High levels of macrolide resistance-associated mutations in Mycoplasma genitalium warrant antibiotic susceptibility-guided treatment

R. H. T. Nijhuis1*, T. T. Severs2, D. S. J. M. Van der Vegt1,3, A. A. Van Zwet1 and J. G. Kusters2

1Laboratory for Medical Microbiology and Immunology, Rijnstate, Velp, The Netherlands; 2Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands; 3Laboratory of Medical Microbiology, Stichting PAMM, Veldhoven, The Netherlands

*Corresponding author. Tel: +31-88-0055455; E-mail: rht.nijhuis@outlook.com

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Objectives: Mycoplasma genitalium is a sexually transmitted pathogen, and infection with it is usually treated with macrolides. Unfortunately, emerging resistance to the macrolides has been associated with mutations in region V of the 23S rRNA gene. The aim of this retrospective study was to describe the incidence of macrolide resistance-associated mutations in M. genitalium from patients in the Netherlands.

Methods: All urogenital samples obtained from patients visiting a general practitioner or hospital in the east of the Netherlands that tested positive using the routine M. genitalium real-time PCR (February 2012–November 2014) were included. Following a PCR targeting the 23S rRNA gene, sequencing of the PCR fragments was performed to identify possible macrolide resistance-associated mutations.

Results: Forty-eight of the 153 samples (31.4%) included in this study contained a mutation in the 23S rRNA gene. This was reduced to 44/146 (30.1%) if only samples from unique patients were included. The predominant mutations identified were A2058G (16/44; 36.3%), A2059G (14/44; 31.8%) and a unique high proportion of A2058T (12/44; 27.3%). Treatment failure was observed in four patients initially infected with M. genitalium containing macrolide resistance-associated mutations, while in one of these patients subsequent treatment with moxifloxacin resulted in a microbiological cure.

Conclusions: This study shows that macrolide resistance-associated mutations in M. genitalium occur with a high frequency. In contrast to studies from other regions, Dutch M. genitalium isolates carry the A2058T mutation at high frequency. Our data confirm the need for prospective detection of macrolide resistance-associated mutations prior to treating patients.

Keywords: antimicrobial resistance, STDs, azithromycin, 23S rRNA

Introduction

Mycoplasma genitalium is a sexually transmitted pathogen that can cause urethritis, cervicitis, pelvic inflammatory disease and possible infertility in women.1 In men, M. genitalium is mostly responsible for non-gonococcal urethritis (NGU), which eventually can turn into chronic NGU.2,3 M. genitalium infections should be treated with the macrolide antibiotic azithromycin, preferably using the 1.5 g extended course (500 mg once, followed by 250 mg/day for 4 days), since a 1 g single-dose treatment has been shown to lead to a greater development of resistance to macrolide antibiotics.4–6

As with many other bacteria, macrolide resistance in M. genitalium is usually the result of an SNP mutation in the 23S rRNA gene.7 In 2008, Jensen et al.8 were the first to describe a failure in treatment of M. genitalium infections using azithromycin and reported that this was associated with mutations at positions 2058/2059 (Escherichia coli numbering) in region V of the 23S rRNA gene. Today, the prevalence of azithromycin resistance-associated mutations is reported to be around 13% in France,9,10 40% in the UK, Australia and Denmark9–13 and even up to 100% in Greenland.14 So far, no data have become available on the prevalence of azithromycin resistance-associated mutations in the Netherlands.

The aim of this study was to describe the mutations found in region V of the 23S rRNA gene of M. genitalium-positive clinical samples in the Netherlands.

Materials and methods

Sample selection

All urogenital samples that were sent for M. genitalium diagnostics and urogenital samples from patients with clinical symptoms related to
possible M. genitalium infections (February 2012–November 2014) sent to the Laboratory for Medical Microbiology and Immunology (Rijnstate, Velp, The Netherlands) were included in this retrospective study. The laboratory provides diagnostic services to general practitioners and five hospitals in the east of the Netherlands and covers a total population of ~900,000 inhabitants. No diagnostic services are provided to any sexually transmitted disease (STD) clinic. For routine M. genitalium diagnostics, DNA was extracted using the NucliSENS EasyMAG system (bioMérieux, Marcy-l’Etoile, France). Subsequently, real-time PCR screening for M. genitalium was performed on an ABI 7500 real-time PCR System (Life Technologies, Bleiswijk, The Netherlands), using the primers and probe listed in Table 1. An aliquot of DNA extract from each of the samples that were positive for M. genitalium (Ct value <45) was stored at −20°C until further processing.

Analysis of the 23S rRNA gene for mutations associated with macrolide resistance

A 283 bp fragment of the 23S rRNA gene of M. genitalium was amplified from the stored DNA of the M. genitalium-positive samples using the primers as listed in Table 1. The presence of correctly sized fragments was confirmed by gel electrophoresis. PCR products were sequenced on an ABI 3730 System (Life Technologies).

Results

During the study period, a total of 3202 samples from 2838 patients were tested in routine diagnostic procedures for the presence of M. genitalium. Of these, 421 samples obtained from 378 individuals (13.3%) were determined to be positive. For 374 samples, a DNA aliquot was available for further testing. Of these, 153 samples (mean Ct value 28.7), derived from 146 different patients (55 males and 91 females), were positive for M. genitalium in the second PCR targeting the 23S rRNA gene and could therefore be included in this study. A total of 48 samples from 44 of the 146 different patients (30.1%) were found to contain a mutation in the 23S rRNA gene. A2058G was detected most often (16/44, 36.3%), followed by A2059G (14/44, 31.8%), A2058T (12/44, 27.3%), A2058C (1/44, 2.3%), A2058A (1/44, 2.3%), and A2062C (1/44, 2.3%).

Follow-up samples

Of the 44 patients who were shown to be infected with M. genitalium containing macrolide resistance-associated mutations, five patients had two samples included in this study, at least one of which contained a mutation. For four of these patients, the primary samples contained macrolide resistance-associated mutations and were followed by M. genitalium-positive samples containing the same mutations, most probably due to a failure of azithromycin treatment (Table 2). Only for Patient 8 could a new sample determining microbiological cure be obtained within a reasonable period of time after the M. genitalium-positive follow-up sample, together with information on the treatment regimen (moxifloxacin). For the fifth patient, Patient 25, there were no mutations in the 23S rRNA gene in the primary sample. The test of cure after treatment with azithromycin was determined to be positive for M. genitalium and the sample contained an A2058G mutation.

Discussion

M. genitalium is an emerging sexually transmitted pathogen that can cause serious infections, which are usually treated with the macrolide antibiotic azithromycin. However, in 2008, Jensen et al. reported azithromycin treatment failure among M. genitalium-positive patients with NGU as a result of a mutation in position 2058 or 2059 (E. coli numbering) of region V of the 23S rRNA gene of the M. genitalium isolates. Since this report, the incidence of mutations associated with resistance to this antibiotic is increasing, as illustrated by the results of a Swedish study among patients visiting an STD clinic that reported an increase from 0% (2007) to ~20% (2011). Since no data are currently available from the Netherlands, this study is the first to report the incidence of macrolide resistance-associated mutations in the 23S rRNA gene of M. genitalium in the Netherlands.

In this retrospective study, a total of 374 samples were analysed for the presence of the 23S rRNA gene; only 40.9% of these (153 samples) were found to be positive by gel electrophoresis and could subsequently be tested for the presence of macrolide resistance-associated mutations. This discrepancy in the number of samples positive for sequencing can be explained by the low load of M. genitalium in the agarose-gel-negative samples. The mean Ct value of the MgPa real-time PCR was 28.7 in the samples that showed amplification in the 23S rRNA gene versus 35.3 in the negative samples. Moreover, the gene for MgPa is a multicopy gene, while that for 23S rRNA is a single-copy gene, resulting in a difference in sensitivity of the assays.

Of the 153 included samples obtained from 146 different patients, 30.1% (44/146) contained M. genitalium with a macrolide resistance-associated substitution. However, this high proportion of 30.1% might be an underestimation of the current situation in the Netherlands since patients from STD clinics were not tested in this study (our laboratory provides diagnostic services to general practitioners and five hospitals in the east of the Netherlands). No diagnostic services are provided to any sexually transmitted disease (STD) clinic.

Table 1. Primers and probe used in this study

<table>
<thead>
<tr>
<th>PCR</th>
<th>Target</th>
<th>Oligo name</th>
<th>Sequence (5’–3’)</th>
<th>Final concentration (nM)</th>
<th>Product size</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycgen</td>
<td>MgPa</td>
<td>Mycgen-fw1</td>
<td>GAGAARTACCTTRAGTGCTGCAAA</td>
<td>333</td>
<td>78 bp</td>
<td>adapted from Chalker et al.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mycgen-fw2</td>
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<td>333</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mycgen-rev</td>
<td>GTAAATACATAAAAGGTCTACGGTTGTTAC</td>
<td>333</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mycgen-FAM</td>
<td>FAM-ACTTTGCAATACAGAAGT-MGB</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MG23S</td>
<td>23S</td>
<td>MG23S-fw</td>
<td>GAAAGTTAAGGAGGAGGTAGCAT</td>
<td>150</td>
<td>283 bp</td>
<td>this study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MG23S-rev</td>
<td>CTACCACTCTCAGATGTTGTTT</td>
<td>150</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aTwo forward primers with wobbles were used in order to detect all possible M. genitalium variants, as discussed by Chalker et al.17
services only to general practitioners and hospitals) and an earlier study showed that the highest rate of macrolide resistance was found in patients tested at STD clinics.13

Similar to other studies,10,12,13 substitutions A2058G and A2059G were the most commonly observed mutations found in patients in this study, at 36.3% and 31.8%, respectively. In contrast to other studies, we found an extremely high proportion of A2058T mutations (27.3%). This high proportion of A2058T mutations might be explained by clonal spread, as previously found for the A2058C substitution.4 However, since no further typing was performed, we can neither confirm nor exclude the possibility of clonal spread of M. genitalium containing this A2058T substitution.

This study is the first to describe an A2062C transversion in a M. genitalium-positive sample. So far, only two other studies have reported an A2062 substitution in M. genitalium. Chisment et al.9 identified an A2062T transversion, but were not able to conclude whether this particular mutation was associated with macrolide resistance. In a more recent study, Guschin et al.16 described the first identification of an A2062G transversion in M. genitalium, which was associated with treatment failure using the macrolide antibiotic josamycin. Although an A2062G substitution in closely related Mycoplasma pneumoniae isolates had earlier been shown to result in phenotypical resistance to josamycin, susceptibility with very low MIC values was determined for azithromycin.16

As shown in Table 2, five patients with follow-up samples were included in this study. At least one of the samples contained M. genitalium 23S rRNA sequences carrying a macrolide resistance-associated mutation. Patient 25 initially showed a WT M. genitalium, while the follow-up sample turned out to contain an A2058G substitution. Although a new infection cannot be excluded, it is most likely that this mutation occurred under pressure of antibiotics. For Patient 8, the results determined, together with the information on treatment that was provided, clearly illustrate that azithromycin treatment failure occurred because of the A2058G mutation. Unfortunately, due to the retrospective character of this study, no correct conclusions can be drawn based on the available data for the other four patients.

This retrospective study shows that almost one-third of the patients are infected with M. genitalium containing macrolide resistance-associated mutations, leading to treatment failure. Because of this high proportion, an accurate prospective analysis of macrolide resistance-associated mutations in M. genitalium would be very useful, if not mandatory, both for the correct treatment of individual patients and to prevent the further spread of the resistant microorganisms.

In conclusion, this study is the first to report the incidence of macrolide resistance-associated mutations in samples testing positive for M. genitalium in the Netherlands. Here, we show that a high number of patients were infected with M. genitalium containing macrolide resistance-associated mutations (30.1%) and a high number of A2058T mutations. Since the macrolide antibiotic azithromycin is currently advised for empirical therapy, treatment failure might occur in almost one-third of patients. In order to prevent treatment failure for individual patients and stop the further spread of macrolide-resistant M. genitalium, it is essential to implement a routine detection of macrolide resistance-associated mutations.

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Transparency declarations
None to declare.

References