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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

Elena Shipitsyna,¹ Tatiana Khusnutdinova,^{1,2} Olga Budilovskaya,^{1,2} Elizaveta Shedko,³ Elena Goloveshkina,³ Guzel Khayrullina,⁴ Anna Krysanova,^{1,2} Kira Shalepo ,^{1,2} Alevtina Savicheva,^{1,2} Magnus Unemo ^{5,6}

For numbered affiliations see end of article.

Correspondence to

Dr Magnus Unemo, Department of Laboratory Medicine, Orebro universitet, SE-701 85 Orebro, Sweden; magnus.unemo@orebroll.se

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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 geq/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 S831 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% ($p_{trend}=0.0009$) and 10% ($p_{trend}=0.0447$), respectively, in 2018–2020. QRAM rate appeared to increase (from 0% to 13%), but significance was not reached ($p_{trend}=0.0605$).

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.¹ MG macrolide resistance levels have rapidly increased internationally.² 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).² Testing MG-positive samples for MRAMs before treatment is recommended internationally.¹ Many laboratory-developed tests and several commercial assays for MRAM detection are available.¹ Recently, SpeDx MG *parC* (beta) PCR assay, the first commercial test for quinolone resistance-associated mutations (QRAMs) detection, displayed high sensitivity and moderate specificity.³

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

Of 198 MG-positive urogenital samples consecutively collected in St. Petersburg during 2007–2008, 2013–2017 and 2018–2020 (all stored at -20°C), 187 (94.4%) remained MG-positive using the AmpliSens *Mycoplasma genitalium*-FL assay



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(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 μ L) 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 \times L Genetic Analyzer (Applied Biosystems, Foster City, USA), and FinchT V.1.4.0 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%). χ^2 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 MG, 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% ($p_{trend}=0.0009$) and 10% ($p_{trend}=0.0447$), respectively, in 2018–2020. QRAM rate appeared to increase (from 0% to 13%), but significance was not reached ($p_{trend}=0.0605$) (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% ($p_{trend}=0.0009$) and 13% ($p_{trend}=0.0605$), 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.⁴ Finally, in 2019, an MRAM and QRAM prevalence of 21.7% and 20.8%, respectively, were described in Moscow.⁵ 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 \times 2, 10 days) for syndromic treatment, or josamycin (500 mg \times 3, 10 days) or ofloxacin (400 mg \times 2, 10 days).⁶ For comparison, the European MG guideline recommends doxycycline (100 mg \times 2, 7 days) for syndromic treatment, and azithromycin (500 \times 1 mg first day, then 250 \times 1 mg on days 2–5) or josamycin (500 mg \times 3 daily, 10 days) as first-line treatment in the absence of MRAMs, moxifloxacin (400 mg \times 1 daily, 10 days) when MRAMs detected or as second-line treatment, and

Table 1 Prevalence and temporal trends of potential macrolide and quinolone resistance-associated mutations in *Mycoplasma genitalium* in St. Petersburg, Russia

Mutations	No. of MG-positive samples* with mutations (CI95%)			P _{trend}
	2007–2008 (n=21)	2013–2017 (n=64)	2018–2020 (n=60)	
Macrolide resistance-associated mutations	0 (0 (0 to 15.5))	5 (7.8 (3.4 to 17.0))	15 (25.0 (15.8 to 37.2))	0.0009
Quinolone resistance-associated mutations	0 (0 (0 to 15.5))	5 (7.8 (3.4 to 17.0))	8 (13.3 (6.9 to 24.2))	0.0605
Macrolide plus quinolone resistance-associated mutations	0 (0 (0 to 15.5))	2 (3.1 (0.6 to 10.7))	6 (10.0 (4.7 to 20.1))	0.0447

*Cervicovaginal swab specimens from women and urethral swab specimens (also some few urine specimens) from men.

pristinamycin (1 g×4 daily, 10 days), minocycline (100 mg×2 daily, 14 days) or doxycycline (100 mg×2 daily, 14 days) as 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 geq/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.⁴ 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,⁷ which is the most common third-line treatment in the European MG guideline.¹ 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.^{2,4,8} 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, S83I, D87N or D87Y are most frequently associated with treatment failure or decreased susceptibility to moxifloxacin.^{2,8} Some *parC* mutations, such as ParC S83I, have shown a stronger association with moxifloxacin treatment failure than other.^{2,8,9} 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 geq/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.

QRAM detection should not routinely inform treatment, but it is valuable for following up moxifloxacin treatment failure, and in epidemiological surveillance and research. International surveillance of resistance-associated mutations in MG, further research into clinical relevance of several *parC* mutations (and also concomitant *gyrA* mutations) and novel treatments are essential.

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.
- ⇒ The prevalence of *M. genitalium* potential macrolide resistance-associated mutation and quinolone resistance-associated mutation (QRAM) in St. Petersburg, Russia dramatically increased between 2007–2008 and 2018–2020.
- ⇒ 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.

Author affiliations

¹Department of Medical Microbiology, D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russian Federation

²Department of Clinical Laboratory Diagnostics, St. Petersburg State Pediatric Medical University, St. Petersburg, Russian Federation

³Laboratory of Molecular Diagnostics and Epidemiology of Reproductive Tract Infections, Central Research Institute of Epidemiology, Moscow, Russian Federation

⁴Group of Development and Implementation of New Technologies and Products, Central Research Institute of Epidemiology, Moscow, Russian Federation

⁵WHO Collaborating Centre for Gonorrhoea and Other STIs, Department of Laboratory Medicine, Orebro universitet, Orebro, Sweden

⁶Institute for Global Health, University College London, London, UK

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ORCID iDs

Kira Shalepo <http://orcid.org/0000-0002-3002-3874>

Magnus Unemo <http://orcid.org/0000-0003-1710-2081>

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ABSTRACT in native language

Задачи Резистентность *Mycoplasma genitalium* (MG) к антимикробным препаратам является глобальной проблемой общественного здравоохранения, недостаточно исследуемой и контролируемой. Мы валидировали первый коммерческий тест, AmpliSens *M. genitalium*-ML / FQ-Resist-FL, для одновременного определения мутаций, потенциально ассоциированных с устойчивостью к макролидам и хинолонам (МАРМ и МАРХ, соответственно), и оценили распространенность МАРМ и МАРХ в Санкт-Петербурге (Россия).

Методы Урогенитальные пробы, положительные на MG (n = 145; получены в 2007-2020) и отрицательные на MG (n = 56 в 2021) при рутинной диагностике, были ретроспективно проанализированы на МАРМ и МАРХ с использованием теста AmpliSens *M. genitalium*-ML / FQ-Resist-FL (Центральный научно-исследовательский институт эпидемиологии, Москва, Россия) и секвенирования по Сэнгеру для валидации результатов.

Результаты Тест AmpliSens *M. genitalium*-ML / FQ-Resist-FL обнаружил потенциальные МАРМ и МАРХ с чувствительностью 100% (CI95% 83,9-100) и 92,3% (CI95% 66,7-99,6) и специфичностью 99,2% (CI95% (95,6-100) и 100% (CI95% 97,2-100), соответственно, в клинических образцах, содержащих ≥ 1000 MG ГЭ/мл. В целом, МАРМ были обнаружены в 13,8% (CI95% 9,1–20,3) образцов, при этом наиболее распространенной была мутация в позиции 23S рРНК A2058G (45,0% [CI95% 25,8–65,8]). МАРХ были обнаружены в 9,0% (CI95% 5,3–14,7%) образцов, и наиболее частой была мутация S83I (53,8% [CI95% 29,1–76,8]). Двойная резистентность была обнаружена в 5,5% (CI95% 2,8-10,5) образцов. Частота мутаций, ассоциированных с резистентностью к макролидам и к обеим группам антибиотиков значительно возросла со временем: с 0% в 2007-2008 до 25% (P_{trend} 0,0009) и 10% (P_{trend} 0,0447) в 2018-2020,

соответственно. Частота мутаций, ассоциированных с резистентностью к хинолонам, возросла с 0 до 13%, но различия не достигли статистической значимости (P_{trend} 0,0605),.

Выводы Быстрый рост резистентности MG к антимикробным препаратам в Санкт-Петербурге, особенно заметный для МАРМ, требует внедрения в России терапии, основанной на результатах определения МАРМ. Первый коммерческий тест для одновременного определения резистентности к макролидам и хинолонам, AmpliSens *M. genitalium*-ML / FQ-Resist-FL, высокочувствителен и высокоспецифичен для обнаружения МАРМ и МАРХ, и может применяться в терапевтических схемах, основанных на результатах определения МАРМ, и, вероятно, для надзора за МАРХ. Необходимы международный мониторинг мутаций в MG, связанных с резистентностью к антимикробным препаратам, дальнейшие исследования клинической значимости ряда мутаций в гене *parC* и разработка новых препаратов.

Ключевые слова: *Mycoplasma genitalium*, мутации, определяющие резистентность к макролидам и фторхинолонам, 23S рРНК, *parC*, тест AmpliSens *M. genitalium*-ML / FQ-Resist-FL.