Direct Detection of carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas spp*. from positive blood cultures

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INTRODUCTION

Carbapenemase-producing Gram-negative bacteria have been recognised as an increasing concern over the past decade, following global spread. The early detection of carbapenemase-producing organisms direct from blood cultures will allow early effective antibiotic therapy, improve antibiotic stewardship and allow infection control procedures to be initiated.

The aim of this project was to adapt and evaluate different commercially available systems, one biochemical and one molecular method, for the rapid direct detection of carbapenamase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa* in positive blood cultures:

Strains

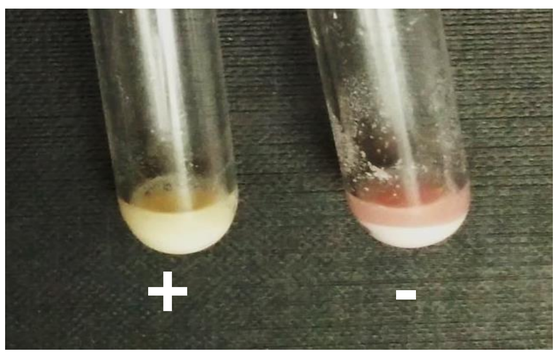
To develop assays experiments were performed on NCTC carbapenemase control strains, and then tested against clinical laboratory strains (KPC n = 3, VIM n = 6, NDM n = 2 and OXA-48 n = 2) comprising *K. pneumoniae* (4) *E. coli* (3) and *P. aeruginosa* (6) isolates.

For the spiked blood cultures bacteria were inoculated into 5 day negative blood cultures to give a final conc. of 10-100 cfu ml^{-1}. Samples were loaded onto the BacTec (Becton Dickinson).and processed when they flagged positive

Rapid CARB Screen

The Rapid CARB screen kit (Rosco Diagnostica), based on the CarbaNP biochemical assay [1], directly detects the hydrolysis of imipenem in the presence of an indicator, with a positive reaction being shown with a red-to-yellow colour change. The Rapid CARB screen has been validated for detection of carbapenemase activity from bacterial colonies, and contains instructions for detection direct from blood cultures. We found that following manufacturer's instruction led to indeterminate results in the Rapid CARB screen assay on up to 40% of samples. We developed a method using an enrichment culture followed by Rapid CARB assay that allowed the reliable detection of carbapenemase activity

1. 10 drops of positive blood culture added to 10 ml nutrient broth containing 10 µg meropenem disk
2. Incubated at 37°C for 3 hours, with vigorous shaking
3. Bacteria collected by centrifugation and washed in PBS
   The following steps are as instructed in the Rapid CARB assay
4. Protein extracted using commercial non-ionic detergent for 30 minutes
5. 50 µl protein extract added to 100 µl saline x 2.
6. Rapid CARB DIATAB and control DIATAB added to tubes
7. Reactions placed at 37°C for up to two hours, and inspected for red→yellow colour change at 30 60 and 120 minutes

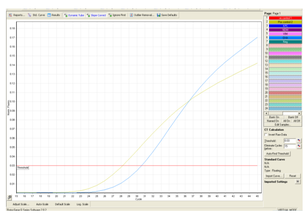


Check-Direct CPE

The Check-Direct CPE assay is a real-time PCR for the simultaneous detection and discrimination of KPC, OXA-48 VIM and NDM carbapenemases. It exists in two formats, the first open to use on any real-time amplification system, the second specifically designed for the BD MAX™ (Becton Dickinson) automated DNA extraction and PCR amplification platform. The Check-Direct assay has been validated for use on bacterial isolates and on rectal swabs [2], but has not been tested on blood cultures.
We developed and optimised sample processing methods for using the Check-Direct CPE assay direct on blood cultures on both the Qiagen Rotorgene Real-Time PCR cycler, and on the BD MAX platform.

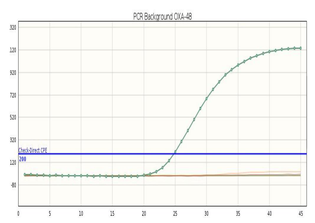
Check-Direct CPE

1. 3 µl positive blood culture added to 1ml sample buffer
2. 100 µl of this added to GeneOhm (Becton Dickinson) sample lysis tube
3. Sample vortexed for 5 minutes
4. Lysed sample diluted 1:100
5. Diluted DNA used as template for PCR following manufacturers instructions.
6. Results of assay interpreted following specified cut-off, threshold and analytical parameters



Check-Direct CPE for BD MAX

1. ~ 10 µl (1 drop) positive blood culture added to 1ml saline
2. 50 µl of this added to Sample extraction tube containing 600 µl water
3. Sample was loaded onto the BD MAX, and Check-Direct CPE mix added to extraction cartridge
4. Automatic DNA extraction performed using the Exk 1 DNA kit (Becton Dickinson), and PCR performed following manufacturers instructions
5. Results automatically analysed by BD MAX software, with presence (Ct value >0) or absence (Ct = -1) for each channel



RESULTS

1. The Rapid CARB screen was able to detect 17/17 carbapenemase strains, following our enrichment culture method. Results were available 4-7 hours after the start of the assay. No false positive reactions were observed with AmpC overproducers, or ESBL-producing strains. The assay was also able to detect a *P. aeruginosa* producing VIM metallo-ß-lactamase, and *A. baumannii* producing OXA-23.
2. The Check-Direct CPE assay performed on diluted blood samples detected 17/17 carbapenemase strains, with results available 3-4 hours after a blood culture became positive. Limit of detection of the assay was ~ 2 x 10^7 cfu per ml, or ~ 60 cfu per reaction. This process required 30 minutes of manual processing for a sample. No false positive reactions were observed.
3. The Check-Direct CPE assay for BD MAX reduced hands on time to around 10 minutes, and had an improved limit of detection ~ 2 x 10^5 cfu per ml, without affecting assay performance

Strain	Check Direct Rotorgene (Ct value)	Check Direct BD MAX (Ct value)	Rapid Carb Screen	Cfu ml^{-1} In Sample
Klebsiella pneumoniae NCTC 13438 KPC	KPC +ve (28.9)	KPC +ve (26.2)	+ve	4.6 x 10^9
Pseudomonas aeruginosa NCTC 13437 VIM	NDM/VIM +ve (32.0)	VIM +ve (23.2)	+ve	6 x 10^9
Klebsiella pneumoniae NCTC 13443 NDM-1	NDM/VIM +ve (35.9)	NDM +ve (29.0)	+ve	1.3 x 10^9
Klebsiella pneumoniae NCTC 13442 OXA-48	OXA-48 +ve (37.1)	OXA-48 +ve (23.6)	+ve	1.7 x 10^9

Table 1: Direct comparison of results produced from processing of spiked blood cultures with NCTC strains

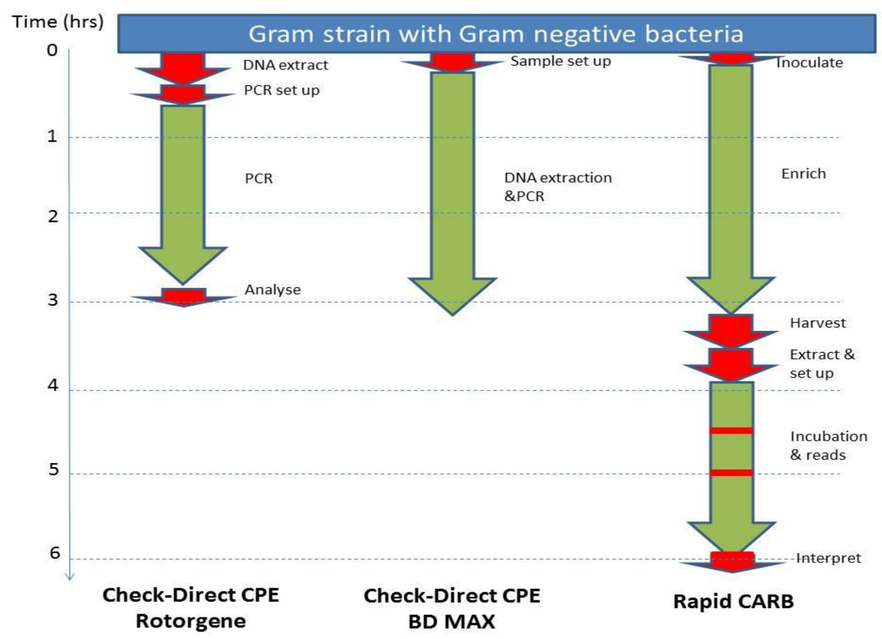


Figure 1: Comparison of the timeline of detection methods. Red signals 'hands-on' sample processing, green represents incubation stages

CONCLUSIONS

- We describe two methods for the direct detection of carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa* direct from blood cultures, with 100 % sensitivity and specificity.
- The Rapid CARB screen offers a simple and inexpensive method able to detect carbapenemase activity regardless of type, but suffers from a long turn-around time, and requires extended hands-on processing and offers no information on type of carbapenemase
- The Check-Direct CPE assay on diluted positive blood samples allows the sensitive detection and discrimination of KPC, VIM/NDM and OXA-48 carrying bacteria within 4 hours of a blood culture becoming positive
- Check-Direct CPE for BD MAX reduces hands-on processing time, and additionally discriminates VIM and NDM strains, with an enhanced limit of detection

REFERENCES [1] Nordmann *et al*, 2012. *Emerg Infec. Dis.* 18(9) 1503-7 [2] Nijhuis *et al*. 2013 Diagnostic Microbiology and Infectious Disease 77 316–320