Detection of macrolide resistance in Mycoplasma genitalium in France

Delphine Chrisment1,2, Alain Charron1,2, Charles Cazanave3, Sabine Pereyre1,2,4† and Cécile Bébéar1,2,4*†

1Univ. Bordeaux, Unité Sous Contrat Mycoplasmal and Chlamydial Infections in Humans, Bordeaux, France; 2INRA, Unité Sous Contrat Mycoplasmal and Chlamydial Infections in Humans, Bordeaux, France; 3Centre Hospitalier Universitaire de Bordeaux, Service Maladies Infectieuses, Bordeaux, France; 4Centre Hospitalier Universitaire de Bordeaux, Laboratoire de Bactériologie, Bordeaux, France

*Corresponding author. Unité Sous Contrat Infections humaines à mycoplasmes et chlamydiae, Université Bordeaux Segalen, 146 rue Léo Saignat, 33076 Bordeaux cedex, France. Tel: +33-5-57-57-16-25; Fax: +33-5-56-93-29-40; E-mail: cecile.bebear@u-bordeaux2.fr
†Equal contribution: S. Pereyre and C. Bébéar co-last-authors.

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Objectives: Mycoplasma genitalium is a sexually transmitted organism associated with non-gonococcal urethritis in men and several inflammatory reproductive tract syndromes in women. Resistance to macrolides has been recently associated with point mutations in the 23S rRNA gene. The aim of this study was to detect these mutations using a large French collection of M. genitalium-positive specimens. We evaluated whether these mutations were related to azithromycin treatment failure and whether macrolide-resistant M. genitalium may be spreading.

Patients and methods: A retrospective study conducted in France between 2003 and 2010 included 156 urogenital clinical specimens from 136 patients that were positive for M. genitalium. Mutations in domain V of M. genitalium 23S RNA were detected using amplification and sequencing. The mutated strains were genotyped by studying single nucleotide polymorphisms in the mgpB gene.

Results: We have detected macrolide resistance-associated mutations in M. genitalium since 2006 at a rate of 13.2%, ranging from 10% to 15.4% of patients per year. Nine mutations at position 2059 as well as two A2058G substitutions, one A2062T substitution and one C2038T substitution (Escherichia coli numbering) were identified in M. genitalium. These patients had treatment failure with azithromycin in 75% (6/8) of cases. For one patient, genotyping showed selection for the mutation during treatment with azithromycin.

Conclusions: For the first time, we describe macrolide resistance for M. genitalium in France and demonstrate that its detection has increased since 2006. Epidemiological surveillance of M. genitalium is necessary to adapt treatments to M. genitalium infections.

Keywords: azithromycin, 23S rRNA mutations, non-gonococcal urethritis

Introduction

Mycoplasma genitalium is a recognized sexually transmitted organism associated with urogenital infections. It is associated with 15%–25% of non-gonococcal urethritis (NGU). In women it is associated with cervicitis, pelvic inflammatory disease and tubal factor infertility.1 M. genitalium is a fastidious species that is difficult to grow from clinical specimens. Thus, nucleic acid amplification tests are necessary for detection and susceptibility testing methods. M. genitalium is intrinsically susceptible to tetracyclines, macrolides and fluoroquinolones.1,2 Treatment trials of M. genitalium-positive NGU have demonstrated that a single 1 g dose of azithromycin is more efficient than doxycycline administered at a 100 mg dose twice daily for 7 days. However, a single 1 g dose of azithromycin is associated with 15%–30% of treatment failures.3,4 An extended 5 day course of azithromycin administered at 500 mg on day 1 followed by 250 mg daily on days 2–5 eradicates M. genitalium from 85%–100% of patients, according to previous studies.1,5 Point mutations at positions 2058 and 2059 (Escherichia coli numbering) in domain V of the 23S rRNA gene, associated with macrolide resistance, have been identified in M. genitalium from patients with chronic NGU or azithromycin treatment failure from Scandinavia, Australia, New Zealand and Japan.2,6–8 This macrolide resistance could be selected during treatment with a single 1 g dose of azithromycin.2,6–8 The aim of this study was to search for mutations associated with macrolide resistance in the 23S rRNA gene from M. genitalium and to determine their prevalence in France using a large collection of M. genitalium-positive specimens. After recording the clinical
history of patients infected with a mutated strain, we evaluated whether these mutations were related to azithromycin treatment failure. Moreover, the types of single nucleotide polymorphisms (SNPs) from mutated M. genitalium were determined to assess the potential for the spread of an M. genitalium macrolide-resistant clone in France.

Materials and methods

One hundred and fifty-six urogenital specimens collected between January 2003 and December 2010 in France were systematically and retrospectively selected in accordance with the guidelines of the Institutional Review Board of the Bordeaux University Hospital, including 53 first-void urine, 18 urethral, 60 vaginal, 19 cervical, 2 rectal, 1 sperm, 1 tubo-ovarian abscess and 1 intrauterine device specimens, as well as 1 liquid from the Pouch of Douglas. These specimens were obtained from 136 patients with a positive diagnosis for M. genitalium; 132 were at Pellegrin Hospital (Bordeaux, France) and 4 were at Saint-Louis Hospital (Paris, France). Among the 136 M. genitalium-positive patients, 71 (52%) were from sexually transmitted diseases (STD) clinics and 65 (48%) were from general practice clinics. These specimens and their DNA extracts were stored at (4°C). Mutations in domain V of the 23S rRNA gene of M. genitalium were detected directly from clinical specimens using amplification and sequencing in accordance with Jensen et al. The mutated bacteria were genotyped by studying SNPs in the mgb8 gene (MG191). Treatment histories from patients infected by M. genitalium with a 23S rRNA mutation were collected from the database of hospital records.

Results and discussion

Among the 136 M. genitalium-positive patients, 80 (59%) were women [median age 25 years (14–57)] and 56 (41%) were men [median age 31 years (20–64)]. M. genitalium infections were more frequent in patients ≤30 years old, who represented two-thirds of the studied population (i.e. 90 patients ≤30 years old out of the 136 M. genitalium-positive patients investigated). Mutations in domain V of the 23S rRNA gene of M. genitalium were detected in 115 patients. Thirteen of these 115 patients (11.3%) were infected with M. genitalium harbouring a 23S rRNA mutation. Among these 13 patients, a similar proportion came from STD clinics (7/13, 54%) and general practice clinics (6/13, 46%). We detected macrolide resistance-associated mutations in M. genitalium since 2006 at a rate of 13.2%, ranging between 10% and 15.4% of patients per year (Figure 1). Before 2006, we likely investigated too few patients to detect these mutations. It should be noted that the difference in the proportion of patients infected with mutated M. genitalium was not significant between 2003–06 and 2007–10 (P=0.19, Yates’s χ² test). A few studies have reported macrolide resistance-associated mutations in M. genitalium, but the number of M. genitalium-positive specimens was too low to accurately assess the prevalence. As a comparison, in the phylogenetically closely related respiratory mycoplasma, Mycoplasma pneumoniae, macrolide resistance-associated mutations emerged in France in 2005 with a similar 10% rate of resistance.

Nine substitutions at position 2059 (six A2059G, two A2059T and one A2059C), two A2058G mutations, one A2062T mutation and one C2038T mutation in the 23S rRNA gene of M. genitalium were discovered (Table 1). Thus, in this study, substitutions at position 2059 were more frequent than mutations at position 2058. Although Jensen et al. recorded an identical number of mutations at positions 2058 and 2059, two recent studies reported substitutions at positions A2059 only.

Substitutions A2058G and A2059G have previously been associated with high-level macrolide resistance in M. genitalium. Substitution A2059C has never been reported in M. genitalium, but it was associated with high-level resistance to macrolides.

![Figure 1](https://academic.oup.com/jac/article-abstract/67/11/2598/707306/Downloaded from https://academic.oup.com/jac/article-abstract/67/11/2598/707306)
in vitro-selected M. pneumoniae mutants. Consequently, the treatment failure in Patient 2 is probably attributable to this substitution. Mutations A2062T, A2059T and C2038T have never been associated with macrolide resistance in a mycoplasma species. Unfortunately, the lack of data on the clinical history of Patients 6 and 8, and the clinical recovery of Patient 9 did not allow the consequences of these substitutions on the macrolide susceptibility of M. genitalium to be assessed. Antimicrobial susceptibility testing using a cell culture-based method with antibiotic dilutions and monitoring by quantitative PCR is under investigation to determine whether substitutions C2038T, A2059T and A2062T are associated with increased macrolide MICs. Mutations in domain II of the 23S rRNA gene and the ribosomal protein L4 and L22 genes were not investigated in this study. In fact, no mutation has been detected in domain II of the 23S rRNA gene in M. genitalium or in the phylogenetically close M. pneumoniae. Mutations in the genes for ribosomal proteins L4 and L22 have been described for M. genitalium, but it was not possible to demonstrate their involvement in macrolide resistance because they were associated with mutations A2058G or A2059G or located outside of the known regions proximal to the macroclide-binding site. More- over, mutations in ribosomal proteins L4 or L22 from M. pneumo- neuriae were associated with lower levels of resistance compared with mutations in domain V of the 23S rRNA gene.

Seven distinct mgpB SNP types were found for the mutated strains (Table 1): four were previously reported (types 1, 2, 4 and 6) and three were newly described in this study (types 89–91; GenBank accession numbers JQ43991–3, respectively). The multiple 23S rRNA substitutions and many mgpB SNP types

<table>
<thead>
<tr>
<th>Patient</th>
<th>Origin, clinic</th>
<th>23S rRNA genotype</th>
<th>SNP type</th>
<th>Before treatment</th>
<th>Prescribed treatment</th>
<th>After treatment</th>
<th>Therapeutic outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Paris, GP</td>
<td>NI</td>
<td>NI</td>
<td>(1) 500 mg of CRO, one injection on day 1, and 200 mg of DOX daily for 18 days</td>
<td>(1) A2059G</td>
<td>(1) 89</td>
<td>(1) clinical failure</td>
<td></td>
</tr>
<tr>
<td>2 Bordeaux, GP</td>
<td>A2059C</td>
<td>4</td>
<td>(1) 1 g of AZM</td>
<td>(1) NI</td>
<td>(1) NI</td>
<td>(2) NI</td>
<td>(2) clinical recovery</td>
</tr>
<tr>
<td>3 Bordeaux, GP</td>
<td>A2058G</td>
<td>6</td>
<td>(1) 200 mg of DOX daily for 7 days</td>
<td>(2) AZM 5 days and 200 mg of CFM daily for 7 days</td>
<td>(2) NI</td>
<td>(2) NI</td>
<td>(2) clinical failure</td>
</tr>
<tr>
<td>4 Bordeaux, STD</td>
<td>A2059G</td>
<td>2</td>
<td>(1) 1 g of AZM daily for 2 days and 200 mg of CFM daily for 7 days</td>
<td>(1) NI</td>
<td>(1) NI</td>
<td>(1) clinical failure</td>
<td></td>
</tr>
<tr>
<td>5 Bordeaux, GP</td>
<td>A2059G</td>
<td>6</td>
<td>(1) NA</td>
<td>(1) NI</td>
<td>(1) NI</td>
<td>(1) NA</td>
<td></td>
</tr>
<tr>
<td>6 Bordeaux, GP</td>
<td>C2038T</td>
<td>1</td>
<td>(1) AMC and OFX daily for 21 days</td>
<td>(1) NI</td>
<td>(1) NI</td>
<td>(1) NA</td>
<td></td>
</tr>
<tr>
<td>7 Bordeaux, STD</td>
<td>A2059G</td>
<td>90</td>
<td>(1) NA</td>
<td>(1) NI</td>
<td>(1) NI</td>
<td>(1) NA</td>
<td></td>
</tr>
<tr>
<td>8 Bordeaux, GP</td>
<td>A2062T</td>
<td>2</td>
<td>(1) NA</td>
<td>(1) NI</td>
<td>(1) NI</td>
<td>(1) NA</td>
<td></td>
</tr>
<tr>
<td>9 Bordeaux, STD</td>
<td>A2059T</td>
<td>ND</td>
<td>(1) AZM 5 days</td>
<td>(1) WT + A2059T</td>
<td>(1) NI</td>
<td>(1) NI</td>
<td>(1) clinical recovery</td>
</tr>
<tr>
<td>10 Bordeaux, STD</td>
<td>WT</td>
<td>ND</td>
<td>(1) 1 g of AZM</td>
<td>(1) NI</td>
<td>(1) NI</td>
<td>(1) NI</td>
<td>(1) clinical failure</td>
</tr>
<tr>
<td>11 Bordeaux, STD</td>
<td>A2059G</td>
<td>4</td>
<td>(1) NA</td>
<td>(1) NI</td>
<td>(1) NI</td>
<td>(1) NA</td>
<td></td>
</tr>
<tr>
<td>12 Bordeaux, STD</td>
<td>WT</td>
<td>2</td>
<td>(1) 1 g of AZM</td>
<td>(1) NI</td>
<td>(1) NI</td>
<td>(1) clinical failure</td>
<td></td>
</tr>
<tr>
<td>13 Bordeaux, STD</td>
<td>NI</td>
<td>NI</td>
<td>(1) 1 g of AZM and 200 mg of CFM daily for 7 days</td>
<td>(1) A2059G</td>
<td>(1) 91</td>
<td>(1) clinical failure</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: (1), first-line treatment; (2), second-line treatment; (3), third-line treatment; AMC, amoxicillin/clavulanic acid; AZM, azithromycin; AZM 5 days, azithromycin administered at 500 mg on day 1 followed by 250 mg daily for days 2–5; CFM, cefixime; CRO, ceftriaxone; DOX, doxycycline; GP, general practice clinic; MXF, moxifloxacin; NA, no data available; ND, M. genitalium was detected, but the SNP type was not determined; NI, M. genitalium was not investigated because no specimen was collected; OFX, ofloxacin; STD, STD clinic; WT, wild-type.
reported herein demonstrate that an M. genitalium macrolide-resistant clone has not spread. Thus, macrolide resistance in M. genitalium in France is likely a polyclonal phenomenon. For two patients (Patients 10 and 12) there was no macrolide resistance-associated mutation before azithromycin treatment, but a substitution in the 23S rRNA gene was observed after treatment. For Patient 12, the SNP type was identical before and after treatment, which demonstrates that this patient was not reinfected by a distinct M. genitalium, but the A2058G substitution was selected during his azithromycin treatments. We confirm herein the risk of macrolide resistance selection by a single 1 g dose of azithromycin, as has been reported by others.2,6–8

Overall, among eight patients infected with a mutated M. genitalium for whom treatment and therapeutic outcomes were available, six patients (75%) (Patients 2, 3, 4, 10, 12 and 13) had a treatment failure with azithromycin at a 1 g or 5 day regimen (Table 1). Interestingly, the extended 5 day course of azithromycin failed in 3/4 of patients (Patients 3, 12 and 13). Thus, in our study, azithromycin regimens were related to many clinical failures in patients infected with a mutated M. genitalium. Moreover, according to the rate of clinical failure with the extended 5 day course of azithromycin, this dosage scheme is not appropriate if M. genitalium has a macrolide resistance-associated mutation. The surprising clinical recovery of patients infected with mutated M. genitalium (Patients 1 and 9) has been reported previously9 and is probably attributable to the immunomodulating properties of azithromycin.10 Moxifloxacin is known to cure patients who have experienced azithromycin treatment failure12 and was successfully used for Patient 12. However, moxifloxacin should be saved for patients with azithromycin failure, because of the risk of the emergence of quinolone resistance. In fact, M. genitalium strains with mutations in the quinolone resistance-determining regions of the parC, parE and gyrB genes as well as strains resistant to both azithromycin and moxifloxacin have emerged.11

In this study, we detected macrolide resistance in M. genitalium since 2006 in France, and showed that the overall prevalence between 2006 and 2010 was 13.2% without spread of a clonal strain. Although a single 1 g azithromycin dose has been recommended as a presumptive treatment for NGU and cervicitis, this regimen could select for macrolide-resistant mutants. Both the single 1 g and the 5 day azithromycin treatments led to a majority of treatment failures for infection by a mutated strain. For these reasons, monitoring the macrolide resistance of M. genitalium strains has both an epidemiological advantage and direct application in clinical practice. It is recommended that the second-line treatment for M. genitalium genital infections is adapted in case of a first azithromycin treatment failure. For enhanced ease in detecting macrolide resistance-associated mutations in M. genitalium, development of a rapid molecular assay is needed.

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Transparency declarations
None to declare.

References