New multiplex molecular diagnostic assay for the detection of clinically relevant antibiotic resistance genes

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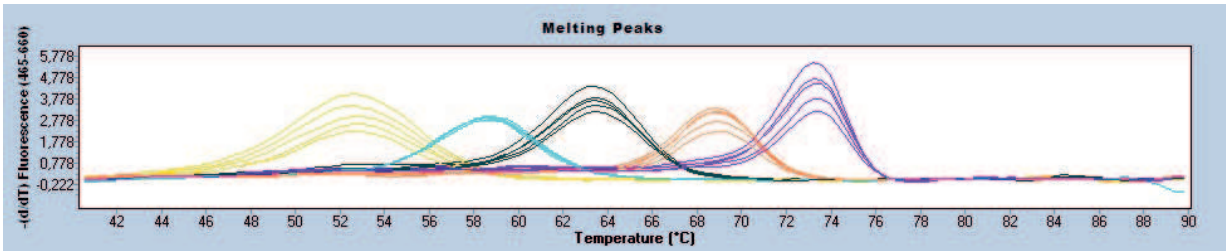
Bacterial resistance to antibiotics is a major global health concern due to the wide range of antibiotic resistance genes found in bacteria [1], and the increasing lack of effective treatment options available [2]. Early recognition of the type of antibiotic resistance genes in clinical samples allows (i) better targeted antibiotic therapy, thereby avoiding the unnecessary use of ineffective antibiotics, and (ii) more effective infection control procedures in healthcare settings, helping reduce the spread of antibiotic resistant pathogens.

During the PneumoNP project, PathoFinder designed and tested a 2-step multiplex amplification in real-time (2SMART) assay [3] that simultaneously detects and distinguishes several antibiotic resistance gene families, including extended-spectrum beta-lactamase (ESBL) (TEM, SHV, CTX-M-1, -2, -8, -9, -25), plasmid-mediated *ampC* genes (DHA, CMY, MOX, FOX), the most important carbapenemases (KPC, OXA48, IMP, VIM, NDM), the recently discovered plasmid-borne gene mediating colistin resistance (*mcr*-1) [4], and a housekeeping gene (hemolysin) of *Klebsiella pneumoniae,* which is being used as a model bacterium for infection studies during the PneumoNP project. The assay is based on fluorescent melt curve analysis and can be performed using standard real-time PCR equipment that many clinical laboratories already have available *in house*. Time to result is 2 ½ hours (excluding nucleic acid extraction) The present prototype assay detects the above mentioned antibiotic resistance genes with a sensitivity of ≤100 copies/reaction.



Example of 2SMART assay output. Analysis of dilution series of (left to right) CTX-M-9, -1, -8, -25 and *mcr-1* at 1^E6 - 1^E2 copies/reaction. Melt peaks for the respective targets are at 53, 58, 63, 69 and 73°C, as expected.

References

1. Philippon, A., et al., Antimicrob Agents Chemother, 2002. **46**(1): p. 1-11.
2. WHO, Antimicrobial resistance: global report on surveillance, 2014.
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4. Liu, Y.Y., et al., The Lancet. Infectious diseases, 2016. **16**(2): p. 161-8.