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Direct detection of carbapenem resistance determinants in clinical specimens using immunochromatographic lateral flow devices

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Sir,

Accurate, rapid, affordable diagnostic tests are critically important in tackling the problem of antimicrobial-resistant infections. Assays that can be used for pathogen detection and drug susceptibility, close to the point of care (POC), have the most potential to influence clinical decisions, antimicrobial prescribing, stewardship and patient outcomes.[1,2] Considerable effort has been invested in the development of assays for the rapid detection of bacterial sepsis and antimicrobial resistance determinants. This includes genotypic (DNA amplification/sequencing) and phenotypic assays targeted towards bacterial strains such as the ESKAPE (*Enterobacter*, *Staphylococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Enterococcus* spp.) group of organisms, but also for specific resistances to key antimicrobial therapies.[3]

The need to detect resistance to carbapenems (ertapenem, imipenem, meropenem) has received considerable attention.[4] Once considered drugs of last resort in the treatment of Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae)*, their efficacy is now compromised by the global spread of carbapenemases (KPC, NDM, VIM, OXA). Carbapenemase genes are usually carried on MDR plasmids that encode enzymes able to degrade or destroy carbapenems and also many other β-lactam antibiotics.[5]

There are myriad methods for the detection of carbapenem resistance genes and for carbapenem-resistant Enterobacteriaceae (CRE). Recently a number of immunochromatographic lateral flow cassettes have been developed (CORIS BioConcept, Gembloux, Belgium) that enable 5 min detection of KPC, NDM, VIM and OXA-48-like carbapenemases directly from cultured bacterial colonies.[6–10] Versions of the assay have been evaluated in simplex (KPC *K*-SeT®, OXA-48 *K*-SeT®) and multiplex (RESIST-3 O.K.N.) forms across different laboratories, with 100% sensitivity and specificity reported when compared with molecular methods of detection.[6–10] In lower limit of detection (LLD) studies the minimum bacterial load required in a sample is quoted as low as 10^4–10^5 cfu/mL.

Experience to date suggests that this technology could be well suited and further adapted for use directly with clinical samples as a POC test. Here we assessed the performance of the RESIST-3 O.K.N. card for the direct, simultaneous detection of KPC, NDM and OXA-48-like carbapenemases in positive blood culture bottles.

Thirty carbapenem-resistant (meropenem MIC >4 mg/L) enterobacterial isolates (*K. pneumoniae* $n = 16$, *E. coli* $n = 8$, *Enterobacter cloacae* $n = 4$, *Citrobacter koseri* $n = 2$) known to produce KPC-2 ($n = 8$), OXA-48/181/232 ($n = 14$), NDM-1/5/7 ($n = 12$) and VIM ($n = 2$) β-lactamases (singly or in combination) were used in the evaluation. Isolates were grown overnight at 37°C in 3 mL of Trypticase soy broth (TSB; Oxoid, Basingstoke, UK), then serially diluted in PBS to a final concentration of 10^2 cfu/mL. Inocula were quantified by viable bacterial counts plated using the Miles and Misra technique on Mueller–Hinton II (MH II) agar. Inocula of 10^2 cfu were aliquoted into Aerobic (FA Plus™) and Anaerobic (SN™) BacT/ALERT blood culture bottles (bioMérieux) followed by the addition of 10 mL of sterile heparinized horse blood (Oxoid). Bottles were incubated aerobically on the BacT/ALERT system for 18 h. Aliquots (0.1 and 0.5 mL) were removed from bottles once flagged positive (<10 h) and centrifuged at 12 000 rpm for 1 min (Biofuge, Heraeus). After discarding the supernatant, 10 drops of lysis buffer (LY-A) were added to the deposit. Three drops of this suspension were added to wells of RESIST-3 O.K.N. cassettes and results read within 15 min. Bottles were subcultured to selective mSuperCARBA agar (bioMérieux) to ensure viable growth of each CRE in both blood culture bottles and that each still produced the target carbapenemase.

All BacT/ALERT FA Plus™ and SN™ bottles spiked with isolates known to produce OXA-48-like or KPC-2 carbapenemases were positive following direct inoculation of the RESIST-3 O.K.N. cards; no additional bands were observed with either of the isolates co-producing VIM. None of the isolates producing NDM carbapenemases gave positive bands on cards inoculated with preparations made from blood culture bottles, despite each strain being strongly positive when taken directly from a TSB culture. To investigate any possible inhibitory effects of the BacT/ALERT blood culture base media on the growth of NDM carbapenemase producers, the experiment with NDM-producing strains was repeated using bottles supplemented additionally with 1 mg/L meropenem and 0.1 M $ZnSO_4$. These were added to control for any growth effects due to sequestration of antimicrobials or divalent cations by components of the culture media or horse blood.

We found the RESIST-3 O.K.N. triplex card to be compatible with the BacT/ALERT blood culture media and highly sensitive in the detection of KPC and OXA-48-like carbapenemases in Enterobacteriaceae. This is in accordance with recent reports that used single immunochromatographic assays (OXA-48 *K*-Set®) for the direct detection of OXA-370 and another blood culture system (VersaTREK®; ThermoFisher, Waltham, MA, USA).[10] We observed

only very weak bands for NDM, in stark contrast to those for OXA-48 and KPC, when applying a mixed KPC/OX-48/NDM culture. Furthermore, the effects of the volume of blood culture media and bottle used in incubation influenced the result. Weak positive NDM bands were obtained using 100 μL from BacT/ALERT FA Plus™ bottles whilst tests using cultures from BacT/ALERT SN™-inoculated bottles were negative with 100 μL of culture and only weakly positive on increasing the material to 500 μL. This was despite supplementation of bottles with meropenem and $ZnSO_4$ to promote growth and limit Zn starvation, which could be due to components of the bacterial culture media or addition of horse blood. Increasing the biomass further (>1.5 mL) risks making the procedure impractical for diagnostic laboratories as the volume exceeds the capacity of most microcentrifuges. Direct detection of NDM from positive blood culture sets will likely require an alternative protocol or further enrichment of the inoculum before it can be recommended for diagnostic use in the detection of NDM.

Rapid identification of KPC- and OXA-48-like-mediated carbapenem resistance from positive blood culture appears to be possible using the RESIST-3 O.K.N. cassettes. This requires minimal technical expertise and only access to a semi-automated blood culture system, common to most diagnostic laboratories. Adaptation of the test as a true POC test may be best pursued by applying it as a tool to either aid patient screening for carriage of CRE or in the diagnosis or empirical treatment of urinary tract infections. Successful identification of OXA-48 directly from pre-incubated (2 h) spiked stool samples has recently been reported with high sensitivity and a lower limit of detection of 10^2 cfu/mL.[10] The same could be expected with urine samples, for which the critical limit of 10^5 cfu/mL bacteria is widely used as a diagnostic criterion to inform treatment.

Further evaluation of rapid immunochromatographic assays for the detection of carbapenem-resistant infections should be undertaken prospectively and in parallel with prevailing tests (e.g. PCR) to assess their utility and economic viability in the management of CRE carriage and infection using real-time patient samples in a setting with a high prevalence of CRE infections. This, along with a cost–benefit analysis, will promote their most effective and diligent use as part of any strategy aimed at combatting antimicrobial resistance.

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