfu/mL) - Figure 1.

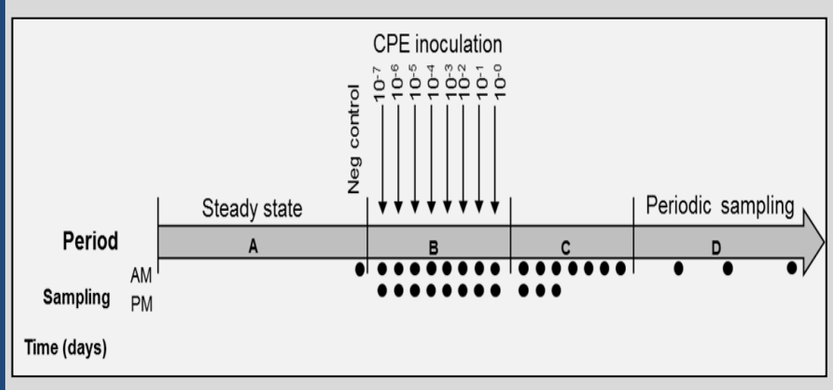

Figure 1. Experimental design. Increasing inoculum with twice daily sampling and follow on period.

Three clinical isolates with distinctive carbapenem genes (KPC, OXA48, NDM) were inoculated into seperate models. We compared the relative sensitivities of: two commercial multiplex real-time PCR assays, Cepheid Xpert® Carba-R (XCR) and the Check-Direct CPE Screen for BD MAX™ (CDCPE); two commercial culture media, chromID® CARBA SMART and chromID® ESBL; and MacConkey agar containing 0.5mg/L imipenem poured in-house (MAC-IMI). Twice-daily sampling was performed for the first week, with subsequent reduction in sampling post-inoculation - Figure 1. Each sample was tested in triplicate using all five screening methods.

Results

A total of 237 samples were tested. CPE were detected after an inoculation of ~4.9 log10 cfu/mL, and populations increased as CPE inocula increased. After inoculation, populations stabilised at 4-6 log10 cfu/mL in vessel 3 - Figure 2.

MAC-IMI agar was inferior to (lower limit of detection {LOD} 1.66log10cfu/ml) and less reliable than the commercial agars, which had similar sensitivity and a LOD of 0.82 log10 cfu/ml - Figure 2.

Sensitivity and specificity of the molecular tests were calculated using the reference method of triplicate positive culture. The results are summarised in Figure 3. XCR showed decreased sensitivity but increased specificity for KPC and OXA48 compared with CDCPE. Both methods had similar sensitivity for NDM, but XCR had higher specificity.

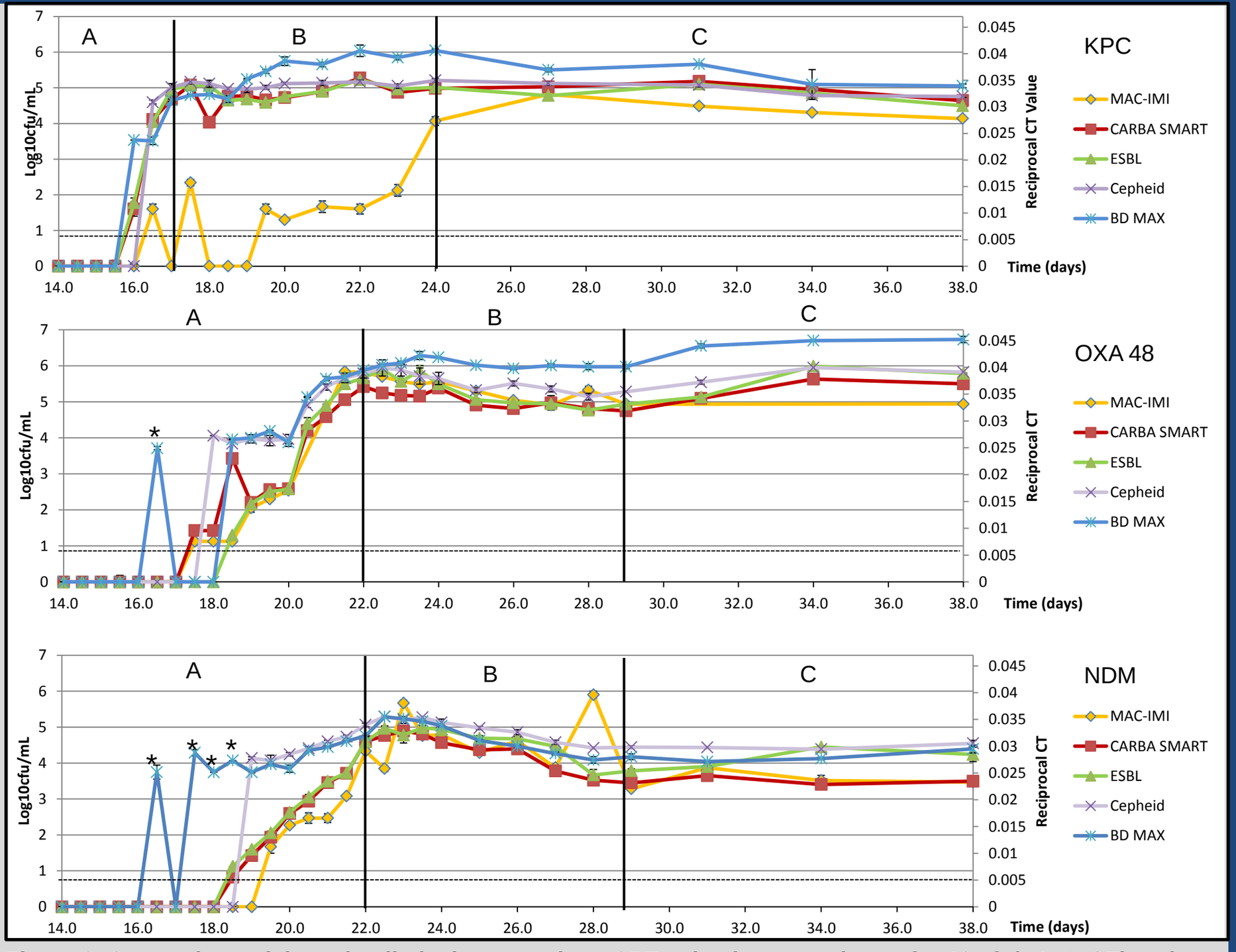

Figure 2. Comparison of detection limits between three CPE selective agars (mean log10 cfu/mL ± SE) and two molecular assays (mean 1/Ct ± SE) for the detection of CP *K. pneumoniae*. The yellow, red and green lines represent the selective agars, where values are read from the primary vertical axis; the purple and blue lines represent the molecular assays, where values are read from the secondary vertical axis; the black dotted line represents the limit of detection for culture media. The black dotted line represents the limit of detection for culture media (growth of a single colony; 0.82 log10cfu/mL); the asterisks indicate false-positive results.

Method	Gene	Sensitivity %	Specificity %
XCR	KPC	83.3 (73.6-90.6)	100 (97.6-100)
	OXA48	87.3 (76.5-94.3)	99.4 (96.9-100)
	NDM	96.5 (87.9-99.6)	99.4 (97-100)
CD CPE	KPC	97.6 (91.7-99.8)	98.0 (94.4-99.6)
	OXA48	92.1 (82.4-97.4)	87.1 (82.1-92.4)
	NDM	98.2(90.6-100)	86.7 (80.8-91.3)

Figure 3. Sensitivity and specificity of molecular screen tests with 95% CI. Calculated against triplicate positive culture as the reference method.

Total number of false-positive results was higher for CD CPE then XCR - Figure 4. A molecular test was considered positive with the detection of at least one CP gene regardless of internal algorithm or CT value, whereas reference methods were culture based.

Method	KPC	OXA 48	NDM	IMP1	VIM
XCR	0	1	1	0	0
CD CPE	3	21	24	0	0

Figure 4. Total number of false positive results for each gene.

Conclusion

- **The in-vitro gut model is a useful approach to measure CPE screening efficacy.**
- **Selective media had the highest sensitivity, but do not provide gene identification and require 24hrs incubation.**
- **CDCPE had lower specificity compared with XCR for individual gene identification – the higher false-positive rate may undermine its use for tracking outbreaks and for infection control interventions.**

References

1 Van Duin D, et al. Virulence 2017;19:460-469
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