P1864 Timely detection of sequence variations of three genes potentially associated with Mycoplasma genitalium treatment failures

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Background. Mycoplasma genitalium (MGEN) infection is a major cause of urethritis in men and is associated with cervicitis, pelvic inflammatory disease, preterm birth, and spontaneous abortion in women. First-line treatment for MGEN infection is the macrolide azithromycin (AZI). SNPs in the 23S rRNA gene of MGEN result in reduced affinity to the 50S ribosomal macrolide target, and are held responsible for the worldwide emergence of macrolide-resistant MGEN. Here, we studied the technical feasibility of determining the 23S rRNA gene sequence of MGEN directly from positive clinical specimens by real-time PCR and melting-curve analysis. In addition, the variability of gyrA and parC gene loci of MGEN were studied as a potential surrogate marker for quinolone resistance.

Materials/methods. The analytical sensitivities (LOD) of all targets were determined using a quantified DNA standard (Vircell). Clinical specimens (urines; urogenital swabs) were screened for the presence of MGEN by the AnyplexII STI-7 assay (Seegene). SNPs in the 23S rRNA gene were analysed using “LightMix Modular Mycoplasma Macrolide” (TibMolbiol). For gyrA and parC published primers were used with a SYBRGreen-PCR and PCR products were subsequently sequenced. Obtained sequences were aligned and compared with database.

Results.

LODs (genome equivalents/PCR) were: 23S rRNA gene, 15; gyrA, 15; and parC, 8.

Screening by multiplex PCR revealed 23 MGEN positive specimens. Of these, 16 (89%) were wild-type and two (11%) showed SNPs in the 23S rRNA gene, respectively. In addition, 19 (95%) revealed wild-type gyrA gene sequences; one specimen showed a silent mutation (C270T). A total of 16 (76%) parC gene sequences were wild-type; 4 (19%) had silent mutations (C234T). One specimen (5%) was a mixed infection (C and T at position 234). One specimen (5%) was not wild-type (G248T with a change in amino acid sequence).

In 2 specimens (9%), that yielded MGEN by the STI-7 assay, no amplification was obtained.

Conclusions: Analytical sensitivities were excellent. Timely confirmation of MGEN wild-type 23S rRNA gene sequence directly from clinical specimens is feasible and may have impact on current treatment guidelines. For quinolones, the relatively high proportion of silent mutations found may limit this approach for predicting treatment failures.