Performance of the first commercial dual resistance assay, AmpliSens Mycoplasma genitalium-ML/FQ-Resist-FL, for detection of potential macrolide and quinolone resistance-associated mutations and prevalence of M. genitalium resistance mutations in St. Petersburg, Russia

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ABSTRACT

Objectives Antimicrobial resistance in Mycoplasma genitalium (MG) is a poorly surveyed and controlled global health concern. We evaluated the first commercial dual resistance assay, AmpliSens M. genitalium-ML/FQ-Resist-FL assay, for detection of potential macrolide and quinolone resistance-associated mutations (MRAMs and QRAMs, respectively) and estimated the prevalence of these mutations in MG in St. Petersburg, Russia.

Methods Urogenital samples positive (n=145 from 2007 to 2020) and negative (n=56 from 2021) for MG in routine diagnostics were retrospectively analysed using the AmpliSens M. genitalium-ML/FQ-Resist-FL assay (Central Research Institute of Epidemiology, Moscow, Russia) and Sanger sequencing for validation.

Results The AmpliSens M. genitalium-ML/FQ-Resist-FL assay detected potential MRAMs and QRAMs with sensitivities of 100% (CI95% 83.9 to 100) and 92.3% (CI95% 66.7 to 99.6) and specificities of 99.2% (CI95% 95.6 to 100) and 100% (CI95% 97.2 to 100), respectively, in clinical specimens with ≥1000 MG gen/mL. In total, MRAMs were detected in 13.8% (CI95% 9.1 to 20.3) of samples, with 23S rRNA A2058G being the most prevalent mutation (45.0% (CI95% 25.8 to 65.8)). QRAMs were found in 9.0% (CI95% 5.3 to 14.7) of samples, with S83I the most frequent mutation (53.8% (CI95% 29.1 to 76.8)). Dual resistance was observed in 5.5% (CI95% 2.8 to 10.5) of samples. Potential MRAM and dual resistance rates significantly increased over time: from 0% in 2007–2008 to 25% (p<0.0009) and 10% (p=0.0447), respectively, in 2018–2020. QRAM rate appeared to increase (from 0% to 13%), but significance was not reached (p=0.0605).

Conclusions The rapid increase in MG antimicrobial resistance in St. Petersburg, especially prominent for MRAMs, necessitates implementation of macrolide resistance-guided therapy in Russia. The first commercial dual resistance assay, AmpliSens M. genitalium-ML/FQ-Resist-FL assay, was sensitive and specific for detection of potential MRAMs and QRAMs and could be valuable in macrolide resistance-guided therapies and possibly for surveillance of QRAMs. International surveillance of antimicrobial resistance-associated mutations in MG, further research into clinical relevance of several parC mutations and novel treatments are essential.

INTRODUCTION

Mycoplasma genitalium (MG) causes urethritis in men and is associated with reproductive tract diseases in women. For uncomplicated MG infections, the European MG guideline recommends macrolides (azithromycin/josamycin) as first-line treatment in the absence of macrolide resistance-associated mutations (MRAMs) and moxifloxacin when MRAMs detected or as second-line treatment. MG macrolide resistance levels have rapidly increased internationally. MG macrolide resistance is primarily caused by 23S rRNA gene mutations, at nucleotide position A2058 or A2059 (Escherichia coli numbering), and moxifloxacin resistance is predominantly mediated by parC mutations, that is, in ParC S83 and D87 codons (MG numbering).

Testing MG-positive samples for MRAMs before treatment is recommended internationally. Many laboratory-developed tests and several commercial assays for MRAM detection are available.

Recently, SpecDx MG parC (beta) PCR assay, the first commercial test for quinolone resistance-associated mutations (QRAMs) detection, displayed high sensitivity and moderate specificity.

We evaluated the first commercial dual resistance assay, AmpliSens M. genitalium-ML/FQ-Resist-FL assay, for detection of potential MRAMs and QRAMs and estimated the prevalence of these mutations in St. Petersburg, Russia.

MATERIAL AND METHODS

Study design, patients and specimens

(Central Research Institute of Epidemiology, Moscow, Russia) and 186 (93.9%) were MG-positive with the evaluated AmpliSens M. genitalium-ML/FQ-Resist-FL assay (Central Research Institute of Epidemiology). Of these 186 samples, 41 (22%) contained <1000 geq/mL of MG DNA (based on MG gyrB quantification), that is, the cut-off for reliable MRAM plus QRAM detection using the evaluated test, and were excluded. Accordingly, urogenital MG-positive samples (n=145; from 2007 to 2008 (n=21), 2013–2017 (n=64), 2018–2020 (n=60)) and MG-negative samples (n=56 from 2021) collected from 58 men, 138 women and 5 patients with sex not reported were included.

DNA isolation
DNA was isolated from sample (100 μL) using the silica-based manual extraction kit DNA-Sorb-AM (Central Research Institute of Epidemiology).

AmpliSens M. genitalium-ML/FQ-Resist-FL assay
The AmpliSens M. genitalium-ML/FQ-Resist-FL assay includes two real-time PCRs: one detecting MG (gyrB) and wild-type 23S rRNA gene, and the other detecting an internal control and wild-type parC. No amplification of wild-type 23S rRNA gene or parC indicates presence of MRAMs or QRAMs (mutations not specified), respectively. The assay was run on a RotorGene instrument (QIAGEN, GmbH, Hilden, Germany), and results interpreted using the AmpliSens M. genitalium-ML/FQ-Resist software (Central Research Institute of Epidemiology).

Detection of resistance-associated mutations using conventional Sanger sequencing
As reference, MRAMs and QRAMs were identified using Sanger sequencing, as previously described,4 on a 3500×L Genetic Analyzer (Applied Biosystems, Foster City, USA), and FinchT V1.4.0 software.

Statistical analyses
GraphPad Prism V9.0.0 (GraphPad Software, San Diego, USA) was used for statistical analyses. Wilson/Brown method calculated 95% confidence intervals (CI95%). χ² test for trend evaluated temporal trends. Significance was set at p<0.05.

RESULTS
The AmpliSens M. genitalium-ML/FQ-Resist-FL assay detected potential MRAMs with 100% (CI95% 83.9–100) sensitivity, 99.2% (CI95% 95.6 to 100) specificity, 95.2% (CI95% 77.3 to 99.8) positive predictive value (PPV) and 100% (CI95% 97.0 to 100) negative predictive value (NPV). Potential QRAMs were detected with 92.3% (CI95% 66.7 to 99.6) sensitivity, 100% (CI95% 97.2 to 100) specificity, 100% (CI95% 75.8 to 100) PPV and 99.2% (CI95% 95.9 to 100) NPV. Accordingly, only two discordant results were obtained (MRAM reported in one sample with wild-type MG 23S rRNA and QRAM missed in one sample with MG ParC S83R). All 56 MG-negative samples were negative for MR, MRAMs and QRAMs.

Potential MRAMs were detected in 13.8% (CI95% 9.1 to 20.3) of samples. 23S rRNA A2058G was the most prevalent mutation (45.0% (CI95% 25.8 to 65.8)), followed by A2058T (25.0% (CI95% 11.2 to 46.9)), A2059G (20% (CI95% 8.1 to 41.6)), A2059T (5.0% (CI95% 0.3 to 23.6)) and A2062G (5.0% (CI95% 0.3 to 23.6)). Potential QRAMs were found in 9.0% (CI95% 5.3 to 14.7) of samples. S83I was the most prevalent mutation (53.8% (CI95% 29.1 to 76.8)), followed by S83N (23.1% (CI95% 8.2 to 50.3), D87N (15.4% (CI95% 2.7 to 42.2)) and S83R (7.7% (CI95% 0.4 to 33.3)). In 5.5% (CI95% 2.8 to 10.5) of samples, potential MRAM plus QRAM were detected: A2059G and S83I (n=3 samples), A2058G and S83N (n=2), A2058G and D87N, A2058G and S83I, A2059T and S83I (n=1). Potential MRAM and dual resistance rates significantly increased over time: from 0% in 2007–2008 to 25% (p<0.0009) and 10% (p=0.0447), respectively, in 2018–2020. QRAM rate appeared to increase (from 0% to 13%), but significance was not reached (p=0.0605) (Table 1).

DISCUSSION
The first commercial dual resistance assay, AmpliSens M. genitalium-ML/FQ-Resist-FL assay, was sensitive and specific in potential MRAM and QRAM detection in clinical specimens with ≥1000 MG geq/mL. From 2007–2008 to 2018–2020, potential MRAM and QRAM prevalence in St. Petersburg increased, that is, from 0% to 25% (p=0.0009) and 13% (p=0.0605), respectively. These findings are supported by previous Russian MG studies. In 2006–2008, MRAMs were lacking in a minor study in Moscow and in 2013–2015, an MRAM and QRAM prevalence of 4.6% (St. Petersburg: 6.8%) and 6.2% (St. Petersburg: 3.9%), respectively, were identified in four Russian cities. Finally, in 2019, an MRAM and QRAM prevalence of 21.7% and 20.8%, respectively, were described in Moscow. Accordingly, MRAM-guided MG treatment needs to be implemented in Russia.

In the Russian MG guideline, recommended first-line antimicrobials for MG infections are doxycycline (100 mg×2, 10 days) for syndromic treatment, or josamycin (500 mg×3, 10 days) or ofloxacin (400 mg×2, 10 days). For comparison, the European MG guideline recommends doxycycline (100 mg×2, 7 days) for syndromic treatment, and azithromycin (500×1 mg first day, then 250×1 mg on days 2–5) or josamycin (500 mg×3 daily, 10 days) as first-line treatment in the absence of MRAMs, moxifloxacin (400 mg×1 daily, 10 days) when MRAMs detected or as second-line treatment, and...
A2062G mutation is detected as an MRAM. Earlier studies specified. Accordingly, as in 0.7% (1/145) of samples (5% of rRNA gene or romycin, which indicates minor differences in the binding with treatment failure or decreased susceptibility to moxifloxacin, S83R, S83I, D87N or D87Y are most frequently associated of mutated MG strains. Consequently, except in moxifloxacin treatment failures and because no ideal third-line treatment. 1 A review of the Russian MG guideline compared with international evidence-based MG guidelines is recommended.

The limitations of the AmpliSens M. genitalium-ML/ FQ-Resist-FL assay include that reliable detection of potential MRAMs and QRAMs requires ≥1000 MG g eq/mL in clinical specimens, and as observed in the present study and previous studies, approximately a quarter of specimens may have a lower MG load. 1 The assay also detects only wild-type 23S rRNA gene or parC, and 23S rRNA or parC mutations are not specified. Accordingly, as in 0.7% (1/145) of samples (5% of potential MRAM samples) in the present study, the 23S rRNA A2062G mutation is detected as an MRAM. Earlier studies have suggested that this mutation results in resistance to josamycin, but not to the more frequently recommended azithromycin, which indicates minor differences in the binding site of these two macrolides. 2 Consequently, the detection of the 23S rRNA A2062G mutation may slightly overestimate the prevalence of azithromycin resistance in epidemiological surveillance and, in a clinical situation, result in unnecessary use of moxifloxacin treatment. Nevertheless, in M. pneumoniae, the 23S rRNA A2062G mutation has been shown to significantly increase the minimum inhibitory concentrations (MICs) of also pristinamycin, 3 which is the most common third-line treatment in the European MG guideline. 4 This may indicate that this mutation is of importance to detect also in countries not using josamycin for treatment. A larger concern with the AmpliSens M. genitalium-ML/FQ-Resist-FL assay is that different parC mutations cannot be distinguished. Accordingly, as in 2.1% (3/145) of samples (23.1% of potential QRAM samples) in the present study, the ParC S83N is detected as an QRAM, and this mutation may not significantly increase the moxifloxacin MIC and it is of unconfirmed clinical significance. 5 6 8 Thus, detection of also ParC S83N, and other mutations in close proximity with unknown clinical significance, 2 4 8 could overestimate the prevalence of resistance to moxifloxacin. Published data have shown that ParC S83R, S83L, D87N or D87Y are most frequently associated with treatment failure or decreased susceptibility to moxifloxacin. 2 Some parC mutations, such as ParC S83L, have shown a stronger association with moxifloxacin treatment failure than other. 2 9 9 However, also for ParC S83I, a lack of association with moxifloxacin treatment failures has been reported in some cases. 8–10 This highlights the potential role of other factors, including MG load, interactions with other ParC or GyrA mutations (eg, GyrA M95I or D99N mutations together with ParC S83I), 8–10 and possible biological fitness of mutated MG strains. Consequently, except in moxifloxacin treatment failures, surveillance and research, the clinical value of QRAM detection is questionable, that is, due to the suboptimal prediction of moxifloxacin in vitro resistance and treatment failures and because no ideal third-line treatment exists. 1 8 Further research into clinical relevance of several parC mutations (including concomitant gyrA mutations) is imperative.

In summary, the first commercial dual resistance assay, AmpliSens M. genitalium-ML/FQ-Resist-FL assay, was sensitive and specific for detection of potential MRAMs and QRAMs in clinical specimens with ≥1000 MG g eq/mL and could be valuable in resistance-guided therapies (MRAM) and epidemiological surveillance (MRAM+QRAM). Macrolide resistance-guided therapy needs to be implemented in Russia.

Key messages

⇒ The first commercial dual resistance assay, AmpliSens M. genitalium-ML/FQ-Resist-FL, was sensitive and specific and could be valuable in macrolide resistance-guided therapies and epidemiological surveillance.


⇒ Implementation of macrolide resistance-guided therapy for M. genitalium in Russia is recommended, but routine QRAM detection to inform treatment is not recommended.

⇒ Enhanced research regarding parC (and concomitant gyrA) mutations, their associations with moxifloxacin in vitro resistance and treatment failures and novel treatments are essential.
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