EV0513
ePoster Viewing
Diagnostic bacteriology – non-culture based, including molecular and MALDI-TOF

Development of rapid isothermal recombinase polymerase amplification assays for the detection of carbapenemase genes

Carrie Turner*[1], Collette Allen[1], Daniele Meunier[2], Katie Hopkins[2], Neil Woodford[3], Mark Sutton[1]

[1]*Public Health England, National Infection Service, Salisbury, United Kingdom*

[2]*Public Health England, National Infection Service, London, United Kingdom*

[3]*Public Health England, London, United Kingdom*

Background: Acquisition of transferable carbapenemase genes has been recognized internationally as a major public health threat. The so called 'big five' carbapenemase families include the KPC and OXA-48-like non-metallo-enzymes and the NDM, VIM and IMP metallo-enzymes. Rapid detection of these is crucial, not only for patient management but also for infection prevention and control of further transmission. Recombinase polymerase amplification (RPA) is an isothermal nucleic acid amplification method that is an alternative to real-time polymerase chain reaction (qPCR). Such assays are amenable to miniaturization on a digital microfluidic platform (Kalsi *et al.* 2015. *Lab Chip*. 15:3065-75). Here we describe the development of rapid RPA assays for the detection of five carbapenemase targets and relate this to sensitivity and specificity of detection using clinical isolates.

Material/methods: RPA assays were developed against KPC, NDM, OXA-48-like, VIM and IMP targets. Assay limit of detection (LoD) and time to positivity (TTP) were established for each of the assays using purified genomic DNA (gDNA) from relevant clinical strains and/or plasmid constructs containing representative genes of the individual carbapenemase families. Cross-reactivity of the assays was determined by testing against a panel of bacteria that are frequently encountered clinically, other carbapenemase/antibiotic resistance genes and background human DNA. Specificity was also assessed by evaluating against a panel of approximately 450 clinical isolates with previously defined carbapenem resistance mechanisms.

Results: All assays reported a LoD of between 10 and 100 genome copies with TTP of less than 20 minutes. No cross-reactivity was observed with non-target bacteria or other antibiotic resistance genes (100% specificity). No false-positives occurred in the presence of human DNA and the assay LoD was unaffected in the presence of a high concentration of non-target DNA. Of the 450 clinical isolates tested, >95% were identified correctly with the relevant assays with no false-positives observed and reasons for discrepant results will be described.

Conclusions: We successfully developed isothermal RPA assays that showed high levels of sensitivity and specificity against the five major carbapenemase families We aim to transfer these tests onto a microfluidic platform as a proof of concept to demonstrate their utility within a point-of-care device which could allow diagnosis of carbapenemase-producing bacteria, potentially allowing

clinicians to manage patient antibiotic treatment options more effectively. The potential for the use of the devices for applied infection control will also be evaluated.