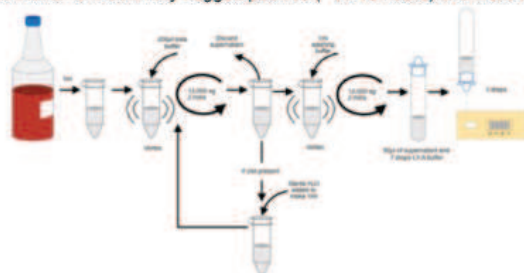


Introduction

- The increasing global prevalence of carbapenemase producing organisms (CPO) are a major public health concern. Rapid detection of organismal genotype and phenotype provides crucial information to inform empiric treatment and outbreak investigation, control and prevention with most current diagnostics achieving one or the other.
- MALDI TOF MS is an ionisation technique that enables rapid biomolecule analysis and identification of bacterial species with minimal sample preparation and low associated costs; consumables and technician time¹. It is one of the most broadly used spectrometry based diagnostics and achieves a result within minutes directly from positive blood cultures, removing the requirement for overnight plated incubations and bacterial isolation techniques².
- The Resist O.K.N test is a single, disposable multiplex lateral flow device (LFD) for the detection of OXA-48, KPC and NDM like carbapenemases in Enterobacteriaceae from solid culture. Utilising an antibody mediated approach, this multiplex LFD offers a simple, rapid and cost effective test (~£15/cartridge)³ where immunological capture of particular OXA-48, KPC and NDM epitopes are immediately readable with a non-enzymatic technique using antibody conjugated to a colloidal gold particle⁴.
- As blood culture diagnostics remains the current gold standard for investigations into bacteremic patients, we aimed to investigate whether the Resist O.K.N could be used directly on positive blood cultures and incorporated into the existing diagnostic workflow for processing positive blood cultures based on MALDI TOF MS analysis.

METHODS

- 80 isolates [*Enterobacteriaceae* (n=54)] and non-fermenters [*Pseudomonas* spp. and *Acinetobacter* spp. (n=26)] were obtained from clinical or screening samples and their CPO status confirmed by the Reference Laboratory (AMRHA, PHE).
- BD BacTec 5 day negative blood cultures (~6 weeks old) were artificially spiked with 10^3 CFU/ml of characterised isolates and incubated on the BacTec instrument until they flagged positive (~10-12 hours) and were then processed.



- * Resist O.K.N was incorporated into the Bruker Sepsityper™ protocol for processing positive blood cultures, outlined above, and MALDI TOF MS run as per the manufacturer's instructions.

RESULTS

- **Table 1. Results from the Resist O.K.N.** In total, all 15 false negatives recorded were NDM isolates and the 4 false positives recorded were from non-fermenters. Examples of accepted reads are demonstrated in Figure 1.

Genotype	n	Resist3 O.K.N	%
NDM	27	12	44.4
KPC	3	3	100
OXA-48	25	25	100
Negative	28	24	85.7

Table 1. NDM, KPC, OXA-48 and Negative results obtained by the Resist O K N and percentage correctly identified.



Figure 1. Photographic representation of each accepted outcome of the Resist3 O.K.N assay

- Overall the sensitivity and specificity was 71.7% and 85.2%, respectively (PPV=90.5% and NPV=60.5%). Exclusion of NDM isolates resulted in sensitivity rising to 100% with specificity remaining unchanged (PPV=86.7% and NPV=100%). Further exclusion of non-fermenters gave sensitivity and specificity of 100%.
- Additionally, MALDI TOF MS generated correct positive ID's for all isolates run directly from blood cultures with accuracy readings in the range of 2.22-2.54.
- **Difficulties in NDM detection:** to enhance detection, the read time was increased from the manufacturer's instructions of, 15 minutes to 5 minute intervals from 10-30 minutes. This enabled the identification of the 12 positive results obtained. However, those detected had notably less visible lines. Neither repeats of increased inoculum nor using 5 day old blood cultures showed any improvement. However, performing the Resist O.K.N on the SepsiTyper final pellet, to be used for the MALDI TOF MS analysis, enabled 6 of the 12 detected to be positively identified.

CONCLUSIONS

- The PPV and NPV of 86.7% and 100% respectively for the detection of KPC and OXA-48 in Enterobacteriaceae and non-fermenters directly from blood cultures is promising. Exclusion of non-fermenters (which Resist O.K.N is not validated for) improved sensitivity and specificity to 100%.
- The combination of MALDI TOF MS and Resist O.K.N enabling a genotypic and phenotypic diagnosis for KPC and OXA-48 within an hour of blood cultures flagging positive could provide a poignant contribution in primary screening of high risk patients, confirmatory diagnostics or rapid point of care testing in the future.

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