DETECTION OF MYCOPLASMA GENITALIUM MACROLIDE RESISTANCE USING THE OPEN CHANNEL OF THE PANTHER FUSION® SYSTEM

Background Current epidemiological studies demonstrate a high prevalence of Mycoplasma genitalium (MG) infections in high-risk groups, especially MSM. Owing to the widespread macrolide resistance the European Guideline on MG infections recommend complementing the molecular detection of MG with an assay capable of detecting macrolide resistance-associated mutations. Macrolide resistance is caused by a single nucleotide polymorphism (SNP) in region V of the 23S rRNA gene. Two nucleotide positions in the 23S rRNA gene have been associated with azithromycin resistance: A2058 and A2059.

Methods The Aptima M. genitalium Assay® was used for the initial detection on the Panther® system. A hydrolysis probe real-time PCR on the Open Channel of the PantherFusion® system for automated reflex testing after MG positive results was established and validated. The Open Channel of the instrument allows the use of custom primers and probes on the PantherFusion® system. Minor groove binder (MGB) hydrolysis probes were used for accurate and reliable SNP discrimination at position 2058/2059. The workflow enables an automated analysis process including DNA extraction, PCR setup, and results interpretation. Using the open channel 300 samples could be genotyped within an 8 h working day.

Results 60 MG positive clinical samples were tested. The laboratory developed test (LDT) was able to detect the wild type variant in 20 samples and the A2058G/A2059G mutations in 22 samples. All results were confirmed by amplicon sequencing and commercially available test system. 18 (30%) samples did not show any typing result either using different LDT or commercial test system.

Conclusion MG positive samples can be typed for macrolide resistance using our LDT on the same platform during one run. The combination of a MG high-throughput test followed by macrolide resistance testing improves the efficiency of large-scale epidemiological resistance surveillance. However, highly sensitive TMA assay might result in a significant number of non-typable samples. Further studies are needed to improve the sensitivity and explanatory power of MG resistance testing.

Disclosure No significant relationships.

SIMPLEPROBE PCR ASSAY FOR DETECTION OF MUTATIONS ASSOCIATED WITH MACROLIDE RESISTANCE IN MYCOPLASMA GENITALIUM SAMPLES

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Background Macrolide-resistant strains of Mycoplasma genitalium are an increasing problem throughout the world, and the implementation of a rapid and sensitive assay for mutation detection to guide treatment is needed. Macrolide-resistant strains have been shown to contain base substitutions in positions 2058 and 2059 (Escherichia coli numbering) in region V of the 23S rRNA gene. In this study, we present a SimpleProbe PCR followed by melting curve analysis to differentiate between macrolide-resistant mutants and wild types.

Methods The assay was performed on 159 Mycoplasma genitalium-positive samples, and the results were compared with DNA sequencing. We also looked at the prevalence of macrolide-resistant strains in a Norwegian population.

Results Of 139 samples characterized successfully by sequencing, 54 (39%) were wild types and 85 (61%) were mutants, consisting of 59 (42%) A2059G, 24 (17%) A2058G, 1 (1%) A2058T, and 1 (1%) A2059C mutation. The melting curve analysis correctly differentiated between wild-type and mutant strains in all cases, but it could not identify the different mutant types.

Conclusion The SimpleProbe PCR proved to be a simple, rapid, and reliable method for the detection of macrolide-resistant isolates of Mycoplasma genitalium in a clinical setting.

Disclosure No significant relationships.

CLINICAL EVALUATION OF THREE COMMERCIAL PCR ASSAYS FOR THE DETECTION OF MYCOPLASMA GENITALIUM AND MACROLIDE RESISTANCE

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Background Because macrolide resistance is increasing worldwide in Mycoplasma genitalium (MG), it is recommended to detect macrolide resistance-associated mutations in MG-positive specimens. Some new commercial kits detect macrolide resistance at the same time as MG detection. The aim the study was to prospectively evaluate the clinical performance of three commercial kits for the detection of MG and macrolide resistance.

Methods Two hundred MG-positive urogenital specimens detected using an in-house real-time PCR are prospectively collected at the Bacteriology Department of Bordeaux University Hospital (France). After DNA extraction of the specimens using the MagNa Pure 96 (Roche), the specimens are submitted to four assays: the SpeeDx ResistancePlus MG assay, the Diagenode S-DiaMGRES assay, the PathoFinder Real accurate TMAcRES and the in-house FRET qPCR assay and 23S rRNA sequencing used as reference. The internal controls of each kits were added in the specimen before extraction and the absence of amplification inhibition associated with the addition of the three internal controls was previously checked.

Results To date, 114 MG-positive specimens have been analyzed. The clinical sensitivity for MG detection was similar for the three commercial kits, ranging between 95.5 and 98.2%. To compare the macrolide resistance detection performance, 90 specimens that have been found MG-positive using the three kits and that could be amplified using the in-house FRET qPCR assay were retained. Therefore, compared