O220

2-hour Oral Session

Real-time PCR with high resolution melting analysis for rapid detection of resistance determinants in *Neisseria gonorrhoeae*

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Objectives: The emergence of resistance to extended-spectrum cephalosporins, azithromycin (AZT) and ciprofloxacin in *Neisseria gonorrhoeae* (*Ng*) represents a public health concern. The primary resistance determinants are mainly acquisition of a mosaic *penA* gene (e.g., type XXXIV with or without substitutions in amino acid position 501 of the PBP2), A2059G or C2611T mutations in 23S rRNA alleles, and Ser91Phe substitution in GyrA, respectively. Currently, resistance testing is mainly based on time-consuming phenotypic culture-based tests. Therefore, there is an urgent need to develop rapid genetic tests to predict resistance, ideally combined with *Ng* detection. This is mainly necessary to inform adequate treatment, which will limit further emergence and spread of resistant isolates. Real-time PCR with High Resolution Melting (HRM) analysis is a sensitive tool for detection of sequence variations. Herein, we have implemented this methodology to detect the most clinically important resistance determinants in *Ng*.

Methods: Overall, 41 *Neisseria* spp. isolates were tested. Twenty-six *Ng* isolates were previously characterized at phenotypic and molecular level. Of these, 22 had the Ser91Phe substitution in GyrA and seven had the mosaic *penA* XXXIV. Moreover, the ceftriaxone-resistant *Ng* strain F89 (*penA* XXXIV, PBP2 Ala501Pro); one AZT high-level-resistant *Ng* strain (AZT-HLR; A2059G mutations in the 23S rRNA alleles); the wild-type reference strain ATCC49226; and twelve commensal and zoonotic *Neisseria* spp., were included in the study. The assay was performed on QuantStudio 7 Flex System with MeltDoctor Master mix (Applied Biosystem). *Ng* identification was based on 16S rRNA and *opa* genes. Primers were designed to flank the mutation site of *gyrA*, 23S rRNA and specifically amplify the *penA* mosaic XXXIV. They generated ~60bp products and operated at the same conditions in single and multiplex reactions.

Results: The identification of *Ng* (n=29) was 100% specific. The *Ng* harboring the Ser91Phe substitution in GyrA generated discernible melting curves compared with wild-type isolates. Similarly, the strain (AZT-HLR) with A2059G mutations in the 23S rRNA alleles generated a unique profile compared with isolates harboring wild-type alleles. All *Ng* with mosaic *penA* XXXIV were amplified (i.e., seven clinical *Ng* and F89) were correctly identified. Furthermore, strain F89 produced a different melting curve compared to isolates with *penA* XXXIV but wild-type 501 PBP2. However, amplification of mosaic *penA* XXXIV also occurred for three non-*Ng* species (*N. meningitis*, *N. mucosa*, *N. macacae*). The methodology was 100% sensitive (i.e., all mutations in the antibiotic resistance determinants were correctly detected).

Conclusions: Our methodology can accurately detect the most frequent mutations generating resistance to antibiotics usually implemented for the management of *Ng* infections. The clinical impact of this rapid diagnostic tool on the outcome of gonococcal infections should be evaluated in the near future directly testing clinical samples.