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

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Objectives

The rapid detection of carbapenemase-producing Gram-negative bacteria from positive blood cultures is critical to implementing optimal antibiotic therapy and appropriate infection control procedures. Currently, detection is based on phenotypic methods that can take 72 hours to complete.

We evaluate two methods for the rapid detection of carbapenemase producing organisms direct from positive blood cultures. Check-Direct CPE [Check-Points] is a commercial multiplex real-time PCR for the simultaneous detection and differentiation of KPC, NDM, VIM and OXA-48 carbapenemases. Rapid CARB screen Diatabs [Rosco Diagnostica] rely on the direct detection of imipenem hydrolysis from bacterial extracts using a colorimetric indicator.

Methods

4 NCTC carbapenemase control strains (KPC n = 1, VIM n = 1, NDM-1 n = 1 and OXA-48 n = 1) and 14 characterised clinical carbapenemase-positive isolates (KPC n = 3, VIM n = 6, NDM n = 2 and OXA-48 n = 2 and IMP = 1) spiked into negative blood cultures were used to optimise conditions and evaluate for both assays.

The Check-Direct CPE assay was evaluated on the Rotorgene Q (using DNA extracted from 3 µl of spiked blood [BD GeneOhm Lysis Kit]) and the BD MAX (using on-board extraction). The PCR was run according to the manufacturer's instructions on both platforms.

Rapid CARB Screen was performed on blood cultures spiked with all carbapenemase positive isolates and 15 AmpC and ESBL positive (carbapenemase negative) isolates initially following manufacturer's guidelines, and subsequently using an enrichment subculture step.

Results

All KPC, VIM, NDM and OXA-48 isolates were detected directly from blood cultures in less than 3 hours on the RotorGene and BD MAX with a detection limit of 1 x 10^5 cfu/ml. This is well below the cfu/ml at which blood cultures flag positive (approximately 1 x 10^9 cfu/ml).

For the Rapid CARB screen assay, we found the manufacturer's instructions to use bacteria direct from blood cultures gave indeterminate results. However, when bacterial cells from a two hour subculture of the blood culture in nutrient broth with imipenem were collected by centrifugation, washed in sterile water, and used as the starting material for the assay this method detected 18/18 carbapenemase producing strains, including 3/3 OXA-48 strains, which can be difficult to detect by phenotypic methods. No false-positive reactions were obtained when tested against ESBL or AmpC producing strains.

Conclusions

We demonstrate two robust and reliable mechanisms for the detection of carbapenemase-producing organisms direct from positive blood cultures.

The detection time of the carbapenemase producing isolates was reduced by >10 fold compared with standard methods. These simple and rapid techniques have the potential to dramatically improve

patient outcome and facilitate rationalisation of both antibiotic use and infection control practices much earlier than currently possible.